Screening mit nicht-invasiven pränatalen Tests (NIPTs) auf fetale Trisomien T21,18, 13

EUnetHTA-Report

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Health Technology Assessment

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Zusammenfassung

Einführung

Das pränatale Screening umfasst Untersuchungen zur Entwicklung des Fetus und zur Gesundheit der Mutter. Pränatale Diagnostik beinhaltet weiterführende bildgebende wie genetische Interventionen, u.a. nach Chromosomenanomalien. Das durchschnittliche Risiko für eine Chromosomenanomalie beträgt etwa 1:1.000, als Hochrisiko wird 1:300 angenommen. Die Rate von allen schweren angeborenen Anomalien liegt bei 3-4%, wovon nur ein Anteil Chromosomenanomalien sind.

Risiko auf Chromosomenanomalie 1:1.000, Hochrisiko 1:300, alle angeborenen Anomalie: 3-4%

Das gängige Ersttrimester-Screening auf Chromosomenanomalien basiert auf drei Parametern (Combined Test):

herkömmliches Ersttrimester-Screening auf Trisomien: Combined Test

- dem mütterlichen Alter,
- einer Ultraschallmessung der Nackentransparenz (der Flüssigkeitsansammlung (Ödem) zwischen der Haut und dem Weichteilgewebe im Nackenbereich) des ungeborenen Fetus,
- der Bestimmung der Hormone PAPP-A (Pregnancy-associated Plasmaprotein A) und Beta-hCG (humanes Choriongonadotropin) im mütterlichen Blut.

mütterliches Alter
Nackentransparenz
Hormone PAPP-A und Beta-hCG

Mit Hilfe eines speziellen Computerprogrammes wird schließlich aus den Ultraschalldaten gemeinsam mit den biochemischen Werten unter der Berücksichtigung des mütterlichen Altersrisikos ein Gesamtrisiko berechnet. Weitere Ultraschalluntersuchung dienen zur Erkennung von Mehrlingen, Wachstumsstörungen, maternalen Risken wie Präeklampsie, Anomalien der Plazentation und Plazentalokalisation sowie von fetalen Fehlbildungen.

Seit einigen Jahren sind zahlreiche neue nicht-invasive pränatale Tests (NIPTs, auch nicht-invasive pränatale Diagnostik [NIPD]) zur Identifizierung von häufigen Chromosomenanomalien erhältlich.

neue nicht invasive Tests am Markt: NIPT

Beschreibung der Technologie und der Komparatoren

Diese neuen In-Vitro-diagnostischen Tests zur Identifizierung von häufigen Chromosomenanomalien basieren auf der Analyse der zellfreien fetalen DNA (Cell-free fetal DNA, cffDNA) aus dem Blut von Schwangeren zur Identifizierung von häufigen Chromosomenanomalien (Trisomie 21 [T21], Trisomie 18 [T18] und Trisomie 13 [T13]) des Fetus. Derzeit werden 21 kommerziell-erhältliche Tests angeboten. Die häufigsten Anbieter sind

NIPT:
T21, T18, T13

21 NIPTs am Markt angeboten

- Ariosa Diagnostics Inc./Roche Sequencing Solutions Inc. (Harmony ®),
- BGI Diagnostics (NIFTY™),
- Igenomix (NACE®),
- LifeCodexx AG (PrenaTest®),
- Natera (Panorama®),
- Sequenom Laboratories (MaterniT® 21 PLUS, nur in den USA vermarktet) und
- Illumina (Verifi™).

Die Techniken von NIPTs sind vielfältig: einige verwenden die Polymerase-Kettenreaktion (PCR) zur Amplifikation der zellfreien DNA (cfDNA), während andere auf molekularer Karyotypisierung (Chromosomale Microarray-Analysen, CMA) beruhen. Darüber hinaus unterscheiden sie sich in den technologischen Verfahren hinsichtlich der Analyse und Interpretation von Screening-Ergebnissen, ebenso wie in unterschiedlichen Qualitätsstandards und Art der Ergebnisberichte. Unabhängig von den Tests benötigen alle einen ausreichenden Anteil an cfDNA im mütterlichen Plasma, um zwischen dem Status der Mutter und dem des Fetus differenzieren zu können. Nicht alle Labore quantifizieren jedoch die fetalen Anteile in einzelnen Proben. Die Untersuchung muss ab der 8. bis 10. Schwangerschaftswoche durchgeführt werden.

verschiedene NIPT Techniken:

PCR, CMA
Unterschiede in Analyse und Interpretation, Qualitätsstandards, Ergebnisberichterstattung

Die meisten NIPTs werden für T21-, T18- und T13- und Aneuploidien der Geschlechtschromosomen angeboten, aber viele Labore haben ihre Analysepalette um andere Trisomien und gängige Mikrodeletionen erweitert. Abhängig von den Tests werden sie für Einlings-, Zwillings-, Eizellspende- oder In-Vitro-Fertilisationsschwangerschaften angeboten.

Erweiterung des Analyse-Spektrums:
Geschlechtsbestimmung
Mikrodeletionen

NIPTs können sowohl im primären wie auch im sekundären Screening zum Einsatz kommen. In den meisten europäischen Ländern werden NIPTs derzeit vor allem durch private Anbieter vermarktet, nicht aber im öffentlichen Gesundheitssystem finanziert. In den meisten dieser Länder hat das Screening auf fetale Aneuploidien als Screening an Frauen mit hohem Risiko anschließend an auffällige oder unklare Befunde begonnen, obwohl einige Länder auch überlegen, die Tests für alle Schwangeren anzubieten.

Einsatzbereiche NIPTs:
primäres Screening oder sekundäres Screening bei auffälligem oder unklarem Befund

Derzeit eingesetzte und – in klinischen Leitlinien identifizierte – Vergleichsinterventionen für nicht-invasive Screening-Optionen umfassen Informationen zum mütterlichen Alter in Kombination mit folgenden Interventionen:

1. Ersttrimester-Screening (vgl. oben Combined Test: fetale Nackenfalten-Messung (NT) und fetale Scheitel-Steiß-Länge (CRL), Informationen zum mütterlichen Alter und mütterliche Serum-biochemische Marker [PAPP-A, Beta-hCG]),
2. Zweittrimester- Screening (Organscreening, Doppler-Ultraschall Triple Test [freies Estriol, Beta-hCG, Alpha-Fetoprotein]), oder
3. Zweistufiges integriertes pränatales Screening: das Serum-Screening im ersten und zweiten Trimester mit oder ohne NT (für „Kontingente“ [alle Hochrisiko Schwangere] oder sequentiell [erst nach positivem Befund]).

in integriertem Screening:
„Kontingente“ (alle Hochrisiko Schwangere) oder sequentiell (nach positivem Befund)

Die Bestätigung eines positiven Ergebnisses erfordert invasive Tests (Chorionzottenbiopsie [CVS] ab der 12. Schwangerschaftswoche oder Amniozentese [AC] ab der 16. Schwangerschaftswoche). Beide Verfahren sind mit einem Fehlgeburtsrisiko verbunden (2-10 von 1.000 Schwangerschaften).

bei bestätigtem positiven Ergebnis:
invasive Verfahren (CVS, AC)

Der hauptsächliche Vorteil von NIPT im Vergleich zum konventionellen Screening-Ansatz besteht in der Einfachheit der Durchführung und der Nicht-Invasivität des Tests sowie in der möglichen Verringerung der falschpositiven [FP] Ergebnisse. Es wird erwartet, dass NIPT unnötige invasive Verfahren (AC und CVS) vermeidet und das Risiko von Komplikationen, Schwangerschaftsverlust und Angstzuständen minimiert. Zudem wird erwartet, dass die NIPTs auch eine frühere Testung erlauben, was den Vorteil hätte, den Schwangeren bzw. den Eltern mehr Zeit zu geben, Entscheidungen für/gegen einen Schwangerschaftsabbruch zu treffen.

hohe Erwartungen an NIPT:
weniger FP-Ergebnisse;
weniger invasive Folgeinterventionen

Das Ziel des vorliegenden EUnetHTA Assessments ist die Bewertung der Wirksamkeit und Sicherheit von nicht-invasiven pränatalen Tests (NIPT) für das Screening auf fetale Trisomie 21 (T21), Trisomie 18 (T18) und Trisomie 13 (T13) bei schwangeren Frauen ab der 8. bis 9. Schwangerschaftswoche.

EUnetHTA: Bewertung zu 5 Einsatzgebieten:

Für die Bewertung der NIPTs werden fünf Einsatzbereiche berücksichtigt:

1. NIPT als Primär-Screening-Test als *Ersatz* für Ersttrimester-Screening/Combined Test (alle Schwangeren)
2. NIPT als Ergänzung (*Add-on*) zu Ersttrimester-Screening/Combined Test (alle Schwangeren)
3. NIPT als Ergänzung (*Add-on*) zu Ersttrimester-Screening/Combined Test für die Hochrisiko-Population
4. NIPT als Ergänzung (*Add-on*) zu Ersttrimester-Screening/Combined Test für Schwangere mit hohem und mittlerem Risiko
5. NIPT als *Ersatz* für invasive Tests

1 Trimester Ersatz - alle
1 Trimester (Add-on) – alle
1 Trimester (Add-on) nur Hochrisiko
1 Trimester (Add-on) Mittel- und Hochrisiko
als Ersatz für invasive Tests

Gesundheitsproblematik

T21 (Down-Syndrom [DS]), T18 (Edwards-Syndrom) und T13 (Pätau-Syndrom) sind die häufigsten Chromosomenstörungen bei Neugeborenen. In Europa liegen die geschätzten Prävalenzen bei 24 (T21), 5,6 (T18) und 2,08 (T13) pro 10.000 Lebendgeburten. Etwa 68,7% der T21-Fälle, 94,3% der T18-Fälle und 93,4% der T13-Fälle werden pränatal diagnostiziert, obwohl die Prävalenz je nach Screening-Strategie (<30% bis ≥ 90%) zwischen den industrialisierten Ländern erheblich variieren kann.

Prävalenzen
T21: 24 in 10.000 Lebendgeburten
T18: 5,6
T13: 2,08

In Europa wird geschätzt, dass mehr als 90% der Menschen mit Down-Syndrom (T21) länger als 20 Jahre leben, und ungefähr 60% sogar ein Alter von 60 Jahren erreichen. Es wird geschätzt, dass in der EU jedes Jahr etwa 5.000 Neugeborene von T21 betroffen sind. Im Gegensatz zu T21 ist die Lebenserwartung von Kindern mit Pätau-Syndrom (T13) und Edwards-Syndrom (T18) aufgrund von Fehlbildungen gering. Die meisten Schwangerschaften enden mit spontanen Fehlgeburten oder Totgeburten, und wenn die Kinder geboren werden, überleben nur wenige mehr als ein Jahr.

Lebenserwartung
T21 bis 60 Jahre
T13, T18 max. 1 Jahr

In den meisten europäischen Ländern werden Ersttrimester-Screenings/Combined Tests für alle schwangeren Frauen in Form von nationalen oder regionalen Schwangeren Screening-Programmen angeboten. In Europa liegt die Schwelle für Tests, die häufig zur Definition eines hohen Risikos verwendet werden, bei 1:250 bis 1:300, obwohl dies von Land zu Land unterschiedlich gehandhabt wird. Klinische Leitlinien empfehlen keine weiteren Tests für Frauen mit niedrigem Risiko. Wenn eine Kontingent-Screening-Strategie angeboten wird, werden Schwangere in Hoch-, Mittel- und Niedrigrisikogruppen klassifiziert. Die Schwelle für mittleres Risiko ist allerdings in den meisten Ländern nicht standardisiert.

in EU-Schwangeren Screening–Programmen
Definition von hohem Risiko: 1: 250 bis 1:300

Klassifizierung in Hoch-, Mittel- und Niedrigrisikogruppen

Die NIPTs sind für alle schwangeren Frauen, die sich für ein pränatales Screening auf T21, T18 und T13 entscheiden, zugelassen. Dies würde bedeuten, dass etwa 5,1 Millionen Schwangerschaften in der EU-28 (EUROSTAT-Fertilitätsstatistik) als mögliche Kandidatinnen für ein NIPT-Screening in Frage kommen. Die genaue Zielpopulation ist jedoch schwer abzuschätzen, da sie sich wesentlich davon unterscheidet, ob eine 1st-line- oder eine 2nd-line-Screening Strategie geplant ist, und auch wo die Risikoschwelle angesetzt wird.

Zulassung der NIPTs: für alle Schwangeren
Hochrechnungen für NIPTs von Screening-Strategie und Risikoschwelle abhängig

Methode

EUnetHTA HTA Core Model®

Das Assessment wurde im EUnetHTA HTA Core Model® REA Version 4.2 erstellt. Eine systematische Suche wurde von Februar bis März 2017 (ohne zeitliche Beschränkung) in 5 Datenbanken durchgeführt:

Literatursuche in 5 Datenbanken

- MEDLINE (PubMed)
- Embase (OVID SP)
- Centre for Reviews and Dissemination (CRD)-Database,
- ISI Web of Knowledge und
- Cochrane Library (Wiley)

sowie Leitlinien

Zusätzlich wurden Leitlinien-Register durchsucht, um relevante Leitlinien zum Schwangeren-Screening zu identifizieren (nach 2010 veröffentlicht).

laufende klinische Studien

Laufende klinische Studien wurden in…gesucht.

- ClinicalTrials.gov
- EU Clinical Trials Register
- International Clinical Trials Registry Plattform (ICTRP) und
- UK Clinical Trials Gateway

ergänzt durch Handsuche im Internet

Allgemeine Internetsuchen und manuelle Recherchen von Zitaten dienten als ergänzende Informationsquellen.

Kontaktierung der Hersteller

Die zum Zeitpunkt der Suche identifizierten Hersteller wurden vom LBI-HTA kontaktiert, um Informationen bezüglich NIPT CE-Kennzeichnung (Art des CE-gekennzeichneten Produkts und Indikationen) und Beschreibungen der unterschiedlichen Technologieeigenschaften zu erhalten.

Ein-/ Ausschluss von Studien nach vordefinierten Kriterien

Zwei AutorInnen von AVALIA-T schlossen relevante Studien nach definierten Kriterien (PICO) basierend auf den Scoping-Fragen ein. Studien, die keine Daten zu relevanten Endpunkten lieferten oder von denen angenommen wurde, dass sie ein inakzeptables Bias-Risiko haben, wurden ausgeschlossen. Die Gründe für den Ausschluss waren folgende:

- gemischte Populationen mit unklaren Auswahlkriterien;
- retrospektive Kohorten- oder Fallkontrolldesigns;
- Mangel an Informationen zum Indextest oder Referenzstandard;
- fehlende unabhängige Bewertung des Indextests/ Referenzstandards;
- Ungeeignete Bezugsgrößen hinsichtlich der Mehrheit der Schwangeren.

unabhängige Auswahl, Datenkontrolle durch 2 AutorInnen

Nicht nur die Studienselektion, sondern auch Bias-Risiko- wie Qualitätsbeurteilung wurde von 2 AutorInnen unabhängig voneinander durchgeführt, und Diskrepanzen wurden im Konsens gelöst. Für die Bereiche klinische Wirksamkeit und Sicherheit wurden die relevanten Daten extrahiert und in Evidenztabellen von einem/r AutorIn von AVALIA-T durchgeführt und von dem/ der Ko-AutorIn überprüft.

Das QUADAS-2-Tool wurde zur Bewertung (RoB – „Risk of Bias“) der diagnostischen Genauigkeits-Studien verwendet. GRADE wurde zur Beurteilung der Qualität der Evidenz herangezogen. Für andere Domänen (organisatorische, ethische und soziale Aspekte) wurde kein Tool zur Qualitätsbewertung verwendet.

RoB Beurteilung mit QUADAS-2

Beurteilung der Qualität der Evidenz: GRADE

Statistische Analysen wurden entsprechend den Empfehlungen der EUnetHTA-Leitlinie „Meta-Analyse von diagnostischen Genauigkeitsstudien“ durchgeführt. Es wurde ein bivariates Random-Effects-Modell verwendet, außer wenn das Modell unzuverlässige Parameterschätzungen lieferte. In diesem Fall wurden zwei univariate Random-Effects-Modelle verwendet.

statistische Analysen mit bi-/univariaten Random-Effects-Modellen

Als Referenztests werden die in klinischen Leitlinien identifizierten Verfahren herangezogen (vgl. oben). Als Referenzstandards werden fetale Karyotypisierung (Untersuchung des gesamten Genoms zur Diagnostik kleinster genomischer Veränderungen) oder Geburtsergebnisse bestimmt durch klinische Untersuchung oder Follow-Up des Neugeborenen verwendet.

Referenztests und Referenzstandards

Die Wirksamkeit der Screening-Verfahren wird ….beurteilt

Wirksamkeitsendpunkte

- in Bezug auf sekundäre Endpunkte (Sensitivität, Spezifität, positiver prädiktiver Wert [PPV] und negativer prädiktiver Wert [NPV]), aber auch
- in Bezug auf primäre Endpunkte, wie die Reduzierung unnötiger invasiver Tests und die Beurteilung der Auswirkungen (Kinder, geboren mit undiagnostizierten T13, T18 und T21, Reduktion der Fehlgeburten im Zusammenhang mit invasiven Tests [AC, CVS], etc.)

Sekundäre: PPV, NPV
Primäre: Reduktion invasiver Tests und von Tot-Fehlgeburten durch CVS+AC

Die Sicherheit wird anhand von … beurteilt.

Sicherheitsendpunkte FP, NP, Testfehler Anstieg von Geburten mit anderen Anomalien Anstieg Schwangerschafts-abbrüche - andere Anomalien

- Falsch-positive Raten (FP),
- Falsch-negative Raten (FN),
- Testfehlern
- Anstieg der Anzahl von Neugeborenen, die mit anderen pränatal nicht nachzuweisenden chromosomalen Anomalien geboren wurden (nicht durch pränatales Aneuploidie-Screening identifizierbar) und
- der Anstieg der elektiven Schwangerschaftsabbrüche bei anderen Chromosomenanomalien mit unsicherer Signifikanz.

Für die Beurteilung der Wirksamkeit und Sicherheit werden randomisierte kontrollierte klinische Studien, nicht randomisierte kontrollierte klinische Studien und diagnostische Genauigkeitsstudien herangezogen. Darüber hinaus werden auch Register für die Beurteilung der Sicherheit herangezogen.

Studiendesigns: RCTs, CTs, diagnostische Genauigkeitsstudien

Qualitative Studien und Konsensdokumente werden für die organisatorischen, ethischen und sozialen Aspekte herangezogen.

qualitative Studien: organisatorische, ethische, soziale Aspekte

Ergebnisse: Verfügbare Evidenz

Direkte Evidenz für die Bewertung der klinischen Wirksamkeit und Sicherheit wurde nur für NIPT als primäre Testmethode gefunden (Ersatz des Combined Tests). Dazu liegen fünf vergleichende diagnostische Genauigkeitsstudien und vier nicht-komparative Studien vor, die bei Einlingsschwangerschaften durchgeführt wurden (vgl. Tabelle 6 in EUnetHTA Bericht). Zu patientenrelevanten Endpunkten liegen keine Daten vor.

Einlinge:
Evidenz für NIPT als primäre Testmethode (Ersatz)
5 komparative, 4 nicht-komparative Studien

Die Frage nach NIPT als Ergänzung zum Combined Test bei Einlingsschwangerschaften mit hohem Aneuploidierisiko (basierend auf Ergebnissen aus Combined Test oder klinischen Informationen) wurde indirekt durch Daten aus 26 Studien beantwortet, die NIPT als abgestufte Strategie untersuchten (vgl. Tabelle 7 in EUnetHTA Bericht). Die Add-on-Strategie für Schwangere mit mittlerem Risiko wurde nur in einer Studie untersucht (vgl. Tabelle 8 in EUnetHTA Bericht).

Evidenz für NIPT als primäre Testmethode (Ergänzung) – Hochrisiko: 26 Studien Mittleres Risiko: 1 Studie

Sechs Studien lieferten Daten zur Genauigkeit von NIPT für Zwillingspopulationen (vgl. Tabelle A5 in EUnetHTA Bericht).

Zwillinge: 6 Studien

Das Szenario des NIPT als Ersatz für invasive Tests wurde nicht berücksichtigt, da derzeit keiner dieser Tests für diesen Zweck zugelassen ist.

keine Evidenz zu NIPT als Ersatz für invasive Tests

Ergebnisse: Klinische Wirksamkeit

Die diagnostische Genauigkeit von NIPT als primäre Testmethode für T21 wurde auf der Basis von 136.544 schwangeren Frauen (885 Aneuploidien und 135.659 Euploidie-Fälle) berechnet. Die Meta-Analyse mit dem bivariaten Random-Effects-Modell ergab eine gepoolte Schätzung der Sensitivität von 99,3% (95% Konfidenzintervall (CI) 97,8%-99,8%) und Spezifität von 99,9% (95% CI, 99,8%-99,9%). Die Ergebnisse unterscheiden sich nicht vom univariaten Modell.

NIPT als primäre Testmethode (Ersatz) bei T21, Ergebnisse der Metaanalyse: 99,3%/ 99,9% (Sensitivität/ Spezifität)

In den vier Vergleichsstudien zeigte NIPT eine höhere Sensitivität im Vergleich zum Standard-Screening (100% vs. 94%, p <0,001). Die Spezifität war ebenfalls signifikant höher. Die PPV in den Studien betrug zwischen 80% und 100%, außer in einer Studie, die einen PPV von 45,5% berichtet. Der NPV betrug in allen in die Bewertung einbezogenen Studien mehr als 99%. Die PPV und NPV waren signifikant höher für NIPT als für den Combined Test in einer der beiden Studien, die eine statistische vergleichende Analyse dieser Ergebnisse (PPV von 80,9% vs. 3,4% und NPV von 100% vs. 99,9%) vorlegte. In einer Studie wurde kein Unterschied gefunden.

NIPT schneidet in allen sekundären Endpunkten (PPV, NPV) besser oder gleich ab

Insgesamt war die Qualität der Evidenz (QoE) für T21 - mit GRADE bewertet - moderat für die Sensitivität und niedrig für die Spezifität. Die QoE für T18 und T13 wurde aufgrund der wenigen Fälle, des Bias-Risikos und/ oder der Ungenauigkeit der Schätzungen als niedrig und sehr niedrig für Sensitivität und Spezifität bewertet.

GRADE: moderate und niedrige Qualität der Evidenz für T21 niedrig bis sehr niedrig T13/ T18

Die Studien, die Daten zur Genauigkeit des NIPT als Zweitstufentest lieferten, betrafen 1.408 Fälle von Aneuploidie und 99.818 Euploidie-Fälle. Die gepoolte Sensitivität unter Anwendung der bivariaten Random-Effekt-Modelle betrug 99,2% (95% CI, 98,6%-99,6%) und die Spezifität 99,9% (95% CI, 99,9%-99,9%).

NIPT als sekundäre Testmethode (Ergänzung) : vergleichbare Ergebnisse wie oben

Somit waren die Testgenauigkeitsergebnisse unabhängig von der Position des NIPT innerhalb der Screening-Strategie sehr ähnlich.

Die Evidenzbasis für Zwillingspopulationen wurde aufgrund der geringen Fallzahl, des Verzerrungspotenzials und der Ungenauigkeit der Schätzungen als sehr niedrig eingestuft. Die erhobenen Studien umfassten 33 T21-Fälle und 1.547 Euploidie-Fälle.

Evidenz bei Zwillingen: geringe Fallzahl, RoB hoch, ungenau

Die eingeschlossenen Studien lieferten keine Daten zu primären Endpunkten wie zur Reduktion invasiver Tests (AC oder CVS).

keine Evidenz zur Reduktion invasiver Tests

Ergebnisse: Sicherheit

Die wichtigsten vergleichenden Informationen zur Sicherheit beziehen sich auf Ergebnisse zu sekundären Endpunkten (FP- und FN-Raten) und sog. „No-Call"-Ergebnisse (Testfehler, Proben geringer Qualität, unklare Befunde). Für T21 waren die FP- und FN-Raten, die ohne Fehlgeburten und „No-Call" Ergebnisse berechnet wurden, bei Combined Tests höher als bei NIPT. Die Studien zeigten auch höhere FP-Raten mit Standard-Screening als mit NIPT für T18 und T13, aber waren in Bezug auf FN-Raten inkonsistent. Die FP- und FN-Raten stiegen an, wenn die „No-Call"-Ergebnisse (zurückgerufene Proben) in die Analyse einbezogen wurden. Der Anteil der Testfehler betrug in den Studien zwischen 0,5% und 3%.

No-Call Ergebnisse: Testfehler, Proben geringer Qualität, unklare Befunde

FP+FN höher bei Standard als mit NIPT, allerdings höher als berichtet, wenn zurückgerufenen Proben in Analyse blieben

Die FP-Rate, die für T21 und T18 beobachtet wurde, war in der überwiegenden Mehrheit der Studien, die NIPT als einen abgestuften (sekundären) Test bewerteten, null oder sehr niedrig (<0,5%). Die FN-Rate war in den meisten Studien ebenfalls null, obwohl die FN-Rate für T13 und T18 in den wenigen Studien, die FN-Fälle berichteten, stark variierte (2,6%-37,5% bzw. 12,5%-100%). Die bei Zwillingsschwangerschaften beobachteten FP- und FN-Raten waren ebenfalls sehr unterschiedlich.

NIPT als Zweitlinientest: 0-0,5% FP/FN bei T21

große Unterschiede bei T13+T18 und bei Zwillingen

Nur eine Studie liefert vergleichende Informationen über andere Chromosomenanomalien (z. B. andere Trisomien, chromosomale Deletionen oder Doppelungen). Diese Studie ergab, dass NIPT allein 8 von 13 Fällen anderer Chromosomenaberrationen *nicht* fand. Die Combined Teststrategie fand nur 4 dieser 13 Fälle *nicht*.

NIPT bei anderen Anomalien: schlechter wie Kombinationstest

Relative Wirksamkeit

Für drei der fünf Forschungsfragen liegen keine Daten vor:

- NIPT als Teil von Ersttrimester-Screening für alle Schwangere
- NIPT als Ergänzung zu Ersttrimester-Screening für die Schwangere mit hohem und mittlerem Risiko;
- NIPT als Ersatz für invasive Tests.

keine Evidenz für 3 von 5 potentiellen Screening-Strategien

Für die verbleibenden zwei Fragen

- NIPT als primäre Screeningmethode als Ersatz für den herkömmlichen Combined Test und
- NIPT als Ergänzung zum Combined Test für die Hochrisikopopulation (1 in 300 Cut-off-Punkt)

aber: Evidenz zu 2 Screening-Strategien

liegen ausreichende Daten aus den diagnostischen Genauigkeitsstudien zur Bewertung von Sensitivität, Spezifität, PPV, NPV und zur Testausfallrate vor.

Aufgrund des Mangels an direkten Daten zu primären Endpunkten wurde eine Simulationsmodellierung durchgeführt, um die Screening-Strategien mit NIPT im Vergleich zur aktuellen Screening-Praxis in Bezug auf Sensitivität und Spezifität und deren Auswirkung auf die Anzahl der für T21 erforderlichen invasiven Tests zu vergleichen. Schätzungen der Genauigkeit des Combined Tests wurden aus einem Cochrane Review übernommen. Die QoE für die Sensitivität und Spezifität für T18 und T13 bei Hochrisiko-Schwangeren wurde als niedrig eingestuft, und eine Modellierung wurde als nicht angemessen erachtet.

Simulations-modellierung für vergleichende Wirksamkeit zu den 2 Screening-Strategien

Daten zum Vergleich aus Cochrane Review

Simulationsmodellierung: NIPT als primäre Screening-Methode als Ersatz für Ersttrimester-Screening auf T21

NIPT als primäre Screening-Methode als Ersatz

Auf der Grundlage der 2 x 2-Testgenauigkeitsdaten des Cochrane Reviews und der Verwendung eines bivariaten metaanalytischen Modells wird die gepoolte Sensitivität des Ersttrimester-Screening für das Risikoniveau von 1 in 300 auf 87,26% (95% CI, 85,18%-89,09%) berechnet. Die gepoolte Spezifität beträgt 95,50% (95% CI, 94,86%-96,05%). Unter der Annahme einer Prävalenz von T21 von 24 in 10.000 würde ein pränatales Screening basierend auf primären NIPTs zu einem PPV von 82,6% gegenüber einem PPV von 4,4% mit Combined Tests mit einer FN-Rate von Null für NIPT im Vergleich zu 0,03% für Combined Tests führen.

Daten aus Metaanalyse PPV 82,6%

Simulationsmodellierung: NIPT als Ergänzung zum Ersttrimester-Screening für Schwangere mit hohem T21-Risiko

NIPT als Ergänzung für Schwangere mit hohem T21-Risiko

Auf der Grundlage der 2 x 2-Testgenauigkeitsdaten des Cochrane Reviews und der Verwendung eines bivariaten metaanalytischen Modells wird die gepoolte Sensitivität des Ersttrimester-Screening für das Risikoniveau von 1 von 300 auf 87,26% (95% CI, 85,18%-89.09%) berechnet. Die gepoolte Spezifität beträgt 95,50% (95% CI, 94,86%-96,05%). Unter Annahme einer Prävalenz von T21 von 24 in 10.000 und unter der Annahme, dass alle Schwangeren, die im kombinierten Ersttrimester-Screening positiv getestet wurden, NIPT unterzogen werden, würde die berechnete Sensitivität der Add-on-Strategie (Ersttrimester-Screening plus NIPT) für eine hypothetische Kohorte von 10.000 auf der Grundlage der gepoolten Hochrisikodaten 86,8% betragen (95% CI, 82,2%- 90,4%). Die Spezifität wäre 100% (95% CI, 99,9%-100%). Der PPV wäre 99,1% (95% CI, 96,7%-99,7%%) und der NPV wäre 100% (95% CI, 99,9%-100%).

Daten aus Metaanalyse PPV 99,1%

Abwägungen bei der Wahl der Screening-Strategie

Vor- wie Nachteile beider Strategien:

Aus den Simulationsmodellen ist es nicht möglich, direkt abzuleiten, wie die mögliche Implementierung von NIPT die Identifizierung von Feten mit T21 und die Inanspruchnahme von invasiven Tests ändern würde. Aus einem Vergleich der beiden Modelle der möglichen Verwendung von NIPT (d.h. NIPT allein vs. Ergänzung) haben beide Modelle Vor- und Nachteile.

NIPT als Ersatz: Reduktion der unentdeckten T21

Die Verwendung von alleiniger NIPT würde die T21 Nichterkennungsraten (Anzahl der unentdeckten Feten mit T21) reduzieren, würde im Vergleich zum derzeitigen Standard (nur Combined Test), jedoch eine größere Anzahl von invasiven Tests erfordern. Kombiniertes Testen (Combined Test + NIPT) würde im Gegensatz zu der alleinigen NIPT-Strategie die Anzahl der invasiven Tests in höherem Umfang reduzieren.

NIPT als Ergänzung zum kombinierten Test: Reduktion invasiver Tests

Erstattung von NIPT

derzeit EU-weit noch keine breite Anwendung im öffentlichen Gesundheitssystem

Derzeit wird NIPT in den meisten europäischen Ländern hauptsächlich durch private Anbieter, noch nicht im öffentlichen Gesundheitssystem (außerhalb des Kontextes von Forschungsstudien) zur Verfügung gestellt.

Betrachtung ethischer und organisatorischer Aspekte

Zu den vier ethischen Grundprinzipien zur Beurteilung von Interventionen gehören

4 ethische Grundprinzipien

- *Respekt vor Autonomie*: Es wird angenommen, dass die frühe Verfügbarkeit von Ergebnissen und die erhöhte Genauigkeit des NIPT als Screening-Test im Vergleich zum Combined Test die informierte Entscheidung einer schwangeren Frau (sowie ihres Partners/ ihrer Partnerin) erleichtert. Informierte Entscheidung erfolgt aber in Abhängigkeit zu vielen Faktoren, einschließlich der Frage, wie die Einwilligung zur Testung und die Beratung zu möglichen Konsequenzen erfolgt.

Respekt vor Autonomie: Informierte Entscheidung: Einwilligung zu NIPT und Beratung

- *Nicht-Schaden und Nutzen wie Fürsorge*: Die Beurteilung von „non-maleficence" und „beneficence" von NIPT ist komplex, da sie sich auf das (individuelle) Werturteil stützt, ob die Früherkennung dieser Trisomien schädlich oder nützlich erlebt werden. Im Wesentlichen erfordert es eine Abwägung der Vorteile und Schäden nicht nur für die schwangere Frau und für ihre PartnerInnen, sondern auch für die Familie und andere Beteiligte. Für NIPT können die Vorteile und Nachteile je nach Perspektive und Implementierungsansatz (Add-on, vollständiger oder teilweiser Ersatz der Combined Tests) erheblich variieren. Der Nutzen wird in Studien primär in der Genauigkeit der NIPT-Ergebnisse und in der Reduktion des verfahrensbedingten Fehlgeburtsrisikos nach invasiver Diagnostik gesehen. Da pränatales Screening wegen seiner Assoziation mit Schwangerschaftsabbrüchen ein sensibles Thema ist, können die ethischen Implikationen von NIPT je nach den für die Gesellschaft und Individuen akzeptablen Zielen und Werten in den verschiedenen Ländern und Kulturen sehr unterschiedlich sein.

Nicht-Schaden und Nutzen wie Fürsorge

Abwägung der Vorteile und Schäden für schwangere Frau, PartnerInnen, Familie

- *Gleichheit und Gerechtigkeit*: Um dem Grundsatz der Gleichheit (gleicher Zugang) gerecht zu werden, sollte sichergestellt werden, dass der NIPT gegenüber dem herkömmlichen Screening kosteneffektiv und öffentlich finanziert ist. Verschiedene gesundheitsökonomische Modelle ergaben, dass NIPT als „kontingenter" Test bei Hochrisiko-Schwangeren mit einem Risiko-Cut-off von mehr als 1 zu 150 kosteneffektiv ist. Es liegen aber keine Vergleichsstudien zu den verschiedenen NIPT-Algorithmen vor, die Aussagen zum „Uptake" nach informierter Entscheidung, zur tatsächlichen Performanz (Reduktion von invasiven Tests und Fehlgeburten, Nachweis anderer Anomalien usw.) machen und wirft wichtige Fragen zur Implementierung in der Praxis auf. Bei privater Finanzierung von NIPT besteht angesichts der höheren Genauigkeit von NIPT die Gefahr, das T21/ Down-Syndrom ein Problem sozial-benachteiligter Familien wird, was eine soziale Stigmatisierung noch verstärken würde.

Gleichheit und Gerechtigkeit

Zugang für alle, die einwilligen

  Das Ausmaß der Umsetzung des NIPT in verschiedenen Ländern steht mit Faktoren wie Bildungsniveau, Einkommen oder Versicherungsschutz in Zusammenhang. Daher ist die NIPT-Aufnahme in Ländern mit hohem Einkommen wahrscheinlicher als in Ländern mit geringen Ressourcen. Diese Ungleichheiten können durch die NIPT-Kosten noch verschärft werden, da der Test hauptsächlich in privaten Einrichtungen durchgeführt wird.

globale und sozio-ökonomische Ungleichheiten

Unter Berücksichtigung des Grundsatzes der Verteilungsgerechtigkeit ist zu prüfen, ob es ethisch vertretbar ist, Ressourcen für Technologien auszugeben, die hinsichtlich ihrer Umsetzung und Ergebnisse noch große Unsicherheiten aufweisen.

Ressourcen für Interventionen mit Unsicherheiten

Nicht-intendierte Auswirkungen

Wenn NIPT bei allen Schwangeren durchgeführt wird, ist mit einer Erhöhung der Nachweisrate von T21, T18 und T13 zu rechnen, was zu einem Anstieg an Schwangerschaftsabbrüchen führen könnte. Vor diesem Hintergrund werden Bedenken geäußert, dass jene Schwangeren/Familien, die sich gegen eine pränatale Testung und/oder gegen einen Schwangerschaftsabbruch entscheiden, stigmatisiert werden und gegebenenfalls sogar der Zugang zu medizinischer Versorgung (Physiotherapie, Ergotherapie oder Schulprogramme) reduziert werden könnte.

Stigmatisierung bei Entscheidungen pro/ kontra NIPT

Da es sich bei NIPT um einen sicheren und einfachen Test handelt, der auch privat verfügbar ist, besteht das Risiko, dass der Test auch für geringe gesundheitliche Beeinträchtigungen oder sogar unerwünschte nichtmedizinische Merkmale (Geschlechtsselektion) verwendet wird.

Abtreibungen bei marginaler Beeinträchtigung, Geschlecht

Die Praxis der Abläufe zu Einwilligungen und zu Beratungsgesprächen - insb. bei einem einstufigen Screening und der Einführung von NIPT als Routinetest - wird öffentliche Gesundheitseinrichtungen vor Herausforderungen stellen. Es besteht die Befürchtung, dass Frauen – in Ermangelung an ausreichenden Beratungskapazitäten – eine gute informierte Entscheidung vorenthalten wird. Umfassende NIPT-Vortest-Beratung zu Vorteilen und Unsicherheiten sowie zum Potenzial zur Entdeckung von Zufallsbefunden könnte erschwert zugänglich werden. Das Gentechnikgesetz (GTG 1994, § 69) regelt, dass Schwangere *vor* einem genetischen pränatalen Test und *nach* dem Vorliegen des Untersuchungsergebnisses zu beraten sind, damit sie zum einen eine persönliche Entscheidung treffen können, ob sie das Screening akzeptieren oder ablehnen, sowie zum anderen *nach* einer Beratung, um positive Ergebnisse mit betroffenen Frauen zu besprechen.

Abläufe zu Einwilligung und zu Beratungsgesprächen zu bedenken

Vor- und Nachberatung bei NIPT in GTG 1994 §69 geregelt

Da das NIPT-Screening andere Implikationen als das Screening mittels Combined Test hat, sollten klare und genaue Einwilligungsformulare entwickelt werden, die den KlinikerInnen Schulungsmaterialien zur Erklärung des Zwecks des Testens und der potenziellen Risiken und Vorteile zur Verfügung stellen. Im Jahr 2017 wurde das IQWIG mit der Erstellung solcher Materialien für Deutschland beauftragt.

IQWIG erarbeitet derzeit Infomaterial und Einwilligungsformulare

Organisatorische Aspekte und Kostenbewertungen

Wie oben erwähnt, können je nach NIPT-Screening-Strategie für die beteiligten Gesundheitsfachkräfte im Ablauf und Aufwand – aufgrund des Bedarfs nach zusätzlicher Vor- und Nachtestberatung zur informierten Entscheidungsfindung – Veränderungen im Ablauf und Mehraufwand erforderlich werden. Im Einklang mit den Ergebnissen einer kürzlich in den USA durchgeführten Studie sollte auch den Einwilligungsdokumenten besondere Aufmerksamkeit geschenkt werden, damit den bestehenden psychosozialen Abwägungen angemessen Aufmerksamkeit geschenkt wird.

Veränderungen im Arbeitsablauf und Arbeitsaufwand

durch zusätzliche Beratung

Wenn NIPT als Ersatz im Ersttrimester Screening eingesetzt werden soll, ist zu erwarten, dass die Arbeitslast in Labors sowie beim Ultraschall deutlich abnimmt, wenngleich Tests für andere Gesundheitsbedrohungen (fetale Wachstumsbeschränkung oder Präeklampsie) noch erforderlich sind.

NIPT als Ersatz: Verringerung der Arbeitslast in Labors und bei Ultraschall

Auch ist die qualitätsgesicherte Handhabung der Proben beim Versand oder Transport wie bei der Analyse von Bedeutung. Spezifische, von Herstellern unabhängig entwickelte Mindeststandards, Qualitätskontrollen, Laboranforderungen sind für NIPT noch nicht entsprechend entwickelt. Auch der Schutz der Vertraulichkeit von Patientinneninformationen ist einzuhalten.

qualitätsgesicherte Handhabung der Proben

Datenschutz

In europäischen Ländern (basierend auf Angaben zu Österreich, Deutschland, Großbritannien, Rumänien, Spanien und der Schweiz) belaufen sich die Kosten des NIPT auf rund 447 - 992 €. Verschiedene gesundheitsökonomische Evaluierungen befassten sich mit der Kosten-Effektivität von NIPT in der Diagnostik von T21 in europäischen Ländern (d.h. in den Niederlanden, Belgien und Spanien).

Kosten-Effektivität

Ein rezentes (August 2017) gesundheitsökonomisches Modell für Österreich wurde durch den Hauptverband der Sozialversicherungen (HVB) durchgeführt (http://bit.ly/2DSvDEp). 3 Screening-Strategien wurden für die Endpunkte Detektionsraten, vermiedene invasive Untersuchungen und Kosten modelliert.

HVB Modell 2017: Vergleich zu 3 Screening Strategien

Diskussion

Die hauptsächliche Einschränkung der Aussagekraft der NIPT Studien zu T21 in der Allgemeinbevölkerung bezieht sich auf das hohe Verzerrungspotenzial: Das Follow-Up war in den meisten Studien unvollständig, und zwei der Studien, die (aufgrund der Stichprobengröße) am meisten zu den Ergebnissen beitragen, zeigen hohe „lost-to-follow-up" Raten von 16,4% bzw. 23%. Die Überprüfung negativer NIPT-Fälle wurde in den meisten Studien durch Sichtung von Krankenakten, Informationen von AllgemeinmedizinerInnen und durch Telefoninterviews durchgeführt, was ebenfalls wichtige Bedenken hinsichtlich der Vollständigkeit und Zuverlässigkeit der Spezifitätsdaten aufwirft (GRADE: Low-QoE).

Einschränkungen der Studien zu T21 in der allgemeinen Population

hohes Verzerrungspotential; hohe lost-to-follow-up Raten

Die Evidenz für T21 in Hochrisikopopulationen basiert ebenfalls auf Daten von mäßiger Qualität. Der Ausschluss von Fällen von Fehlgeburten, Totgeburten und Fällen ohne oder mit unsicheren Ergebnissen kann zu einer Überschätzung der Spezifität und der PPV sowohl in der Allgemeinbevölkerung als auch in der Hochrisikopopulation führen. Ein niedriger fetaler Anteil (in den Proben) oder andere Qualitätsprobleme können auch die aktuellen Schätzungen der Sensitivität und Spezifität verändern. Im Allgemeinen waren die T18- und T13-Studien aufgrund der geringen Stichprobengröße unzureichend aussagekräftig.

Einschränkungen der Studien zu T21 in Hochrisikopopulation

Ausschluss von Fällen und Qualitätsprobleme

Eine wichtige Einschränkung der Studien ist der Mangel an Informationen bezüglich des Nachweises von Neuralrohrdefekten und anderen wichtigen chromosomalen Anomalien im Vergleich zum Combined Test. Obwohl NIPT eine große Anzahl von größeren Anomalien nicht identifiziert, die zufällig durch invasive Tests diagnostiziert werden, ist das Ausmaß dieses Mangels an Nachweisen von anderen Anomalien unbekannt. In diesem Sinne scheint es wesentlich zu sein, dass Schwangeren, die sich einer cfDNA-Analyse unterziehen, auch eine Ultraschalluntersuchung angeboten wird. Unsicherheiten bestehen bezüglich der Implikationen zufälliger Befunde, die nicht im

Einschränkung von NIPT

Mangel an Information zu anderen Anomalien

spricht für NIPT als Zusatz zum bestehenden Screening

Fokus der NIPT Studien standen (z.B. Aneuploidien der Geschlechtschromosomen, Mikrodeletionen).

Die Beurteilung der Performanz verschiedener Teststrategien in der Praxis bedarf der Berücksichtigung aller Auffälligkeiten, Abtreibungen, Fehlgeburten und anderer patientInnenbezogener Ergebnisse. Wichtige Unsicherheiten bestehen bezüglich der besten Screening-Strategie. Wie die EUnetHTA Modellierungsergebnisse (aber auch jene vom HVB) zeigen, müssen EntscheidungsträgerInnen eine Abwägung der verschiedenen Ziele des NIPT machen:

Unsicherheiten zur besten Screening-Strategie : EntscheidungsträgerInnenmüssen Ziele abwägen

- Das Ziel der Erkennung aller T21-Fälle könnte mit einer etwas höheren Rate invasiver Tests erreicht werden;
- Das Ziel, die Rate der invasiven Tests zu reduzieren, hat andererseits den Nachteil, dass nicht alle Fälle von T21 erfasst werden.

Ziel
Erkennung möglichst aller T21-Fälle
oder
Reduktion invasiver Tests

Dennoch ist das Modell allein keine ausreichende Grundlage für eine Entscheidung, da es auf mehreren Annahmen und Vereinfachungen beruht.

Fazit

Die vorliegenden Daten belegen, dass die Sensitivität vom NIPT bei T21 signifikant höher ist als im herkömmlichen Ersttrimester-Screening und dass diese Form des Screenings zu einer erheblichen Verringerung unnötiger invasiver Tests führen würde. Aufgrund der nicht ausreichenden Überprüfung negativer Ergebnisse bestehen jedoch weiterhin Unsicherheiten hinsichtlich der Spezifität der NIPTs. Daten zu den wichtigsten Sicherheitsergebnissen fehlen ebenfalls (Zunahme der Anzahl der Neugeborenen, die mit anderen Anomalien geboren wurden, elektiver Schwangerschaftsabbruch bei anderen unbestätigten Chromosomenanomalien mit unsicherer Signifikanz usw.).

Nachweise für höhere Sensitivität von NIPT bei Ersatz (Erstlinie) und …

im Vergleich zu herkömmlicher Teststrategie

viele Daten fehlen: Einfluss auf geborene Kinder mit anderen Anomalien

Einschränkungen bei Studienlage zu

anderen Strategien

keine Aussagen zu T13+T18

Zwillingsschwangerschaften

bester Screening-Strategie möglich

- Es liegen keine Daten vor, um die Genauigkeit von NIPT als Teil des bisherigen Ersttrimester-Screenings zu beurteilen.
- Die verfügbaren Daten deuten darauf hin, dass die Verwendung von NIPT als Add-on für das Ersttrimester-Screening von T21-Hochrisikopopulationen zu einer erheblichen Reduzierung von invasiven Tests führen könnte, obwohl dies erst mit Versorgungsdaten bestätigt werden muss. Die Durchführung des Tests (Testversagen, unsichere Ergebnisse) und die Aufnahme wie Akzeptanz (Uptake) von NIPT-Screening gehören zu den Faktoren, die dazu beitragen könnten, dieses Verhältnis in der realen Praxis zu ändern.
- Es fehlen Daten, um die Verwendung von NIPT als Ergänzung zum Ersttrimester-Screening für Populationen mit hohem und mittlerem Risiko zu beurteilen.
- Die niedrige QoE für T18 und T13 erlaubt keine Rückschlüsse auf diese Trisomien für eine der Screening-Strategien.
- Es gibt keine ausreichenden Nachweise für die Genauigkeit der NIPT bei Zwillingsschwangerschaften.
- Um die Performanz der verschiedenen Teststrategien beurteilen zu können, sind entsprechend konzipierte prospektive Vergleichsstudien erforderlich, in denen alle Auffälligkeiten, Aborte, Fehlgeburten und andere patientInnenbezogene Ergebnisse erfasst werden.
- Wichtige Unsicherheiten bestehen bezüglich der besten Screening-Strategie.

EUnetHTA Joint Action 3 WP4

**Rapid assessment of other technologies using the HTA Core Model®
for Rapid Relative Effectiveness Assessment**

SCREENING OF FETAL TRISOMIES 21, 18 AND 13 BY NONINVASIVE PRENATAL TESTING

Project ID: OTCA03

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The assessment represents a consolidated view of the EUnetHTA network members and is in no case the official opinion of the participating institutions or individuals.

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LIST OF ABBREVIATIONS

ACOG	American College of Obstetricians and Gynaecologists
ACP	American College of Physicians
ART	assisted reproductive technology
cfDNA	cell-free DNA
cfDNA	cell-free fetal DNA
CI	confidence interval
CRD	Centre for Reviews and Dissemination
CRL	crown–rump length
CVS	chorionic villus sampling
DOR	diagnostic odds ratio
DS	Down syndrome
DTA	diagnostic test accuracy
EU	European Union
EUnetHTA	European Network for Health Technology Assessment
EUROCAT	European Surveillance of Congenital Anomalies
FCT	first-trimester combined testing
FN	false negative
FP	false positive
GIN	Guidelines International Network
GRADE	Grading of Recommendations, Assessments, Development and Evaluation
hCG	human chorionic gonadotropin
β-hCG	β subunit of human chorionic gonadotropin
HSROC	hierarchical summary receiver operating characteristics
ICTRP	International Clinical Trials Registry Platform
IVF	in vitro fertilisation
LR+	positive likelihood ratio
LR–	negative likelihood ratio
MeSH	Medical Subject Headings
NIPT	noninvasive prenatal testing
NGS	next-generation sequencing
NPV	negative predictive value
NT	nuchal translucency
PAPP-A	pregnancy-associated plasma protein A
PCR	polymerase chain reaction
PICO	population, intervention, comparison and outcome
PPV	positive predictive value
QoE	quality of the evidence

SNP	single nucleotide polymorphism
T13	trisomy 13
T18	trisomy 18
T21	trisomy 21
WGS	whole genome sequencing

SUMMARY OF RELATIVE EFFECTIVENESS OF NONINVASIVE PRENATAL TESTING FOR FETAL ANEUPLOIDIES

Scope

The aim of this collaborative assessment is to evaluate the relative effectiveness and safety of noninvasive prenatal testing (NIPT) for the screening of fetal trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) in pregnant women of at least 8–9 weeks' gestation. Five screening pathways are considered for the purpose of NIPT assessment:

1. NIPT as a primary screening test (total replacement of first trimester combined testing (FCT))
2. NIPT as part of FCT (replacement of serum testing)
3. NIPT as an add-on to FCT for the high-risk population
4. NIPT as an add-on to FCT for the high- and intermediate-risk population
5. NIPT as a replacement for invasive testing

Specific interventions and population subanalyses are outlined in Section 1.

The comparator, chosen by application of EUnetHTA criteria [1], is first-trimester serum screening (pregnancy-associated plasma protein A (PAPP-A) and β subunit of human chorionic gonadotropin (β-hCG)) and/or an ultrasound scan to measure fetal nuchal translucency (NT) or fetal crown-rump length (CRL) and maternal age. Fetal karyotyping or birth outcomes determined through clinical examination or follow-up of the newborn are considered the reference standards.

The effectiveness of the screening processes is evaluated in terms of secondary outcomes (sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)) but also in terms of primary outcomes, reducing unecessary invasive tests, assessing the impact on children born with undiagnosed T13, T18 and T21, natural miscarriages or stillbirths, and miscarriages related to invasive testing (amniocentesis or chorionic villus sampling (CVS)). False positive (FP) rates, false negative (FN) rates and test failures were chosen as critical safety issues. The increase in the number of children born with other major prenatally undetected chromosomal conditions/ anomalies (not targeted by prenatal aneuploidy screening) and the increase in elective pregnancy termination for other chromosomal anomalies with uncertain significance were considered important safety issues. Several organisational, ethical and social outcomes were also considered of relevance (see Section 1).

Randomised controlled clinical trials, nonrandomised controlled clinical trials and diagnostic test accuracy (DTA) studies on the index test, the comparator and the reference standard (cross-sectional studies) are included for the effectiveness and safety domain. In addition, registries are included for the safety domain and qualitative studies and consensus documents are included for the organisational, ethical and social domains.

Introduction

Description of technology and comparators

Noninvasive prenatal tests are in vitro diagnostic tests that use cell-free DNA (cfDNA) from maternal blood of pregnant women for the identification of common chromosomal anomalies of the fetus, including T21, T18 and T13. Though commonly referred to as cell fetal free DNA, the DNA does not derive from the fetus but originates from the cytotrophoblast layer of the chorionic villi (the outer placental cell layer) [2].The search identified 21 commercialised assays, although many others might exist given the externalisation of the technology. The most common providers are Ariosa Diagnostics Inc./Roche Sequencing Solutions Inc. (Harmony®), BGI Diagnostics Technology Col Ltd. (NIFTY™), Igenomix SL (NACE®), LifeCodexx AG (PrenaTest®), Natera® (Panorama®), Sequenom Laboratories (MaterniT® 21 PLUS) and Illumina Inc. (Verifi™) . Of these, the MaterniT® 21 PLUS test is commercialised only in the USA [3] (**B0001**).

The landscape of NIPT is diverse: some tests adopt polymerase chain reaction (PCR) for amplification of the cell-free DNA (cfDNA) before next-generation sequencing (NGS), whilst others rely on other methods of quantification such as chromosomal microarray analysis. The NGS approach and the software for analysis and interpretation of screening results differ between tests, using these different quality standards and reporting results in different ways. Regardless of the tests, all require a sufficient proportion of cfDNA in the maternal plasma to be able to cfDNA-differentiate between the status of the mother and the fetus. However, not all laboratories quantify fetal fraction in individual samples. Testing has to be performed from 8 to 10 weeks' gestation. All assays can be confounded by several biological and maternal factors, including confined placental mosaicism, maternal copy number variations, and fetal mosaicism [4]. Failure to obtain an NIPT result could also be due to different technical/statistical reasons [5, 6] (**B0001**).

Currently available prenatal screening options include maternal age combined with one of the following: (1) first-trimester screening (NT, maternal age and maternal serum biochemical markers), (2) second-trimester serum screening (maternal age and maternal serum biochemical markers), or (3) two-step integrated screening, which includes first- and second-trimester serum screening with or without NT (integrated prenatal screening, serum integrated prenatal screening, contingent and sequential) [7-9]. Confirmation requires invasive testing (amniocentesis or CVS). Both procedures are associated with a risk of miscarriage, which seems to differ substantially depending on the skills of the operator and the number of procedures performed [10, 11].

Most noninvasive prenatal tests are offered for T21, T18 and T13 and sex chromosome aneuploidies but many laboratories have expanded their panels to include other trisomies and common microdeletions. Depending on the assays, they can be available for singleton, twin, egg-donor or in vitro fertilisation (IVF) pregnancies.The proposed indication is primary or secondary screening (see Table 3). In most European countries, noninvasive prenatal tests are delivered mainly through private providers, not yet being available in publicly funded antenatal services outside the context of research studies. In most of these countries, screening for fetal aneuploidies has started as contingent screening with high-risk women, although some countries have started offering the tests for all pregnancies (**B0003**, **B0009**, **A0021**).

The main claimed benefit of NIPT in relation to conventional screening approaches resides in the simplicity and noninvasiveness of the test, as well as in the potential reduction in FP results. It is claimed that NIPT avoids unnecessary invasive procedures (amniocentesis and CVS), minimising the risk of complications, pregnancy loss and anxiety. It is claimed that the assays might also allow earlier testing, which would have the advantage of giving parents more time to make decisions .

Reimbursement

Currently, NIPT is delivered mainly through private providers, not yet being available in publicly funded antenatal services outside the context of research studies in most European countries (**A0021**). The information provided by manufacturers on the reimbursement status/recommendations in Europe can be found in Tables A14–A15 in Appendix 2**.**

Health problem

T21 (Down syndrome, DS), T18 (Edwards syndrome) and T13 (Patau syndrome) are the most common chromosomal disorders among newborns. In Europe, the estimated prevalences are 24, 5.6 and 2.08 per 10,000 live births, respectively [12]. Around 68.7% of T21 cases, 94.3% of T18 cases and 93.4% of T13 cases are diagnosed prenatally, although prevalence can differ substantially between countries depending on the uptake of screening programmes (<30% to ≥90%) (**A0002**, **A0003**, **A0004**).

In Europe, more than 90% of individuals with T21 are expected to survive beyond the age of 20 years, and approximately 60% reach the age of 60 years [13-16] (**A0003**). Individuals are characterised by physical growth delay and mental retardation [17, 18], and are commonly affected by many comorbidities (congenital heart diseases, hearing and vision problems, neurobehavioural and psychiatric disorders, etc.) and premature ageing chronic diseases [19, 20]. Assessment, monitoring, prevention and guidance will be required from birth [18, 21]. It is estimated that with the current screening approximately 5000 newborns are affected in the EU each year (**A0003**, **A0004**, **A0005**, **A0006**).

In contrast to T21, T13 and T18 are lethal conditions characterised by major structural malformations. Most pregnancies will end in spontaneous abortions or stillbirths, and if born, few children survive beyond the first year [22]. Most newborns have severe impairments, and although little follow-up information exists, it has been reported that mental delay ranges from marked to profound. Most individuals do not achieve expressive language or walk independently (**A0005**).

In most European countries, combined testing is offered to all pregnant women in the form of national or regional population screening programmes, although some countries (e.g., Ireland, Austria and Malta) still have no official prenatal screening policies [23]. In Europe, the threshold for testing frequently used to define high risk is 1 in 250 to 1 in 300, although this differs between countries. Guidelines recommend no further testing for low-risk patients . If contingency screening is available, women will be classified into high-, intermediate- and low-risk groups, and the intermediate-risk group will be offered second-trimester screening, although the threshold for intermediate risk is not standardised in most countries. For explicit information regarding NIPT recommendations, see Table A1 in Appendix 1 (**A0025**).

As far as authorisation is concerned, the target population is all pregnant women who choose to have prenatal screening for T21, T18 and T13. This would mean that around 5.1 million pregnancies would be possible candidates for NIPT in the EU-28 (EUROSTAT fertility statistics) [24]. However, the precise target population is difficult to estimate because it will differ substantially depending on whether will a first-line or a second-line test will be used, and also on the risk threshold used (**A0025**, **A0007**, **A0011**).

Methods

The selection of assessment elements was based on the HTA Core Model® for Rapid Relative Effectiveness Assessment version 4.2 [25]. Additional elements were added from the HTA Core Model® Application for Diagnostic Technologies version 3.0 and the HTA Core Model® Application for Screening Technologies version 3.0 [26]. The checklist for potential ethical, organisational, patient and social, and legal aspects was used to establish the relevance for assessment of these domains.

A systematic search of the scientific literature was performed in February to March 2017 in MEDLINE (PubMed), Embase (OVID SP), the Centre for Reviews and Dissemination (CRD) database, Web of Science and the Cochrane Library (Wiley). Guideline repositories were used to identify relevant guidelines published after 2010. No limitations were applied in terms of the timing or type of studies for any of the domains. Ongoing clinical trials and research projects were found through ClinicalTrials.gov, the EU Clinical Trials Register, the International Clinical Trials Registry Platform (ICTRP) and the UK Clinical Trials Gateway. General Internet searches and manual searches of citations were complementary sources of information. Detailed tables containing the search strategies can be found in Appendix 1.

Manufacturers identified at the time of the search were contacted by the Ludwig Boltzmann Institute for Health Technology Assessment for information related to NIPT CE mark (type of CE-marked product and indications) and technology characteristics. For feasibility reasons, only four manufacturers which were identified to have relevant peer-review publications were asked for submission files. Additional information related to NIPT CE mark (type of CE-marked product and indications) and specific characteristics of the technologies were requested from all manufacturers identified during the assessment scoping phase on 23rd December 2016. Manufacturers were also asked if their product was commercialised in Europe, if their companies produced other technologies relevant for the assessment and if they were aware of any other relevant CE-marked devices on the market for the respective technology and indication. Finally, they were asked to provide additional information/data that they considered relevant/differentiating (studies, etc.). In this way, it was ensured that no key information was missed. General Internet searches were performed to complement information sent by manufacturers in all cases.

Two authors from avalia-t reviewed and selected relevant abstracts according to the population, intervention, comparison and outcome (PICO) question. The full text of potentially relevant articles was read, and studies were included/excluded on the basis of scoping questions. Studies which did not provide data on relevant outcomes or were considered to have an unacceptable risk of bias or applicability concern were excluded. The reasons chosen for exclusion were as follows: mixed populations with unclear patient selection criteria; retrospective cohort or case-control design; lack of information on the index test or reference standard; lack of independent assessment of index test/reference standard; inappropriate reference standard in most of the population. Selection was done independently, and discrepancies between the authors were resolved by consensus. For the clinical effectiveness and safety domains, the relevant data were extracted and recorded in evidence tables by one author from avalia-t and reviewed by another. Both of these steps were checked by the co-author.

The QUADAS-2 tool was used to assess the risk of bias of DTA studies [27]. The level of confidence/certainty in the evidence was evaluated with use of the Grading of Recommendations, Assessments, Development and Evaluation (GRADE) system [28]. No quality assessment tool was used for other domains. A descriptive analysis of data was provided for all relevant outcomes in these domains. Quality evidence assessment was performed by the two authors independently of each other. Discrepancies were resolved by consensus. The whole process was reviewed by the co-author.

Statistical analyses were performed according to recommendations described in the European Network for Health Technology Assessment (EUnetHTA) guideline “Meta-analysis of Diagnostic Test Accuracy Studies” [29]. A bivariate random-effects model was used except when then the model failed to converge or provided unreliable parameter estimates, in which case two univariate random-effects models were used.

Results

Characteristics of the available evidence

Direct evidence for the clinical effectiveness and safety domains was found only for NIPT as a primary testing method (total replacement of FCT). This evidence derives from five paired DTA comparative studies and four noncomparative studies performed in singleton pregnancies. No data exist on patient-relevant outcomes. Characteristics and accuracy results of individual studies are provided in Table 6**.**

The question regarding NIPT as an add-on to FCT in singleton women with high-risk of aneuploidies was answered indirectly from pooled data derived from 26 retrieved studies which assessed the performance of NIPT as a second-tier test (Table 7). The add-on strategy for intermediate-risk patients was addressed in only one study. Six studies provided data on the accuracy of NIPT for twin populations (Table 9). No evidence was found regarding the performance of these tests in combination with and/or NT. The scenario of NIPT as a replacement for invasive testing was not considered because none of these tests are currently indicated for this purpose.

Test accuracy

The diagnostic accuracy for T21 NIPT as a primary testing method was calculated on the basis of 136,544 pregnant women (885 aneuploidy and 135,659 euploidy cases). The meta-analysis using the bivariate random-effects model yielded a pooled estimate of sensitivity of 99.3% (95% confidence interval (CI) 97.8%–99.8%) and specificity of 99.9% (95% CI 99.8%–99.9%), which did not differ from the univariate model (Figure 8). In the four paired comparative studies, NIPT showed a higher sensitivity in comparison with standard screening (100% vs. 94%, respectively, $p<0.001$). The specificity was also significantly higher. The PPV in the studies included ranged from 80% to 100%, except in the study of Bianchi et al. [30], which reported a PPV of 45.5%. The NPV was more than 99% in all studies included in the assessment. The PPV and NPV were significantly higher for NIPT than for FCT in one of the two studies which provided a statistical comparative analysis of these outcomes (PPV of 80.9% vs. 3.4% and NPV of 100% vs. 99.9%) [31]. No difference was found in the other study [30]. Overall, the quality of the evidence (QoE) for T21 assessed with the GRADE approach was moderate for sensitivity and low for specificity. The QoE for T18 and T13 was rated to be low and very low for sensitivity and specificity because of the sparse cases, risk of bias and/or imprecision of the estimates (**D0024**).

The 24 studies which provided data on the accuracy for 21 NIPT as a second-tier test involved 1408 aneuploidy cases and 99,818 euploidy cases. The pooled sensitivity with application of the bivariate random-effect models was 99.21% (95% CI 98.59%–99.56%) and specificity was 99.95% (95% CI 99.93%–99.96%) (Figure 11). Thus, the test accuracy results were very similar regardless of the position of NIPT within the screening strategy. In a similar way to what occurred in general population, the QoE for T18 and T13 was rated to be low and very low (**D0024**).

The evidence base for twin populations was also rated to be very low because of the scarce number of cases, risk of bias and imprecision of the estimates. Retrieved studies involved 33 T21 cases and 1547 euploidy cases.

The studies included failed to provide data regarding the reduction in invasive testing (amniocentesis or CVS).

Comparative performance

No data are available to address three of our five research questions: NIPT as part of FCT; NIPT as an add-on to FCT for the high- and intermediate-risk population; NIPT as a replacement for invasive testing.

For the remaining two questions – NIPT as a primary screening method as a replacement for FCT and NIPT as an add-on to FCT for the high-risk population (1 in 300 cut-off point) – there are sufficient data from diagnostic accuracy studies to assess sensitivity, specificity, PPV, NPV and rate of test failures.

Because of the lack of direct data on primary outcomes, simulation modelling was used to compare screening strategies with NIPT versus current screening practice in terms of sensitivity and specificity and their impact on the number of invasive tests required for T21. Estimates of the combined test's accuracy were provided by Cochrane review. The QoE for the sensitivity and specificity for T18 and T13 in high-risk pregnant women was rated to be low, and modelling was not considered appropriate (**D1002**).

NIPT as a primary screening method as a replacement for FCT for T21

On the basis of the 2×2 test accuracy data of the Cochrane review and the use of a bivariate meta-analytic model, the estimated pooled sensitivity of FCT for the risk level of 1 in 300 is estimated to be 87.26% (95% CI 85.18%–89.09%). The estimated pooled specificity is 95.50% (95% CI 94.86%–96.05%). Assuming a prevalence of T21 of 24 in 10,000 (European Surveillance of Congenital Anomalies (EUROCAT) data [12], prenatal screening based on primary NIPT would result in a PPV of 82.6%% versus a PPV of 4.4% with FCT, with a zero FN rate for NIPT compared with 0.03% for combined testing. The accuracy measures were calculated based on the hypothetical scenarios presented in Figure 16 and Figure 17.

NIPT as add-on to FCT for the population with high risk of T21

On the basis of the 2x2 test accuracy data of the Cochrane review and the use of a bivariate meta-analytic model, the estimated pooled sensitivity of FCT for the risk level of 1 in 300 is estimated to be 87.26% (95% CI 85.18%–89.09%). The estimated pooled specificity is 95.50% (95% CI 94.86%–96.05%). Assuming a prevalence of T21 of 24 in 10,000 (EUROCAT data) [12] and assuming that all women testing positive with FCT would undergo NIPT (pooled sensitivity of 99.24%, 95% CI 98.64%–99.58%, and specificity estimate of 99.95%, 95% CI 99.93%–99.96%), the estimated sensitivity of the add-on strategy (FCT plus NIPT) for a hypothetical cohort of 10,000 estimated on the basis of the pooled high-risk data would be 86.8% (95% CI 82.2%–90.4%). Estimated specificity would be 100% (95% CI 99.9%–100%). The PPV would be 99.1% (95% CI 96.7%–99.7%%) and the NPV would be 100% (95% CI 99.9%–100%). The hypothetical scenario used to estimate sensitivity and specificity of NIPT as an add-on test is shown in Figure 15.

Comparison of NIPT screening pathways

From the simulation models it is not possible to directly estimate how the possible implementation of NIPT would change key outcomes, such as T21 detection and invasive testing. From a comparison of the two models of using NIPT (i.e., NIPT only vs. FCT and NIPT), both models have advantages and disadvantages. Using only NIPT would reduce the number of undetected cases but would require a larger number of invasive tests. FCT would fail to increase the T21 detection rate, but would allow – as opposed to the NIPT-only strategy – the number of invasive tests to be reduced to a larger degree. These estimations can change if no-call results are included in the analysis. Because of the lack of data on the rate of missed cases among no-call results, this modelling could not be done (Figure 16 and Figure 17).

Safety

The main comparative information relates to intermediate safety outcomes (FP and FN rates) and no-call results (test failures, low-quality samples, undeterminate results). For T21, FP and FN rates calculated excluding miscarriages and no-call results were higher with combined testing than with NIPT (Table 12). Studies also showed higher FP rates with standard screening than with NIPT for T18 and T13 but were inconsistent regarding FN rates. FP and FN rates increased when no recall cases were included in the analysis. The proportion of no-call results ranged from 0.5% to 3% (**C0008**).

The FP rate observed for T21 and T18 was zero or very low (<0.5%) in all of the studies which assessed NIPT as a second-tier test (Table 13). The FN rate was also zero in most of the studies, although the FN rate for T13 and T18 differed widely in the few studies which did report FN cases (2.6%–37.5% and 12.5%–100%, respectively). The FP and FN rates observed in twin pregnancies were highly variable (Table 14) (**C0008**).

Only one study provides comparative information on other chromosomal anomalies (e.g., other trisomies, chromosomal deletions or duplications). This study found that NIPT alone missed 8 of 13 cases of other chromosomal aberrations. FCT missed only 4 of these 13 cases.

Table 1: Summary of the available evidence on the performance of noninvasive prenatal testing

Outcome	No. of studies and no. of patients	Study design	Factors that may decrease QoE					Performance, % (95% CI, %)	Test accuracy, QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias			
GENERAL POPULATION										
Trisomy 21										
Sensitivity (TP+FN)	23 studies 28,188 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.33 (97.82–99.80)	⊕⊕⊕◯ Moderate	Critical
Specificity (FP+TN)	23 studies 28,188 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.93 (99.85–99.97)	⊕⊕◯◯ Low	Critical
Trisomy 18										
Sensitivity (TP+FN)	20 studies 25,972 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely[b]	97.43 (94.41–98.84)	⊕◯◯◯ Very low	Critical
Specificity (FP+TN)	20 studies 25,972 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.94 (99.87–99.97)	⊕⊕◯◯ Low	Critical
Trisomy 13										
Sensitivity (TP+FN)	15 studies 22,650 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely[b]	99.81 (1.14–100)	⊕◯◯◯ Very low	Critical
Specificity (FP+TN)	15 studies 22,650 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.95 (99.94–99.97)	⊕⊕◯◯ Low	Critical
HIGH-RISK PREGNANT POPULATION										
Trisomy 21										
Sensitivity (TP+FN)	23 studies 28,188 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.21 (98.59–99.56)	⊕⊕⊕◯ Moderate	Critical
Specificity (FP+TN)	23 studies 28,188 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.95 (99.93–99.96)	⊕⊕◯◯ Low	Critical

Outcome	No. of studies and no. of patients	Study design	Factors that may decrease QoE					Perfor-mance, % (95% CI, %)	Test accuracy, QoE	Im-portance
			Risk of bias	Incon-sistency	Indirectness	Imprecision	Publication bias			
Trisomy 18										
Sensitivity (TP+FN)	20 studies 25,972 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely[b]	96.86 (88.35–99.21)	⊕◯◯◯ Very low	Critical
Specificity (FP+TN)	20 studies 25,972 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.97 (99.93–99.98)	⊕⊕◯⊕ Low	Critical
Trisomy 13										
Sensitivity (TP+FN)	15 studies 22,650 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely[b]	97.67 (59.69–99.91)	⊕◯◯◯ Very low	Critical
Specificity (FP+TN)	15 studies 22,650 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	99.98 (99.92–99.99)	⊕⊕◯◯ Low	Critical
HIGH- OR INTERMEDIATE-RISK PREGNANT POPULATION										
Trisomy 21										
Sensitivity (TP+FN)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	97.7 (88.2–99.6)	⊕⊕⊕◯ Moderate	Critical
Specificity (FP+TN)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	99.9 (99.8–100)	⊕⊕⊕◯ Moderate	Critical
Trisomy 18										
Sensitivity (TP+FN)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely	87.5 (69.0–95.7)	⊕⊕◯◯ Low	Critical
Specificity (FP+TN)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	99.8 (99.8–100)	⊕⊕⊕◯ Moderate	Critical

Outcome	No. of studies and no. of patients	Study design	Factors that may decrease QoE					Perfor-mance, % (95% CI, %)	Test accuracy, QoE	Im-portance
			Risk of bias	Incon-sistency	Indirectness	Imprecision	Publication bias			
Trisomy 13										
Sensitivity (TP+FN)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Very[c] serious	Unlikely	50 (15.0–85.0)	⊕⊕◯◯ LOW	Critical
Specificity (FP+TN)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	99.8 (99.7–100)	⊕⊕⊕◯ Moderate	Critical
TWIN PREGNANCY POPULATION										
Trisomy 21										
Sensitivity (TP+FN)	6 studies 1985 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[C]	Unlikely	99.19 (44.71–99.99)	⊕◯◯◯ Very low	Critical
Specificity (FP+TN)	6 studies 1985 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely	99.75 (98.97–99.94)	⊕⊕◯◯ Low	Critical

Abbreviations: CI=confidence interval; DTA=diagnostic test accuracy; FN=false negative; FP=false positive; QoE=quality of the evidence; TN=true negative; TP=true positive.

[a] Many studies presented a high or unclear risk of bias due to the use of an inappropriate reference standard or important follow up losses; for sensitivity estimations, risk of bias was very serious for some studies due to the lack of confirmation of negative noninvasive prenatal test cases.

[b] Possibility of publication bias not excluded but not considered sufficient to downgrade QoE.

[c] Many studies showed wide CIs of sensitivity.

Discussion

The main limitation of general population T21 studies relates to the high risk of bias related to the flow and timing and reference standard domains. Follow-up was incomplete in most studies, and two of the studies which contribute most to the results given the sample size have losses as high as 16.4% and 23%, respectively. The verification of negative NIPT cases was done in most studies by review of medical records, general practitioner databases and telephone interviews, raising also important concerns regarding the completeness and reliability of the specificity data (low QoE GRADE approach).

The findings for T21 in high-risk populations were also based on moderate-low quality evidence. Excluding cases of miscarriages, stillbirths and cases with no or uncertain results could have led to an overestimation of the specificity and PPV in both the general population and the high-risk population. The low fetal fraction or other quality issues could also change current estimates of sensitivity and specificity. In general, T18 and T13 studies were insufficiently powered because of the small sample size, and this could have greatly contributed to the imprecision observed and could also explain why many of the studies failed to show FN or FP cases for any of the three types of aneuploidy.

An important limitation of the studies is the lack of information regarding the detection of neural tube defects and other major chromosomal anomalies in relation to FCT screening. Although NIPT will miss a large number of major anomalies that are incidentally diagnosed by invasive testing, the extent of these losses is relatively unknown. In this sense, it seems to be essential that patients who are undergoing cfDNA analysis should be offered maternal serum fetoprotein screening or ultrasound evaluation. Uncertainties remain regarding the implications of incidental findings on sex chromosomal aneuploidies and other conditions which are not being targeted.

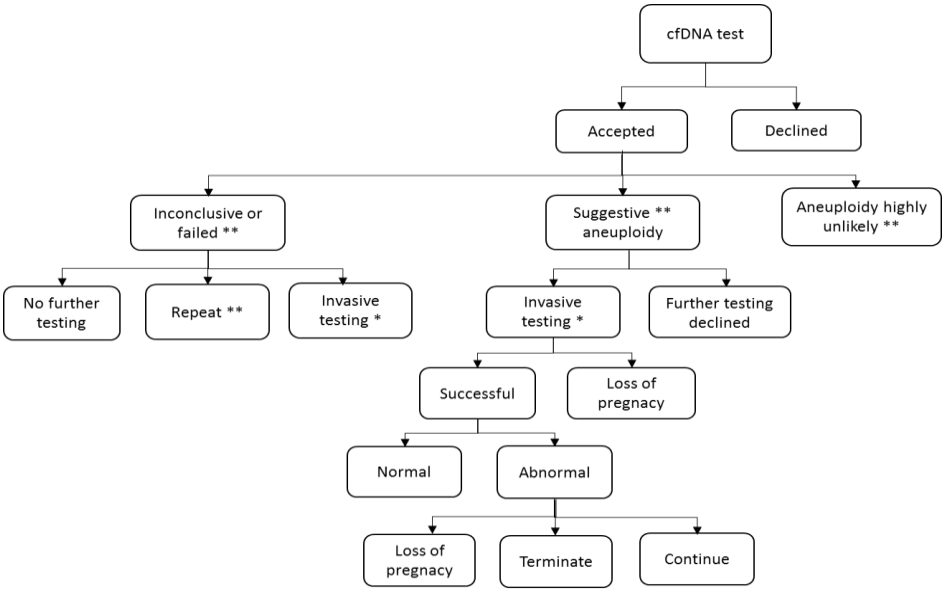
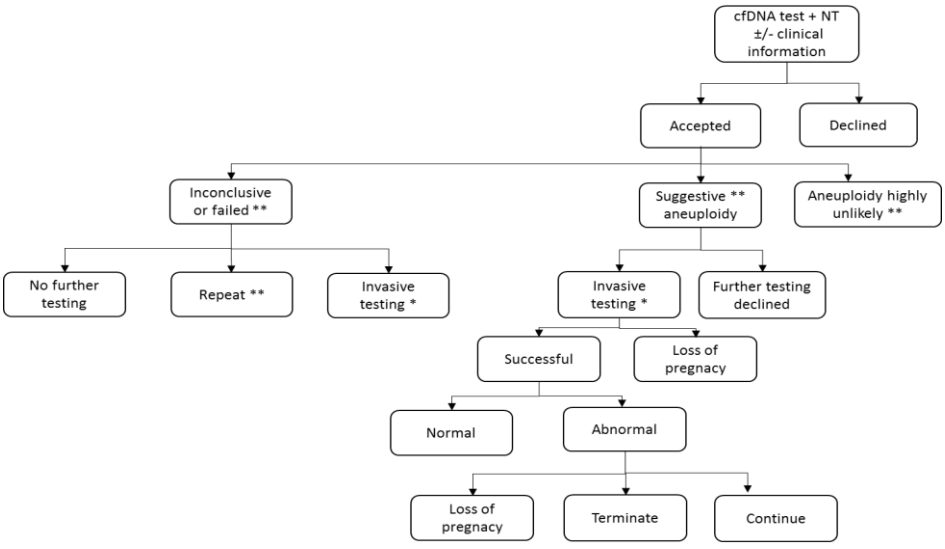
It is essential to highlight that although paired study designs have advantages over randomised controlled trials, given that they may be more feasible and would require fewer patients, they are inappropriate for assessing trade-offs between different screening approaches. To determine if NIPT would serve as a replacement, triage or add-on requires more information than the accuracy of the test. It needs assessment of the performance of the different test strategies, taking into account detection of all anomalies, abortions, miscarriages and other patient-related outcomes. Important uncertainties remain regarding the best screening pathway. As illustrated by the modelling results, however, it is necessary for decision makers to find the right balance between the different aims of using NIPT: the aim of detecting all T21 cases might be achieved with a slightly higher rate of invasive testing; the aim of reducing the rate of invasive testing, on the other hand, comes with the disadvantage of not detecting all cases of T21. Nevertheless, the model alone is an insufficient basis for any decision, as it is based on several assumptions and simplifications.

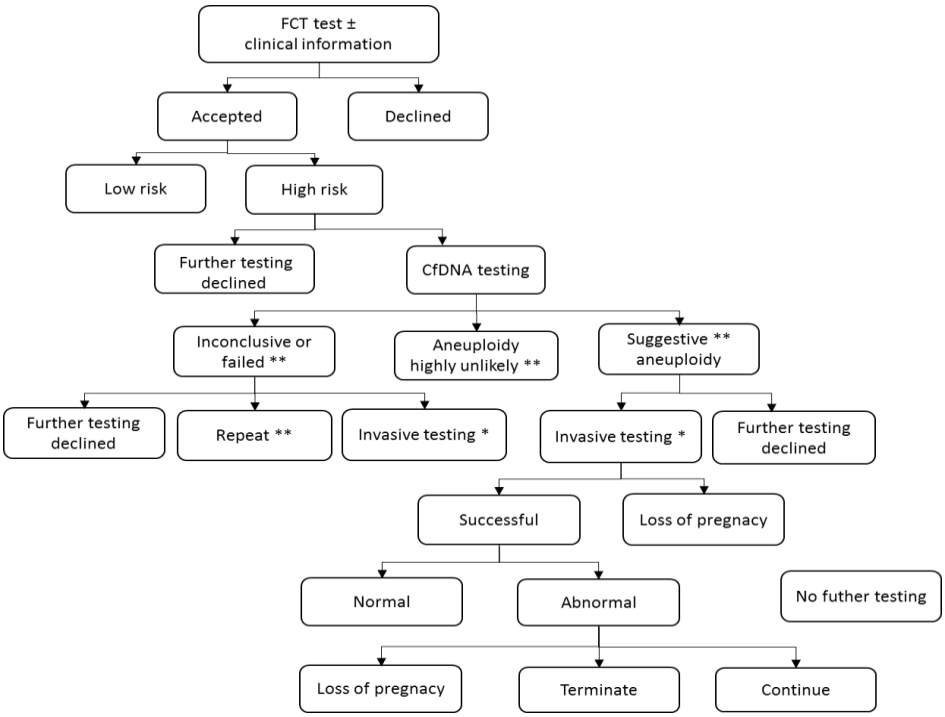
Conclusion

- Existing moderate quality evidence supports that the detection of T21 cases is higher when NIPT replaces FCT as a primary screening test and that this replacement would lead to a reduction in unnecessary invasive testing. However, important uncertainties remain regarding the under-reporting of missed cases given the inappropriate verification of negative results. Data regarding key safety outcomes are also lacking (increase in the number of children born with major anomalies, elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance, etc.). The generalisability of the PPV and NPV is limited by the fact that the prevalence of T21 found in the studies included is not representative of that found in the general pregnant population.
- No data exist to assess the accuracy of NIPT offered as part of FCT.
- Available data suggest that the use of NIPT as an add-on to FCT for screening of the high-risk T21 population could also lead to substantial reductions in unnecessary invasive testing, although this needs to be confirmed with real-world data. The performance of the test (test failures, uncertain results) and the uptake of NIPT screening are among the factors that could contribute to changing this ratio in real practice.
- There is a lack of data to assess the use of NIPT as an add-on to FCT for high- and intermediate-risk T21 populations.
- The low QoE for T18 and T13 does not allow conclusions to be drawn on these trisomies for any of the screening pathways.
- There is insufficient evidence to establish the accuracy of NIPT for twin pregnancies.
- Appropriately designed prospective comparative studies are required to be able to assess the performance of the different test strategies, taking into account detection of all anomalies, abortions, miscarriages and other patient-related outcomes. Important uncertainties remain regarding the best screening pathway.

1 SCOPE

Description	Project scope
Population	Pregnant women of at least 8–9 weeks' gestation undergoing routine primary screening for fetal aneuploidies. Three types of population are considered in this assessment: 1. Pregnant women classified as at high risk of fetal aneuploidies by FCT or assessed as high risk as a result of other risk factors such as family history of genetic or chromosomal anomaly, or previous aneuploid pregnancy history. The cut-off value for defining high-risk women is 1 in 300. 2. Pregnant women classified as having intermediate risk of fetal aneuploidies by FCT. The threshold cut-off value for defining intermediate risk is 1 in 300 to 1 in 1000. 3. General pregnant population without any predefined fetal aneuploidy risk factor. Singleton and twin populations were analysed independently given the claimed differences in performance of NIPT in these populations. Rationale: According to guidelines from the National Society of Genetic Counselors [9] and the American College of Obstetricians and Gynecologists (ACOG) [32] and position statements from the International Society for Prenatal Diagnosis [33] and the (ACOG and the Society for Maternal–Fetal Medicine [34], NIPT could be offered to pregnant women at high risk of aneuploidy or the general obstetric population. The International Society for Prenatal Diagnosis considers NIPT could be used as a primary method, offered secondary to a high-risk assessment or contingently to women ascertained as having high or intermediate risk by conventional screening. NIPT would not be applicable in triplet and higher-order pregnancies.
Target condition	ICD-10 codes: • Trisomy 21: ICD-10-CM diagnosis code Q90 (Q90.0, Q90.1, Q90.2 y Q90.90) • Trisomy 18: ICD-10-CM diagnosis code Q91 (Q91.0-3) • Trisomy 13: ICD-10-CM diagnosis code Q91 (Q91.4-7) MeSH terms: aneuploidy, trisomy 21, trisomy 18, trisomy 13, Down syndrome, Edward syndrome and Patau syndrome

Intervention	Five types of interventions are assessed: 1. Prenatal screening based on NIPT to estimate the risk of fetal aneuploidies, followed – for women testing at risk – by invasive diagnostic tests (NIPT as a primary screening test; total replacement of FCT)  2. Prenatal screening based on NT, NIPT and other clinical information (family history of chromosomal anomaly, previous aneuploid pregnancy history, etc.) to estimate the risk of major fetal anomalies, followed – for women with low risk of aneuploidies – by invasive diagnostic tests (NIPT as part of FCT; partial replacement of FCT)  3. Prenatal screening based on the FCT and/or clinical information (family history of chromosomal anomaly, previous aneuploid pregnancy history, etc.) to estimate the risk of fetal aneuploidies, followed – for women estimated to be at high risk – by NIPT, followed – for women having risk confirmed by NIPT – by invasive diagnostic tests (NIPT as an add-on to FCT and other risk factors)

Description	Project scope
	 4. Prenatal screening based on the FCT to estimate the risk of fetal aneuploidies, followed — for women testing as having intermediate to high risk of aneuploidies – by NIPT, followed – for women having risk confirmed by NIPT– by invasive diagnostic tests (NIPT as add-on to FCT and/or other factors) 5. Prenatal screening and diagnosis with NIPT without confirmation by invasive diagnostic tests (NIPT as a replacement for invasive diagnostic tests) Noninvasive prenatal tests are based on the analysis of cfDNA in the maternal plasma and are performed with one of the following techniques: • Next-generation sequencing: ○ Whole genome sequencing ○ Targeted genome sequencing: chromosome-specific sequencing or single nucleotide polymorphism-based method • Chromosomal microarray analysis Noninvasive prenatal test trademarks identified: Genatal 1, Genatal 2, Genatal +, Verifi™ prenatal test, MaterniT® 21 PLUS, MaterniT® GENOME, VisibiliT™, Harmony® prenatal test, Panorama® prenatal screening test, IONA® test, Vanadis SMART™ NIPT, Prendia START, Prendia EXTEND, VERACITY™, BambniTest, NACE®, NACE® amplified, PrenatalSafe®, Prenataltest®, Aurora, Clarigo™, PrenaTest® or PraenaTest®, informaSeq™ test, TrisoNIM® Advance, Trisonim® Premium, NIFTY™ test. MeSH terms: cell-free fetal DNA, massively parallel sequencing, single nucleotide polymorphism-based method. Intended use of technology: prevention

Description	Project scope
Comparison	Routine first-trimester screening for fetal aneuploidies based on the risk estimated by the standard FCT and other risk factors, followed – for women considered to be at risk – by invasive diagnostic tests. The FCT relies on: • maternal first-trimester serum screening (pregnancy-associated plasma protein A and β subunit of human chorionic gonadotropin); • and an ultrasound scan to measure fetal NT or fetal crown–rump length; • and maternal age. Rationale: comparators were chosen by application of EUnetHTA criteria [1]
Reference standard	• Fetal karyotype through invasive testing such as amniocentesis or CVS • Outcome at birth through clinical examination or follow-up of the newborn or by karyotyping in the case of miscarriage or fetal loss
Outcomes	The intervention assessed is prenatal screening (with different positioning of NIPT) aimed at informing women about the risk of trisomies 13, 18 and 21. The claimed benefit of the tests is to provide information that is more accurate than that from currently used screening tests. The effectiveness of the screening process is evaluated in terms of accuracy (intermediate outcomes), as invasive tests are already a decision based on noninvasive prenatal test results, but also in terms of how these screening strategies could change the management of prenatal aneuploidies and thereby impact on patient-relevant outcomes. • **Safety of NIPT for trisomies 13, 18 and 21** o False negative rate o False positive rate o Increase in the number of children born with other major prenatally undetected chromosomal conditions/anomalies (not targeted by prenatal aneuploidy screening) o Increase in elective pregnancy termination for other chromosomal anomalies with uncertain significance (not targeted by prenatal aneuploidy screening) o Test performance: test failure rate, uncertain results rate • **Effectiveness of NIPT for trisomies 13,18 and 21** o Sensitivity and specificity o Positive predictive value o Negative predictive value • **Effectiveness of prenatal screening with NIPT versus screening without NIPT with regard to patient-relevant outcomes for the different screening strategies** o Reduction in children born with undiagnosed trisomies 13, 18 and 21 o Reduction in the number of miscarriages or stillbirth of individuals affected by trisomies 13, 18 and 21 o Reduction in the number of miscarriages related to invasive testing (amniocentesis or CVS) o Reduction in uptake of invasive testing • **Organisational, ethical and social issues of aneuploidy screening** o Completion of the diagnostic pathway by the 15th week of gestation o Genetic counselling before and after aneuploidy screening o Process-related costs

Description	Project scope
	• **Other important patient outcomes** ○ Anxiety Rationale: outcomes are identified from the documents mentioned above [9, 32, 33] based on EUnetHTA guidelines about selection of endpoints for relative effectiveness assessment [35].
Study design	• **Safety of prenatal screening with NIPT:** randomised controlled clinical trials, nonrandomised controlled clinical trials, DTA studies on the index test, comparator and reference standard (cross-sectional studies) and registries • **Effectiveness of prenatal screening with NIPT:** randomised controlled clinical trials, nonrandomised controlled clinical trials and DTA studies on the index test, comparator and reference standard (cross-sectional studies) • **Organisational, ethical and legal issues and patient outcomes:** reviews/consensus documents and qualitative studies.

Abbreviations: cfDNA=cell-free fetal DNA; CVS=chorionic villus sampling; DTA=diagnostic test accuracy; EUnetHTA=European Network of Health Technology Assessment; FCT=first-trimester combined testing; ICD-10=International Classification of Diseases, 10th revision; ICM-10-CM=International Classification of Diseases, 10th revision, clinical modification; MeSH=Medical Subject Headings; NIPT=noninvasive prenatal testing; NT=nuchal translucency.

2 METHODS AND EVIDENCE INCLUDED

2.1 Assessment team

The tasks assigned to the agencies were:

Avalia-t (authors):

- Developed the first draft of the EUnetHTA project plan
- Performed the literature search and study selection
- Conducted the assessment (extraction, analysis, synthesis and interpretation of findings)
- Sent the first draft to dedicated reviewers, compiled feedback, answered comments and performed changes according to reviewers' comments
- Sent the second draft to external experts, compiled feedback, provided answers to reviewers and were responsible for undertaking corresponding changes
- Sent the second draft to manufacturers for fact checking, compiled feedback and performed changes
- Prepared the final assessment and wrote a final summary of the assessment

Regione Emilia-Romagna (co-author):

- Collaborated in the development of the EUnetHTA project plan
- Checked and approved all steps (e.g., literature selection, data extraction, assessment of risk of bias) and provided methodological support
- Reviewed the first and second draft assessment, proposed amendments where necessary (performed additional hand search when needed) and provided written feedback
- Collaborated in the elaboration of conclusions, which were discussed and agreed on

Dedicated reviewers:

- Reviewed and discussed the EUnetHTA project plan (scoping meeting)
- Reviewed and provided comments on the first draft assessment, as well as methodological support when needed
- Guaranteed quality assurance
- Reviewed and agreed on the conclusions

2.2 Scoping phase/patient involvement

During the scoping phase the assessment team, external experts, manufacturers and a user representative (pregnant women from Alvaro Cunqueiro Hospital, Spain, who had undergone NIPT) were consulted and asked to provide written feedback regarding the Population, Intervention, Comparator and patient related Outcomes (PICO). The assessment team and external experts were asked about the threshold used in their respective countries for risk classification. A scoping meeting was organised before the start of the assessment to discuss the PICO question where the assessment team, clinicians and geneticists (i.e. external experts) were present. All agreed on the PICO as described in the assessment.

2.3 Source of assessment elements

The selection of assessment elements for the description and technical characteristics of technology domain, the health problem and current use of the technology domain, the clinical effectiveness domain and the safety domain was based on the HTA Core Model® for Rapid Relative Effectiveness Assessment version 4.2 [25]. Additional elements were added from the HTA Core Model® Application for Diagnostic Technologies version 3.0 and the HTA Core Model® Application for Screening Technologies Version 3.0 [26]. The checklist for potential ethical, organisational, patient and social, and legal aspects of the HTA Core Model® for Rapid Relative Effectiveness Assessment was used to identify if ethical, organisational, social or legal aspects were deemed relevant for assessment. For the purpose of the report, critical issues were chosen from the ethical analysis, organisational aspects, and patients and social aspects domains of the HTA Core Model® Application for Diagnostic Technologies version 3.0. General questions referring to selected issues were translated into specific answerable questions, which were answered individually or grouped together.

2.4 Search

A systematic search of the scientific literature was performed between February and March 2017 in the following databases:

- CRD database
- Cochrane Library Plus
- MEDLINE (PubMed)
- Embase (OVID SP)
- Web of Science

Specific search strategies were designed for each of the databases to identify studies for the safety and clinical effectiveness domains. Additional strategies were defined to recover information for relevant ethical, organisational, and patient and social issues. Searches in PubMed, Embase and Web of Science were performed for each of the health problem and current use of the technology domain issues and the description and technical characteristics of technology domain issues. Guideline repositories were used to identify relevant guidelines (Guidelines International Network (GIN), National Guideline Clearinghouse, TRIP database, Australian Clinical Practice Guidelines, American College of Physicians (ACP) Website, CPG Infobase). As far as the searches were concerned, no limitations were applied in terms of the type of studies for any of the domains. However, specific searches of guidelines, reviews/health technology assessment reports and accuracy or qualitative studies were limited to the year 2010 when the first studies on NIPT were published. General Internet searches and manual searches of citations were complementary sources of information for all domains; that is, ongoing clinical trials and research projects were located through ClinicalTrials.gov, ICTRP and the UK Clinical Trials Gateway.

Detailed tables containing the search strategies can be found in Appendix 1.

Manufacturers identified at the time of the search were contacted by the EUnetHTA Joint Action 3 WP4 project manager (Ludwig Boltzmann Institute for Health Technology Assessment). For feasibility reasons, and given that general information regarding NIPT approaches is common to all tests, only four manufacturers who were identified to have peer-reviewed publications were asked

for submission files. The short version of the submission files was sent to these manufacturers by the EUnetHTA JA3 WP4 project manager on 18th November 2016. Duly completed submission files were received from Illumina Inc., Roche/Ariosa Diagnostic Inc. and Premaitha Health. General Internet searches were performed to complement technical information sent by manufacturers. Additional information related to NIPT CE mark (type of CE-marked product and indications) and specific characteristics of the technologies were requested from all manufacturers identified during the assessment scoping phase on 23rd December 2016. Manufacturers were asked if their product/s had a CE mark certificate or equivalent regulatory approval for the indications under assessment and if they did, the manufacturers were asked to provide additional information regarding the type of CE mark (research only, etc.) and product for which the CE mark was applicable (software, kit or both). Manufacturers were also asked if their product was commercialised in Europe, if their companies produced other technologies relevant for the assessment and if they were aware of any other relevant CE-marked devices on the market for the respective technology and indication. Finally, they were asked to provide additional information/data that they considered relevant/ differentiating (studies, etc.). In this way, it was ensured that no key information was missed.

2.5 Literature selection and data extraction

Two independent authors from avalia-t selected the relevant abstracts pertaining to the PICO question. The two authors read the full text of potentially relevant articles independently and included/excluded original studies according to the scope predefined eligibility criteria (inclusion/ exclusion). Studies which did not provide data on relevant outcomes, reported on duplicated data or were judged a priori to have a high risk of bias or important concerns regarding applicability issues because of the following reasons were excluded:

- Unclear selection criteria and/or indications
- Case-control design
- Lack of information on the index test or reference standard
- Uncertainty regarding independent assessment of the index test/reference standard
- Inappropriate/no reference standard in more than 80% of cases

Congress abstracts and studies published in languages other than English, Spanish, French or Italian were also excluded. The discrepancies in the study selection process were resolved by consensus. Excluded studies and reasons for exclusion can be found in Appendix 1.

The identification of relevant outcomes was done in accordance with the EUnetHTA guidelines about endpoints used in relative effectiveness assessment [35]. The preliminary proposal of outcome variables developed by the authors and the co-author was discussed with the dedicated reviewers and external experts during the scoping phase. The authors and the co-author evaluated the relative importance of outcomes according to the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system [28].

For the clinical effectiveness and safety domains, the relevant data were extracted and recorded in evidence tables by one author from avalia-t and reviewed by another. Both of these steps were checked by the co-author. Figure 1 displays the study selection flow chart.

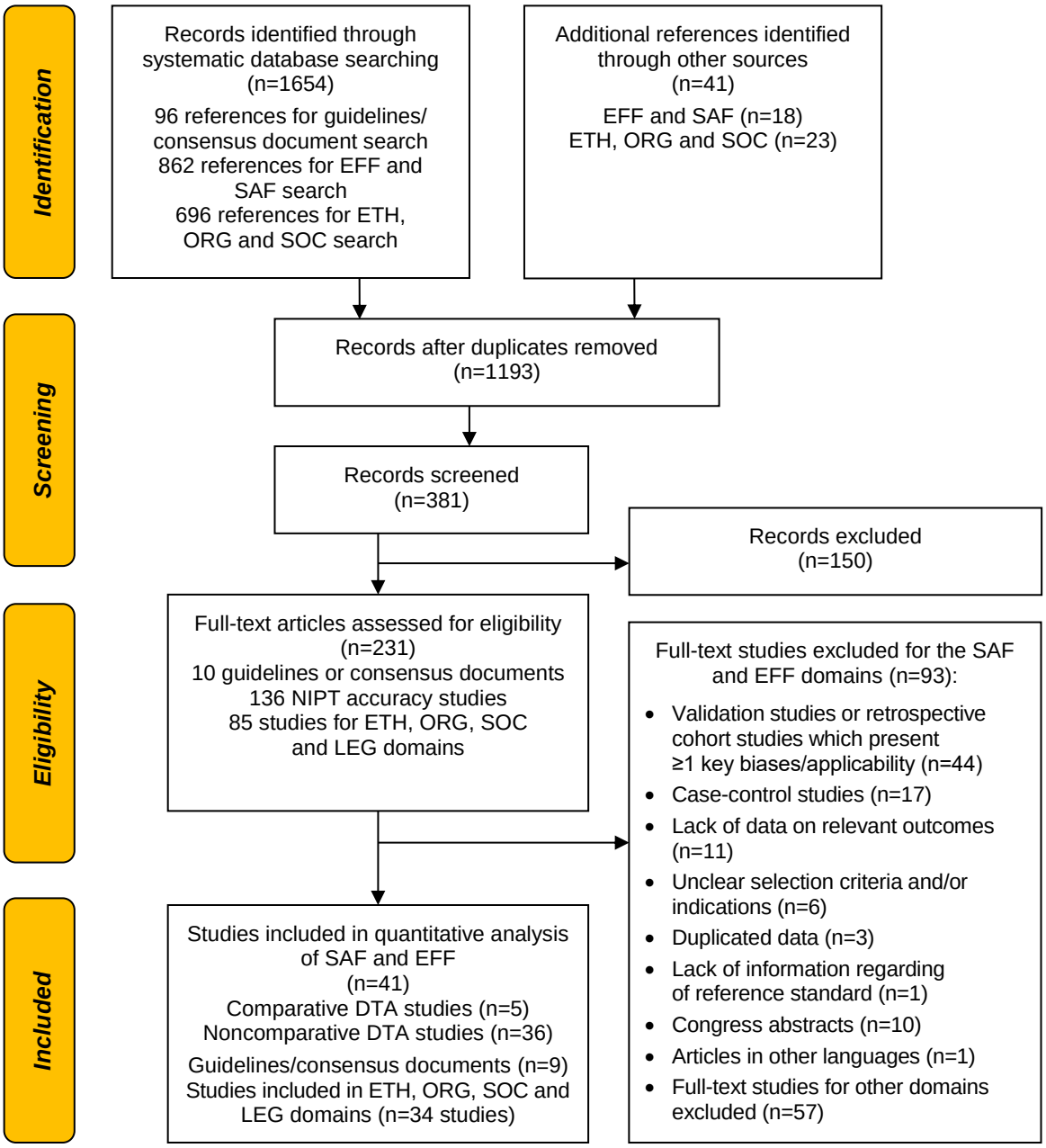


Figure 1: Study inclusion flow chart.

Abbreviations: EFF=clinical effectiveness; SAF=safety; ETH=ethical analysis; ORG=organisational aspects; SOC=patients and social aspects; LEG=legal aspects; NIPT=noninvasive prenatal testing, DTA=diagnostic test accuracy

A total of 1654 abstracts of original research articles/guidelines were retrieved from the systematic bibliographic searches and 41 additional relevant were publications identified by means of the manual search. Overall, 219 were considered potentially relevant and were selected for full-text analysis. After elimination of duplicates and studies which did not comply with the eligibility criteria, 41 original studies were included for the assessment of clinical effectiveness and safety. Nine guidelines were considered for the purpose of the description and technical characteristics of technology and the health problem and current use of the technology domains and 34 studies were analysed for the ethical analysis, organisational aspects, patients and social aspects, and legal aspects domains. Moreover, the search identified five systematics reviews and/or meta-analyses and five health technology assessment reports.

2.6 Quality rating of studies

The QUADAS-2 tool was used to assess the risk of bias of DTA studies [27]. Following QUADAS group recommendations, “if a study is judged as low on all domains relating to bias or applicability, then it is appropriate to have an overall judgment of low risk of bias or low concern regarding applicability”; and “if a study is judged high or unclear in 1 or more domains, then it may be judged at risk of bias or as having concerns regarding applicability” [27]. The level of confidence/certainty in the evidence was evaluated with the GRADE system [28].

No quality assessment tool was used for the description and technical characteristics of technology and the health problem and current use of the technology domains. Information from different sources (manufacturers, bibliography searches, official Web pages and general Internet searches) was compared and contrasted to cross-check it for validity. Information was synthesised in a descriptive manner. A descriptive analysis of data was provided for all relevant outcomes in the other domains (i.e., ethical analysis, organisational aspects, patients and social aspects, and legal aspects domains).

The two authors carried out the Quality of evidence assessment for the description and technical characteristics of technology and the health problem and current use of the technology domains, independently of each other. Discrepancies were resolved by consensus. The whole process was reviewed by the co-author.

2.7 Statistical analysis

Statistical analyses were performed according to recommendations described in the EUnetHTA guideline “Meta-analysis of Diagnostic Test Accuracy Studies” [29].

Statistical analyses were mainly conducted with use of the metandi command in STATA 13. This command fits both hierarchical summary receiver operating characteristics (HSROC) and bivariate random-effects models. Forest plots and measures of variability (variances and covariance of logit sensitivity and logit specificity across studies) were used to assess between-study heterogeneity. A bivariate random-effects model was used to estimate mean sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR–) and diagnostic odds ratios (DORs) with 95% CIs for each trisomy to provide aggregate result for all three trisomies. When the bivariate random-effects model failed to converge or provided unreliable parameter estimates, two univariate random-effects models were used [36].

To summarise overall test performance, the HSROC curve was constructed.

Publication bias was assessed by funnel plots representing DOR versus effective sample size [37]. This analysis was performed only when more than 10 studies were available.

2.8 Description of the evidence used

The guidelines/consensus articles considered for the description and technical characteristics of technology and the health problem and current use of the technology domains were elaborated by different medical colleges or societies from the UK, USA, Australia, etc. (see Appendix 1, Table A1). Only one was evidence based [38].

The clinical effectiveness and safety were assessed for five screening pathways:

1. NIPT as a primary screening test as a replacement for FCT
2. Prenatal screening based on NIPT as part of FCT
3. NIPT as an add-on to FCT for the high-risk population
4. NIPT as an add-on to FCT for the high- and intermediate-risk population
5. NIPT as a replacement for invasive testing

Direct evidence for the clinical effectiveness and safety domains was found only for the first pathway (total replacement of FCT). This evidence derives from five paired comparative studies and four noncomparative studies performed in singleton pregnancies. Moreover, two studies on twin pregnancies offered NIPT as a primary screening strategy to some of the women included, although results were reported jointly with those of women offered NIPT as a second-tier test (high-risk pregnancies). The question regarding NIPT as an add-on to FCT in women with high risk of aneuploidies was answered indirectly from pooled data derived from 26 retrieved studies on singleton pregnancies which assessed NIPT as a second-tier test in these populations. The add-on strategy for intermediate-risk patients was addressed in only one study. Six studies provided data on the accuracy of NIPT for twin populations. No evidence was found regarding the performance of these tests in combination with FCT and/or NT assessment. The scenario of NIPT as a diagnostic test will not be considered as none of the tests are currently indicated for this purpose. No data exist regarding patient-relevant outcomes (Table 2). Detailed information about studies included in the assessment can be found in Appendix 1 (Tables A2–A5).

The evidence included in the ethical analysis, organisational aspects, patients and social aspects, and legal aspects domains comes from 14 quantitative surveys, questionnaires or interviews and two systematic reviews. One of the systematic reviews, which focused on factors affecting the clinical use of noninvasive testing, used a mixed method approach to identify key features of the studies included. The other used thematic analysis to explore Internet advertising of NIPT.

Ongoing clinical trials and research projects identified can be found in Appendix 1 (Table A6).

The search identified five systematics reviews and/or meta-analyses and five health technology assessment reports which were not included because they did not comply with the PICO question. Detailed information about these documents can be found in Appendix 1 (Table A7).

Table 2: Main characteristics of studies included for the clinical effectiveness and safety domains

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
Sarno et al. [39], 2016	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	10,698	Index test trademark: Harmony® prenatal test Comparator: no intervention Reference standard: fetal karyotype (method used not specified)	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Comas et al. [40], 2015	Prospective DTA trial (cross-sectional design) Trisomy 21	333	Index test trademark: Harmony® prenatal test or Panorama™ test Comparator: no intervention Reference standard: CVS or amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Norton et al. [31], 2015	Prospective, multicentre comparative DTA trial (cross-sectional design) NIPT analysis blinded to clinical information Trisomies 21, 18 and 13 and other an-euploidies (45,X maker chromosomes, unbalanced translocations, unbalanced translocations, 7p deletion, 5p deletion/duplication, 1q41 deletion and isochromosome Yp)	18,955	Index test trademark: Harmony® prenatal test Comparator: standard screening (NT and biochemical analytes, i.e., PAPP-A and total hCG or β-hCG Reference standard: CVS, products of conception or neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV. *Effectiveness of prenatal screening with NIPT vs. screening without NIPT* Reduction in children born with undiagnosed trisomies 13, 18 and 21 Reduction in uptake of invasive testing
Pérez-Pedregosa et al. [41], 2015	Prospective comparative DTA trial (cross-sectional design) Trisomies 21 and 18	582	Index test trademark: Harmony® prenatal test Comparator: standard screening, i.e., NT with serum biochemical assays (PAPP-A and total hcG or β-hCG) Reference standard: CVS or amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV *Effectiveness of prenatal screening with NIPT vs. screening without NIPT* Reduction in uptake of invasive testing
Quezada et al. [42], 2015	Prospective, comparative DTA trial (cross-sectional design) Trisomies 21, 18 and 13	2905	Index test trademark: Harmony® prenatal test Comparator: standard screening, i.e., NT and fetal CRL with serum biochemical assays (PAPP-A and total hCG or β-hCG) Reference standard: CVS or amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV *Effectiveness of prenatal screening with NIPT vs. screening without NIPT* Reduction in children born with undiagnosed trisomies 13, 18 and 21

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
				Reduction in uptake of invasive testing
Zhang et al. [43], 2015	Prospective, multicentre DTA trial (cross-sectional design) Trisomies 21, 18 and 13	147,314	Index test trademark: NA (Illumina HiSeq200 platform) Comparator: no intervention Reference standard: CVS or amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Bianchi et al. [30], 2014 **CARE study**	Prospective, blinded, multicentre DTA trial (cross-sectional design) NIPT analysis blinded to clinical data and outcomes Trisomies 21, 18 and 13	2042	Index test trademark: Verifi™ prenatal test Comparator: standard screening, i.e., serum biochemical assays in the first trimester (PAPP-A and total hCG or β-hCG) or second trimester (maternal serum α-fetoprotein, hCG, unconjugated oestriol and inhibin A) with or without NT Reference standard: CVS or amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV *Effectiveness of prenatal screening with NIPT vs. screening without NIPT* Reduction in children born with undiagnosed trisomies 13, 18 and 21 Reduction in uptake of invasive testing
Pergament et al. [44], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13 and monosomy X	1064	Index test trademark: NA (SNPs) Comparator: no intervention Reference standard: amniocentesis, CVS, products of conception or genetic testing of umbilical cord blood, buccal sample or saliva	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Song et al. [45], 2013	Prospective, comparative DTA trial Trisomies 21, 18 and 13 and SCA (45,X and 47,XXy syndrome)	1916	Index test trademark: NA (Illumina HiSeq2000 platform) Comparator: triple serum screening in the second trimester (α-fetoprotein, free β-hCG and unconjugated oestriol) Reference standard: amniocentesis, CVS, cordocentesis or neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV *Effectiveness of prenatal screening with NIPT vs. screening without NIPT* Increase in the number of children born with other unconfirmed chromosomal anomalies Reduction in children born with undiagnosed trisomies 13, 18 and 21 Reduction in the number of miscarriages or stillbirths of individuals affected by trisomies 13, 18 and 21 Reduction in uptake of invasive testing

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
Kim et al. [46], 2016	Prospective DTA trial (cross-sectional design) Trisomy 21	101	Index test trademark: NA (Ion semiconductor-based sequencing) Comparator: no intervention Reference standard: amniocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Ma et al. [47], 2016	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	2439	Index test trademark: NA (BGISEQ-1000/ combinatorial probe-anchor ligation sequencing-cPAL platform) Comparator: no intervention Reference standard: CVS or amniocentesis or cordocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Oepkes et al. [48], 2016 **TRIDENT study**	Prospective multicentre DTA trial (cross-sectional design) Trisomies 21, 18 and 13	1390	Index test trademark: NA (Illumina HiSeq2500 platform or Life Technologies 5500 W SOLID) Comparator: no intervention Reference standard: CVS or amniocentesis, ultrasound data, genetic testing in products of conception and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Persico et al. [49], 2016	Prospective, multicentre DTA trial (cross-sectional design) Trisomies 21, 18 and 13	259	Index test trademark: NA (SNPs) Comparator: no intervention Reference standard: CVS or amniocentesis and/or aCGH	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Zhang et al. [50], 2016	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13 and Turner syndrome	87	Index test trademark: Verifi™ Prenatal Test Comparator: no intervention Reference standard: amniocentesis, neonatal blood karyotyping and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Benachi et al. [51], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	900	Index test trademark: NA (Illumina HiSeq1500 platform) Comparator: no intervention Reference standard: CVS or amniocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
Hernández-Gómez et al. [52], 2015	Prospective DTA trial (cross-sectional design) Trisomy 18 and monosomy X	42	Index test trademark: Harmony® prenatal test Comparator: no intervention Reference standard: amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Ke et al. [53], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	2340	Index test trademark: NA (Illumina HiSeq2000 platform) Comparator: no intervention Reference standard: amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Lee et al. [54], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	93	Index test trademark: NA (MiSeq and NextSeq (Illumina)) Comparator: no intervention Reference standard: amniocentesis, CVS cordocentesis, neonatal peripheral blood or products of conception	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Sago et al. [55], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	7740	Index test trademark: MateriniT PLUS Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Sánchez-Usabiaga et al. [56], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	270	Index test trademark: NA (SNPs) Comparator: no intervention Reference standard: CVS or amniocentesis and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Song et al. [57], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13 and SCA	213	Index test trademark: NA (Illumina HiSeq 2000 platform) Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
Wang et al. [58], 2015	Prospective DTA trial (cross-sectional design) Trisomies 21 and 18	917	Index test trademark: NA (Illumina HiSeq2000 platform) Comparator: no intervention Reference standard: standard karyotyping, FISH and neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Jeon et al. [59], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21 and 18	155	Index test trademark: NA (Ion Proton™ system) Comparator: no intervention Reference standard: amniocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Korostelev et al. [60], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	1968	Index test trademark: NA (SNPs) Comparator: no intervention Reference standard: amniocentesis, chromosomal microarray analysis or neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Porreco et al. [61], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13 and SCA (Turner syndrome, trisomy X, Klinefelter syndrome and 47,XYY syndrome)	4170	Index test trademark: NA (Illumina HiSeq2000 platform) Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Stumm et al. [62], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	522	Index test trademark: NA (Illumina HiSeq2000 platform) Comparator: no intervention Reference standard: amniocentesis, CVS or cordocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Willems et al. [63], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	3000	Index test trademark: Harmony® prenatal test Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Zhou et al. [64], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	7705	Index test trademark: NA Comparator: no intervention Reference standard: amniocentesis or neonatal follow-up	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
Liang et al. [65], 2013	Prospective DTA trial (cross-sectional design) Trisomies 21, 18, 13 and 9 and SCA (Turner syndrome, XXX, XXY or XYY)	435	Index test trademark: NA (Illumina HiSeq2000 platform) Comparator: no intervention Reference standard: amniocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Nicolaides et al. [66], 2013	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13 and Turner syndrome	242	Index test trademark: NA (SNPs) Comparator: no intervention Reference standard: CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Verweij et al. [67], 2013	Prospective DTA trial (cross-sectional design) Trisomy 21	595	Index test trademark: NA Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Lau et al. [68], 2012	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13 and SCA (Turner syndrome and Klinefelter syndrome)	108	Index test trademark: Verifi™ prenatal test Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Norton et al. [69], 2012	Prospective DTA trial (cross-sectional design) Trisomies 21 and 18	4002	Index test trademark: Harmony® prenatal test Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Ehrich et al. [70], 2011	Prospective DTA trial (cross-sectional design) Trisomy 21	480	Index test trademark: NA (GAIIx sequencer; Illumina) Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Gil et al. [71], 2016	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	4012 (460 classified as high risk and 3552 classified as intermediate risk)	Index test trademark: Harmony® prenatal test Comparator: no intervention Reference standard: CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV

Authors and year or study name	Study type and target condition	Number of women enrolled	Intervention(s)	Main endpoints
Fosler et al. [72], 2017	Prospective DTA trial (cross-sectional design) Trisomy 21	487	Index test trademark: Verifi™ prenatal test Comparator: no intervention Reference standard: amniocentesis, CVS or ultrasound findings	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Sarno et al. [39], 2016 ***This study was mentioned for the general pregnant population***	Prospective DTA trial (cross-sectional design) Trisomies 21, 18 and 13	467	Index test trademark: Harmony™ prenatal test Comparator: no intervention Reference standard: fetal karyotype (not specified method used)	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Tan et al. [73], 2016	Prospective DTA trial (cross-sectional design) Trisomy 21	565	Index test trademark: NA (MPS) Comparator: no intervention Reference standard: amniocentesis or CVS	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Bevilacqua et al. [74], 2015	Prospective DTA trial (cross-sectional design) Trisomy 21	515	Index test trademark: Harmony™ prenatal test Comparator: no intervention Reference standard: amniocentesis, CVS or neonatal blood examination	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Huang et al. [75], 2014	Prospective DTA trial (cross-sectional design) Trisomies 21 and 18	189	Index test trademark: NA Comparator: no intervention Reference standard: amniocentesis, CVS or cordocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV
Lau et al. [76], 2013	Prospective DTA trial (cross-sectional design) Trisomy 21	12	Index test trademark: NA Comparator: first- or second-trimester screening and/or first-trimester ultrasound marker screening (NT, fetal nasal bone and Doppler assessment of the tricuspid valve and ductus venosus) Reference standard: amniocentesis, CVS or cordocentesis	*Safety and effectiveness of NIPT:* FN, FP, test failure rate, S, Sp, PPV, NPV

Abbreviations: aCGH=microarray-based comparative genomic hybridisation; CARE=Comparison of Aneuploidy Risk Evaluations; CRL=crown–rump length; CVS=chorionic villus sampling; DTA=diagnostic test accuracy; FISH=fluorescence in situ hybridisation; FN=false negative; FP=false positive; hCG=human chorionic gonadotropin; β-hCG=β subunit of human chorionic gonadotropin; MPS=massive parallel sequencing; NA=not available; NIPT=noninvasive prenatal test; NPV=negative predictive value; NT=nuchal translucency; PAPP-A=pregnancy-associated plasma protein A; PPV=positive predictive value; S=sensitivity; SNP=single nucleotide polymorphism; Sp=specificity; TN=true negative; TP=true positive; SCA=sex chromosome aneuploidy.

2.9 Deviations from project plan

- Delay in the deliverable due to the extension of the scoping and assessment phase.
- Only four manufacturers who had peer-reviewed publications were asked for submission files for feasibility reasons. All were contacted to provide information regarding reimbursement.
- The search was restricted to 2010 given that the first noninvasive prenatal test was launched in 2011.
- Studies were restricted to those published in English, Spanish, French, Italian and Portuguese.
- Subgroup analysis could not be performed because of the lack of data and was deleted from the project plan.
- One additional assessment element was identified for the description and technical characteristics of technology domain (**B0018**).

3 DESCRIPTION AND TECHNICAL CHARACTERISTICS OF TECHNOLOGY

3.1 Research questions

Element ID	Research question
B0001	What are noninvasive prenatal tests and the comparators?
B0002	What is the claimed benefit of NIPT in relation to the comparators?
B0003	What is the phase of development and implementation of NIPT and the comparators?
B0004	Who administers NIPT and the comparators and in what context and at what level of care are they provided?
B0008	What kind of special premises are needed to use NIPT and the comparators?
B0009	What equipment and supplies are needed to use NIPT and the comparators?
B0018	Are the reference values or cut-off points clearly established?
A0020	For which indications has NIPT received marketing authorisation or CE marking?
A0021	What is the reimbursement status of NIPT in prenatal screening?

3.2 Results

Features of the technology and comparators

[B0001] – What are noninvasive prenatal tests and the comparators?

General characteristics of NIPT

Noninvasive prenatal tests are in vitro diagnostic products that use cfDNA from maternal blood of pregnant women. Though commonly referred to as cell fetal free DNA, the DNA does not derive from the fetus but originates from the cytotrophoblast layer of the chorionic villi (the outer placental cell layer). It comprises around 10%–15% of the total cfDNA in the maternal circulation during the late first and early second trimester [2]. Testing can be performed between 8–10 weeks' gestation.

Since the development of the first commercialised NIPT in 2011, which was designed for the detection of T21, T18 and T13, different laboratories have developed different assays, expanding the panel of chromosomal anomalies detected to other common chromosomal anomalies. Table 3 illustrates the features of the commercialised tests which were identified by the search, although many laboratory-developed tests with local redesigned workflows also exist given the transfer of existing technology to many laboratories worldwide. According to an international survey from 28 countries performed in 2015, the most common providers of noninvasive prenatal tests are currently Ariosa Diagnostics Inc./Roche Sequencing Solutions Inc. (Harmony®), BGI Diagnostics Technology Co. Ltd., (NIFTY™ test), Illumina Inc. (Verifi™, VeriSeq NIPT Solution and Serenity (Verifi™ and VeriSeq NIPT Solution)), Igenomix (NACE®), LifeCodexx AG (PrenaTest®), Natera (Panorama®) and Sequenom (MaterniT 21 PLUS test). Of these, the MaterniT 21 PLUS test is a laboratory-based test which is commercialised only in the USA [3].

The landscape of NIPT is diverse: some tests adopt PCR for amplification of the cfDNA before next-generation sequencing, whilst others rely on other methods of quantification such as chromosomal microarrays. All technologies thereafter use proprietary algorithms for chromosomal aneuploidy risk analysis. Regardless of the tests, all require a sufficient proportion of cfDNA in the maternal plasma to be able to cfDNA-differentiate between the status of the mother and the fetus. Initially many manufacturers established a minimum fetal fraction of 4% for NIPT evaluation, although with further development of NIPT technologies and analysis methods, this limit has been lowered. It has been highlighted that specimens containing excessive amounts might also affect the performance of the test (Harmony submission file) [77]. However, not all laboratories quantify fetal fraction in individual samples. Fetal fraction can be influenced by factors such as fetal aneuploidy, gestational age and maternal body mass index and weight, although failure to obtain an NIPT result could also be due to different technical/statistical reasons [5, 6]. In cases of a low fetal fraction, a redraw can be requested.

The results of NIPT can be confounded by several biological factors such as confined placental mosaicism, maternal copy number variations, maternal mosaicism, fetal partial trisomy or translocations, fetal mosaicism, fetal structural chromosomal anomalies other than trisomy, intrauterine fetal demise and disappearing twin [4]. Other factors which are liable to confound results are recent maternal transfusions, maternal organ or bone marrow transplants, maternal immunotherapy or stem cell therapy or maternal malignancy [77-79].

Specific features of the process and tests

NGS technologies can be broadly categorised as whole genome sequencing (WGS) or targeted sequencing (chromosome-specific sequencing and single nucleotide polymorphism (SNP) analysis) [80]:

- WGS analyses the whole genome and generates DNA sequence reads from all chromosomes nonspecifically. This method analyses random sequences and can allow millions of short DNA fragments to be sequenced rapidly in a single run. It can allow screening of more conditions than T21, T18 and T13 in extended screening programmes. The depth of sequencing or coverage (number of reads giving information about a base present at a set position in the reference sequence, or the number of times a base is represented within all the reads) will determine the resolution achievable; hence more sequencing will increase performance and allow the test to be more specific and sensitive.

- Targeted sequencing as the name indicates targets a subset of genomic regions. This approach differs from the whole genome approach by selectively amplifying and sequencing specific genomic regions, significantly reducing the total number of analysed reads. The region can include certain chromosomal loci (chromosome-specific sequencing) or SNPs of interest. The genome region will be predetermined by the specific test used, and the test would need to be modified and validated to provide information about other regions of the genome.

Most existing tests use first-generation quantitative WGS approaches or counting methods (Table 3) to determine the percentage of cfDNA compared with cell-free maternal DNA. Practically all of these use the Illumina platforms (Illumina Inc., San Diego, CA, USA) for multiple parallel sequencing. Accessing results commonly takes from around 3 to 10 days. The IONA® test (Premaitha Health plc, Manchester, UK) uses another platform, the Ion Proton™ ion semiconductor sequencing platform (Thermo Fisher Scientific), which is categorised by a total turnaround time, from the start of sample processing to a result, of 3 days.

Illumina Inc. has developed a new-generation noninvasive prenatal test, the VeriSeq NIPT Solution, which has been commercialised with different brand names (Table 3). VeriSeq NIPT Solution uses paired end sequencing. This allows the enrichment of a sample for placental cfDNA and as such removes the need for presequencing PCR. The turnaround time from sample accessing to results for the VeriSeq NIPT Solution is 26 hours. Natera has commercialised an NGS approach which specifically targets SNPs to determine ploidy (Panorama®). This second-generation approach, used also by other companies such as *Imegen-Instituto de Medicina Genómica*- (Spain), in comparison with other first-generation tests, has the ability to differentiate between cell-free maternal DNA and cfDNA, and in addition to identifying T21, T18, T13, monosomy X, and sex chromosome trisomies, it can identify the presence of a vanishing twin and maternal duplications. Currently it is being validated for common microdeletion conditions, including 22q11.2 deletion syndrome, 1p36 deletion syndrome, cri du chat syndrome, Prader-Willi syndrome and Angelman syndrome. Several other tests such as TrisoNIM and Prendia also target other trisomies and microdeletions.

The Harmony® test, initially commercialised as a laboratory NGS test based on Illumina technology, changed in 2014 to use an alternative microarray sequencing process that operates on the Affymetrix technology platform. This targeted amplification process, termed *digital analysis of selected regions* amplifies a set of universal PCR products from genomic intervals on chromosomes 1–13, 18, 21, X and Y and determines the fetal fraction by also measuring SNPs. These can be analysed to distinguish between cfDNA and cell-free maternal DNA. No data have been published regarding the Vanadis™ NIPT system, which is an innovative approach based on fluorescence. This system has still not been placed on the market in the European Union (EU).

Existing noninvasive prenatal tests have different software for analysis and interpretation of screening results with differentiating features, algorithms and quality standards. For example, not all tests measure fetal fraction to ensure adequate DNA analysis, or if done, include it in the analysis of fetal risk. VeriSeq NIPT Solution tests use a quality control metric to ensure that samples have sufficient coverage to make a confident call, eliminating the need for a set fetal fraction percentage cut-off point. The neoBona®, for example, integrates sequencing depth on each chromosome, the percentage of fetal fraction and size of the fragments to calculate risks [81]. The Harmony® prenatal test establishes standards thresholds for all quality control metrics. This test uses an analysis algorithm termed *FORTE* to compute the probability of trisomies and probability of fetal sex chromosomes. The algorithm takes into account the total cfDNA quantification from chromosomes measured with digital analysis of selected regions, the amount of fetal DNA in a sample as measured by NIPT, maternal age and gestational age-related risk for trisomy in calculating the probability score [82].

Table 3: Features of the intervention

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Illumina Inc. (San Diego, CA, USA)	Verifi™ prenatal test (https://www.illumina.com/)	Illumina Verifi™	NGS (WGS)	T21, T18, T13 and sex chromosome aneuploidies in singleton pregnancies T21, T18, T13 and presence of Y for women with twins through natural or reproductive methods Additional indications: T9, T16 and microdeletions (Di George, Prader-Willi/ Angelman, Cri-du-Chat, Wolf-Hirschhorn and 1p36 deletion)	3–5 days	All pregnant women ≥ 10 weeks gestation who have chosen to have T13, T18 and T13 prenatal screening Not intended to be used in isolation from other clinical findings and tests results Single, twin or egg donor pregnancies
Illumina Inc. (San Diego, CA, USA)	VeriSeq NIPT Solution Includes: the VeriSeq NIPT Workflow Manager for the VeriSeq NIPT Microlab STAR, the VeriSeq NIPT Sample Prep Kits, and the VeriSeq Onsite Server with the VeriSeq NIPT Assay Software.	Illumina VeriSeq NIPT Solution	NGS paired end (WGS)	T21, T18, T13 and sex chromosome aneuploidies	1 day (26 hours)	Intended for use in pregnant women of at least 10 weeks gestation The product must not be used as the sole basis for diagnosis or other pregnancy management decisions
Genesis Genetics (London, UK)	Genesis Serenity prenatal test (http://genesisgenetics.co.uk/genesis-serenity/)	Illumina Verifi™ and VeriSeq NIPT Solution	NGS paired end (WGS)	T21, T18, T13 and sex chromosome aneuploidies	—	Single or twin pregnancies

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Labco Quality Diagnostics/ Synlab International GmbH (München, Germany)	NeoBona® NeoBona® Advanced NeoBona® Advanced+ (http://www.neobona.es/)	Illumina VeriSeq NIPT Solution	NGS paired end (WGS)	**NeoBona:** T21, T18, T13 **NeoBona Advanced:** T21, T18, T13 and sex chromosome aneuploidies (singleton pregnancy only) **NeoBona Advanced+:** T21, T18, T13, T16, T9, sex chromosome aneuploidies and microdeletions (singleton pregnancy only) **Prenatal Test Extended Panel:** T21, T18, T13, sex chromosome aneuplodies and microdeletions (DiGeorge, Angelman/Prader - Willi, 1p36 deletion, Wolf -Hirschhorn y Cri-du-chat) (singleton pregnancy only) **Prenatal Test Extended Panel +** All chromosomes: T21, T18, T13, sex chromosome aneuplodies, microdeletions (DiGeorge, Angelman/Prader -Willi, 1p36 deletion, Wolf -Hirschhorn y Cri-du-chat) and all chromosome aneuploidies. (singleton pregnancy only)	10 days	Can be used in pregnancies ≥ 10 weeks of gestation Single, twin, IVF and egg donor pregnancies
Sequenom Laboratories (San Diego, CA, USA)	VisibiliT™ MaterniT 21® PLUS test (previously MaterniT 21) MaterniT® GENOME (https://www.sequenom.com/)	Illumina	NGS (WGS)	**VisibiliT™:** T21 and T18 **MaterniT® 21 PLUS:**T21, T18, T13, sex chromosome aneuploidies and 7 microdeletions (T21, T18, T13, sex chromosome aneuplodies, T16, T22 and microdeletions (Di George, Prader-Willi/ Angelman, Cri-du-Chat, Wolf-Hirschhorn, Jacobsen, Langer-Giedion and 1p36 deletion) **MaterniT® GENOME:** All chromosomes and deletions or duplications of chromosome material 7 Mb or larger, as well as seven clinically micro-deletion regions less than 7 Mb in size (Di George, Prader-Willi, Cri-du-Chat, Wolf-Hirschhorn, Jacobsen, Langer-Giedion and 1p36 deletion)	5 days	Can be utilized in pregnant women ≥ 10 weeks gestation MaterniT21 Plus is relevant for pregnancies at increased risk of fetal anomalies

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Natera® (San Carlos, CA, USA)	Panorama® (http://www.panoramatest.com/)	Illumina	NGS (SNP)	T21, T18, T13, sex chromosome aneuploidies and most common microdeletions, including 22q11.2 deletion syndrome, 1p36 deletion syndrome, cri du chat, Prader-Willi and Angelman	9 days	Panorama could be useful for the general pregnant population ≥ 9 weeks gestation Single pregnancies
Premaitha Health PLC (London, UK)	Iona® test (http://www.premaitha.com/the-iona-test)	Thermo Fisher Scientific	NGS (WGS)	T21, T18 and T13	3–5 days	Suitable for all pregnant women ≥ 10 weeks of gestation Intended to be used by a clinician in combination with other risk factors to estimate the risk of affected pregnancies Single, twin, surrogate or in-vitro fertilization pregnancies
Ariosa Diagnostics Inc./Roche Sequencing Solutions Inc. (San Jose, California, USA)	Harmony® prenatal test	Affymetrix[a] F. Hoffman-La Roche, Ltd)	Chromosomal micro-arrays	T21, T18, T13, Monosomy X*, sex chromosome aneuploidies* and 22q11.2 deletion syndrome *singleton pregnancy only	≤7 days	Intended for use in pregnant women ≥ 18 years of age, of ≥ 10 weeks´ gestation, and with ≤ 2 foetuses
LifeCodexx AG (Germany)	PrenaTest® (https://lifecodexx.com/)	Illumina	NGS (WGS)	T21, T18, T13, sex chromosome aneuploidies and 22q11.2 deletion syndrome	A few days	Available for pregnant women ≥ 9 weeks Primary diagnostic procedures in combination with ultrasound in pregnant women who are at high risk of fetal aneuploidies (≥ 35 years old, increased risk based on screening methods, ultrasound anomalies, prior pregnancies with aneuploidy, family risk, other medical reasons) Single or twin pregnancies

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Berry Genomics Co. Ltd. (Beijing, China)	BambniTest	Illumina	NGS (WGS)	—	—	—
BGI Diagnostics Technology Co. Ltd. (Shenzhen, China)	NIFTY™ test (http://www.niftytest.com/)	Illumina and Thermo Fisher Scientific	NGS (WGS)	T21, T18, T13, sex chromosome aneuploidies and most common microdeletions, including 2q33.1, 1p36, cri du chat, Prader-Willi, Angelman, Jacobsen, DiGeorge and van der Wonde	10 days	Available for any pregnant women ≥ 10 weeks gestation but particularly suitable for ≥ 35 years, fetal ultrasonographic findings indicative of increased risk of aneuploidy, reassurance following screening results, contraindication for invasive testing, prior pregnancies with trisomy, received In-Vitro Fertilization (IVF) treatment or suffered habitual abortion Single, twin, egg-donor and IVF pregnancies
Igenomix SL (Valencia, Spain)	NACE® test NACE® amplified test (http://nace.igenomix.es/)	Illumina	NGS (WGS)	**NACE®:**T21, T18, T13 and sex chromosome aneuploidies **NACE® amplified:** additionally T9 and T6 and six common microdeletions: 1p36, cri du chat, Prader-Willi, Angelman, Wolf-Hirschhorn and DiGeorge (singleton pregnancy only)	NACE®: 3 days NACE® amplified: 15 days	Available for pregnant women ≥ 10 weeks of gestation Specially indicated for women with abnormal 1st trimester test results, previous T21 pregnancies or suspicious ultrasonographic findings Single, twin, egg-donor and IVF pregnancies Any age, with independence of BMI
NIM Genetics (Madrid, Spain)	TrisoNIM® Advance TrisoNIM® Premium (https://www.nimgenetics.com/trisonim/)	Illumina and Thermo Fisher Scientific (NIFTY™ technology)	NGS (WGS)	**Trisomy Advance:** T21, T18, T13, sex chromosome aneuploidies and 3 microdeletions (1p36, 2q 33.1 and cri du chat) **TrisoNIM Premium:** T21, T18, T13, sex chromosome aneuploidies, T9, T16, T22 and 7 microdeletions (1p36, 1q32-q41 (van der Woude), 2q33.1, 5p (cri du chat), 10p14-p13 (DiGeorge 2), 11q (Jacobsen) and 16p12.2-p11.2	5–7 days	Can be used in pregnancies ≤ 10 weeks Single, twin, egg-donor pregnancies and IVF pregnancies

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Imegen Instituto de Medicina Genómica (Valencia, Spain)	Genatal 1 Genatal + Genatal 2 (Genatal Twin (https://www.imegen.es/test-prenatal-no-invasivo/)	Illumina Panorama® technology	NGS (SNP)	**Genatal 1:** T21, T18, T13 and sex chromosome aneuploidies **Genatal +:** T21, T18, T13, triploidy, sex chromosome aneuploidies and 5 microdeletions **Genatal 2** (exclusive for twin or multiple pregnancies: T21, T18 and T13)	7 days	Can be used in pregnancies ≥ 9 weeks of gestation Single, twin, egg donor pregnancies and multiple pregnancies (depending on the tests)
Genoma Laboratories (Rome and Milan, Italy)	PrenatalSafe® PrenatalSafe® Kario PrenatalSafe® Kario Plus PrenatalSafe® 5 PrenatalSafe® 3 PrenatalSafe® Plus (http://www.prenatalsafe.it/)	Illumina	NGS (WGS)	**PrenatalSafe® 3:**T21, T18 and T13 **PrenatalSafe® 5:** T21, T18, T13 and sex chromosome aneuploidies **PrenatalSafe® Plus:** T21, T18, T13, T9, T16 and 6 microdeletions (1p36, cri du chat, Prader-Willi, Angelman, Wolf-Hirschhorn and DiGeorge) **PrenatalSafe® Kario:** All chromosomes of fetal karyotype **PrenatalSafe® Kario Plus:** All chromosomes of fetal karyotype and 9 microdeletions (1p36, cri du chat, Prader-Willi, Angelman, Wolf-Hirschhorn, DiGeorge, Jacobsen, Langer-Giedion and Smith-Magenis)	3 days	Can be performed in all pregnant women ≥ 10 weeks of gestation Single, twin, egg-donor pregnancies and IVF pregnancies
Sorgente Genetica S.r.l. (Milan, Italy)	Aurora (http://www.testprenataleaurora.it/)	Illumina Verifi™	NGS (WGS)	T21, T18, T13, sex chromosome aneuploidies, T9, T16 and 5 microdeletions (1p36, cri du chat, Prader-Willi/Angelman, Wolf-Hirschhorn and DiGeorge)	10 days	Can be performed in all pregnant women but particularly recommendable for maternal age > 35 years, positive screening test for the first/second quarter, suggestive fetal US findings, contraindication for invasive testing, personal/family history of chromosomal anomalies Single, twin, egg-donor pregnancies and IVF pregnancies
Ebios Futura S.r.l. (Cuneo, Italy)	Prenataltest®	Illumina	NGS (WGS)	T21, T18, T13 and sex chromosome aneuploidies	<10 days	Can be performed in pregnant women ≥ 10 weeks gestation Single or twin pregnancies

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Multiplicom NV, Belgium (Agilent Technologies, CA, USA)	Clarigo™ (http://www.multiplicom.com/)	Illumina	NGS (targeted technology)	T21, T18 and T13	6–10 days	Can be used in pregnant women ≥ 8 weeks gestation Single pregnancies
Genesupport, FASTERIS, Swiss Institute for Bioinformatics (Switzerland)	Prendia START Prendia EXTEND (http://www.prendia.ch/fr/)	—	NGS (WGS)	**Prendia START:** T21, T18 and T13 **Prendia EXTEND:** sex chromosome aneuploidies, rare chromosomal anomalies (6, 7, 14, 15, 16) and structured chromosomal anomalies of other autosomes	7–14 days	Can be performed in pregnant women ≥ 10 weeks gestation Single, twin, egg-donor pregnancies and IVF pregnancies
LabCorp Inc. (North Carolina, USA)	InformaSeq℠ (https://www.integratedgenetics.com/)	Illumina	NGS (WGS)	T21, T18, T13 and sex chromosome aneuploidies (optional)	5–7 days	Can be performed in pregnant women ≥ 10 weeks gestation Single and twin pregnancies
Vanadis Diagnostics, Perker Elmer Inc. (Sweden)	Vanadis™ NIPT system http://www.vanadisdx.com/	—	Microplate-based technology	T21, T18 and T13	2–3 days	Vanadis™ NIPT is under development. The system does not conform to 98/79 EC In Vitro Diagnostic Medical device directive and cannot beplaced on the market or put into service in EU until they have been made to comply.
NIPD Genetics (Nicosia, Cyprus)	VERACITY™ test (https://www.nipd.com/)	Illumina	NGS (targeted enrichment technology)	T21, T18 and T13 and sex chromosome aneuploidies	A few days	Can be performed in pregnant women ≥ 10 weeks gestation Single and twin pregnancies

Abbreviations: IVF=in vitro fertilisation; NGS=next-generation sequencing; SNP=single nucleotide polymorphism; T6=trisomy 6; T9=trisomy 9; T13=trisomy 13; T16=trisomy 16; T18=trisomy 18; T21=trisomy 21; US=ultrasound; WGS=whole genome sequencing.

[a] Illumina WGS technology before 2014.

Comparators for T13, T18 and T21 screening

Screening tests

Multiple prenatal screening strategies exist for first- and second-trimester T21, T18 and T13 prenatal screening. The most commonly used approaches involve the measurement of serum proteins via a blood draw and ultrasound assessment. The FCT, which uses NT, PAPP-A and beta human chorionic gonadotropin (ßhCG) measurements, is the standard of practice in most European countries [7-9]. The test is usually performed between 10 weeks and 13 weeks 6 days to provide information about risk in early pregnancy. Differences in levels of the proteins have been observed in patients carrying a fetus with T21 and certain other chromosomal anomalies. Risk is calculated with use of different complex statistical population-derived algorithms which are based on these measurements and other maternal factors, such as maternal age, history of aneuploidy, weight, race and number of fetuses. NT can be used independently as a risk marker because, measured between 11 weeks and 13 weeks 6 days of gestation, it can be associated with other numeric chromosomal anomalies and major fetal anomalies, such as cardiac defects and diaphragmatic hernia, and a number of single gene disorders [38]. The translucent area disappears after 14 weeks' gestational age, when it becomes more echogenic. A recent Cochrane review established that the sensitivity for T21 detection, estimated at a 5% FP rate, was 71% (66%–75%) with the NT and maternal age strategy and 87% (86%–89%) with the combined NT, PAPP-A, hCG and maternal age strategy. Adding other ultrasound markers (nasal bone, fetal heart rate, frontomaxillary facial angle, etc.) increased sensitivity to more than 90% and specificity to more than 95% [83]. The detection rate for T18 and T13 has been shown to as high as 97% and 92% in some large studies [84].

Second-trimester screening for T18 and T21 is also used for women who present at more than 14 weeks' gestation [8, 9, 85], as this test can be performed at around 15–20 weeks' gestation. Women can be offered tests which use double, triple or quadruple serum markers. The most recommended is the quadruple test, which measures levels of hcG, alpha fetoprotein, dimeric inhibin A and unconjugated oestriol, in combination with maternal factors such as age, diabetes and plurality. On the basis of existing studies, it is acknowledged to provide an adjusted risk assessment for T21 and T18 similar to that of first-trimester screening and in addition identifies the risk of open neural defects [38], but delays reassurance and/or restricts women's options. These markers are not reliable for other forms of aneuploidy such as T13 or Klinefelter syndrome. In comparison with first-trimester tests, they do not require specialised ultrasonographic measurements [7-9].

The combination of first- and second-trimester ultrasound markers and serum analytes constitutes an alternative to one-step screening. With integrated screening, the patient undergoes NT and PAPP-A measurements in the first trimester and quadruple screening in the second trimester, receiving a single test result in the second trimester. In stepwise sequential screening, patients who are stratified into the high-risk group on the basis of the first-trimester screening NT and serum markers test are informed of the result and offered diagnostic testing, whilst those estimated to be of low risk proceed with the second-trimester quadruple test, being given a risk based on the combined results. Conventional screening via contingency screening is another two-step screening option, where patients are divided into low-, moderate- and high-risk groups on the basis of FCT. The overall detection rate for contingency screening is 91%–92% for T21 and 91%–96% for T18.

Diagnostic tests

Both NIPT and the comparators require confirmatory invasive testing (amniocentesis or CVS). Confirmation can be done by either karyotyping or chromosomal microarray, which might provide different levels of information. During CVS, a sample of cells is extracted from the placenta by either transabdominal CVS, via a needle, or transcervical CVS, using forceps or a catheter inserted through the cervix. CVS is usually performed between 10 and 14 weeks of pregnancy. Amniocentesis involves the insertion of a needle through the placenta to extract a sample of amniotic fluid. In comparison with CVS, amniocentesis is usually performed after 15 weeks. Both procedures are associated with a risk of miscarriage, which seems to differ substantially depending on the skills of the operator and the number of procedures performed. Miscarriage risks of up to 1% were reported in a Cochrane review [11] of 16 randomised studies but a recent systematic review and meta-analysis of controlled studies excluding those describing fewer than 1000 procedures showed lower added risk of 0.1% for amniocentesis and 0.2% for CVS, suggesting that the risk of miscarriage in specialist centres performing a large number of procedures is considerably lower than the figures currently given [10]. Results are normally available within 3 days (rapid test for chromosomal anomalies) or 10 days (full karyotype of the 23 pairs of chromosomes or array).

[B0002] – What is the claimed benefit of NIPT in relation to the comparators?

The main claimed benefit for the patient relates to the simplicity and noninvasiveness of the test, as well as the increased accuracy and decrease in FP rates in comparison with conventional screening. Whilst traditional FCT screening which involves NT, PAPP-A and hCG measurements and a maternal age strategy is the standard of care for T21 screening in many countries, it is associated with a 5% FP rate and fails to identify an important number of cases. According to a recent Cochrane review the estimated sensitivity for T21 was 85% and the specificity was 95% at a cut-off risk of 1 in 250. Adding other ultrasound markers (nasal bone, fetal heart rate, frontomaxillary facial angle, etc.) increased sensitivity to more than 90% and specificity to more than 95% [83] but these ultrasound measurements require referral to a skilled specialist to provide an accurate scan, and there is variation in the experience of the operators. Integrated screening has also shown detection rates as high as 91%–92% for T21 and 91%–96% for T18 but first-trimester results must be withheld until the test has been completed and the patient is not given the option of early diagnostic testing [85].

The higher accuracy of NIPT as a primary test could increase the confidence in the negative results and facilitate the informed consent decision, whilst minimising anxiety related to further testing. With the reduction in FP rates, unnecessary invasive procedures (amniocentesis and CVS) would be reduced, minimising the risk of complications such as iatrogenic loss of pregnancy, rupture of membranes followed by preterm delivery and fear of fetal trauma, as well reducing the anxiety to the [87].The reduction of unnecessary invasive procedures could also have advantages for the system as it could result in a reduction in the burden associated with the number of patient visits and reworks. In comparison with FCT, NIPT measurements can be performed at any time after 8–10 weeks. In this sense, the assays which allow earlier testing would have the advantage of giving women more time to make decisions.

Another potential benefit of NIPT as a primary test relates to the fact that unlike serum protein measurements and ultrasound assessment, which uses indirect and nonspecific markers, NIPT can target specific trisomies. This could potentially facilitate genetic counselling because women could be given precise information about the condition being screened.

When NIPT is used in a contingent approach (add-on to FCT) the same advantages are not applicable. In this case, the potential benefits of NIPT reside in reducing unnecessary invasive testing. NIPT could also constitute an alternative for women who are not willing to undertake invasive screening but would like to have more information to prepare for childbirth.

[B0003] – What is the phase of development and implementation of NIPT and the comparators?

Traditional first-line screening for T21, which involves the measurement of serum proteins and ultrasound assessment, is the current standard of care in most European countries [12]. With regard to NIPT, the first test launched, the MaterniT 21 PLUS (Sequenom Inc.), was commercialised in China/Hong Kong and the USA in 2011. Soon after, it was available in many countries of western Europe, the Middle East, South America, Asia and Africa, with samples from these countries being sent to the USA or Hong Kong for testing. Many other companies have commercialised noninvasive prenatal tests, which are offered in more than 60 countries throughout world, although a recent market report shows that North America accounts for 64.5% of global NIPT revenue, followed by Europe. Sequenom Inc., Natera Inc., Roche Diagnostics, Illumina Inc., BGI Diagnostics, LabCorp, LifeCodexx AG and Berry Genomics are the leading companies operating in the global NIPT market, although in many European countries, other companies and their distribution partners offer many other tests (Table 4). All offer testing for the chromosomal aneuploidies T13, T18 and T21, but most have now available many other additional options depending on the tests [88].

Whilst commercial laboratories have not published official data regarding the actual uptake, the estimates for 2016 indicate that more than 3 million pregnancies worldwide were screened by NIPT. According to a research market analysis, the market is expect to grow at a rate of 17.44% per year in 2016–2020 (Research and Markets, Global non-invasive prenatal testing market 2016-2020) [89].

NIPT is currently available in most of the European countries, though in many only privately (Table 5). In some countries like UK and Denmark NIPT is offered as contingent screening for women at high risk women from FCT, in others like Switzerland and France it is considered for intermediate to high risk pregnancies (risk > 1:1000), though only for T21 in this last case. In Belgium and the Netherlands NIPT is now accessable for all pregnant women, although in the latter case as part of the Trident 2 research study. In Belgium only for the trisomy 21.

[B0004] – Who administers NIPT and the comparators and in what context and at what level of care are they provided?

Prenatal screening tests are designed for administration by healthcare professionals. Both traditional serum testing and NIPT involve a simple blood sample, which can be obtained during an outpatient appointment with no special requirements. Ultrasound assessment usually requires referral to a specialist. In both screening approaches, women should be counselled both before and after testing to ensure the test, results and limitations are clearly understood.

[B0008] – What kind of special premises are needed to use NIPT and the comparators?

[B0009] – What equipment and supplies are needed to use NIPT and the comparators?

Samples for traditional serum testing are analysed in standard biochemistry laboratories. NIPT samples are frequently collected locally but shipped to external laboratories, which are equipped to handle cfDNA extraction, if not provided in the technology solution. The local implementation

would require a laboratory suitable for molecular workflows such as a clinical genetic laboratory. General laboratory equipment should be present, such as thermocyclers, a fridge, a freezer, pipettes and an extraction system capable of purifying cfDNA from plasma. PCR-based tests require separated areas for pre-PCR and post-PCR work, which must comply with specific requirements with regard to temperature (20°C–24°C), humidity (30%–80%), pressure and environmental area filtering, water sources and liquid hazardous waste disposal. Both biochemical assays and NIPT assays should be handled by trained laboratory personnel working according to manufacturers instructions and in line with good laboratory practice.

Test companies supply the additional equipment, which, depending on the company, can include reagent kits and analysis and application software (Table 4).

[B0018] – Are the reference values or cut-off points clearly established?

Laboratories report cfDNA results in different ways. Some laboratories define a positive or negative aneuploidy risk based on a cut-off point (*Z* score, *t* score or *L* score), which is used to determine if the percentage of the chromosome considered was increased relative to a reference standard. Others report the chance of aneuploidy by calculating the odds ratio for the trisomy considered on the basis of cfDNA counts and sample fetal fraction applying a likelihood ratio to the a priori trisomy risk based on maternal age and gestational age.

Submission files provided by manufacturers do not report the *Z* score or the trisomy odds ratio. The Harmony test provides both a qualitative (high probability/low probability) and a quantitative result in the form of a probability score. In most studies, a probability score greater than 99% (high probability) or less than 0.01% (low probability) is reported. Most of the studies that performed NIPT using Verifi™ or other noninvasive prenatal tests based on Illumina platforms considered a *Z* score of 3 or greater as high risk for aneuploidies and a *Z* score of less than 3 as low risk for aneuploidies (*Z* score>2 in one study and *Z* score>4 in another one). Five studies [54, 59, 61, 62, 65] performed on the high-risk population established a specific *Z* score for each trisomy, and three studies [73, 75, 76] conducted on the twin pregnancy population fixed two cut-off points (i.e., a *t* score and an *L* score) for classifying the trisomy risk of samples.

[A0020] – For which indications has NIPT received marketing authorisation or CE marking?

Noninvasive prenatal tests are most commonly available as laboratory-developed assays, and as such are liable only for the laboratory accreditation and quality standards that apply in the different countries where they are implemented [88]. Most of the existing tests are offered for screening of T21, T18, T13 and sex chromosome trisomies, although several companies offer expanded NIPT panels for other rare chromosomal anomalies and microdeletion syndromes (Table 3). Depending on the assays, they can be available for singleton, twin, egg-donor or IVF pregnancies, The proposed indication and primary screening protocol proposed for NIPT use differ for the different tests. Whilst most of the tests are proposed for use in all pregnant women who have chosen to have prenatal screening for T21, T18 and T13, some highlight the special relevance for high-risk pregnancies (≥35 years old, increased risk based on screening methods, ultrasound anomalies, prior pregnancies with aneuploidy, family risk, other medical reasons) (Table 3). Several assays, among these IONA® and Verifi™, specify that these tests are not intended as the sole basis for diagnosis [78, 79]. The PrenaTest® indicates that it should be used in combination with ultrasound assessment.

None of the tests are indicated as diagnostic tests.

The following are commonly considered contraindications for the use of NIPT:

- Women of less than ≤ 8–10 weeks' pregnancy (depending on the test)
- Women who have a chromosomal anomaly
- Recent maternal blood transfusion
- Maternal organ or bone marrow transplant
- Maternal surgical procedure
- Maternal radiotherapy
- Maternal immunotherapy or stem cell therapy

In recent years, several companies have commercialised specific reagents, kits or analysis software which offer a solution for implementation in local clinical laboratories using standard laboratory equipment and most of the current massively parallel sequencing systems. In line with Directive 98/70/EC, conformity assessment by the notified body is required only for commercialised products designed for evaluating T21 (Annex II list B). The EC declaration can be done by a declaration of conformity of full quality assurance (Annex IV) or by EC-type examination (Annex V) couplet with EC verification or EC declaration of conformity (production quality assurance) (Annex VI). For all other trisomy determinations, companies are required to comply only with the general requirements for in vitro medical devices (Annex III).

Information regarding CE marking is lacking for many of the existing providers or laboratories. Table 4 summarises the information on the companies which provided information on this aspect. Details of the identified in vitro diagnostic products with CE marking are provided in Table A14 in Appendix 2.

Table 4: Regulatory status of noninvasive prenatal tests

Company	Products with CE certificate	Type of CE certificate (if available)	Organisation issuing approval	Year of approval
Illumina Inc.[a,b] (San Diego, CA, USA)	VeriSeq NIPT Solution	List II Annex B	BSI	2017
Natera®[a] (San Carlos, CA, USA)	Panorama-specific NIPT reagents and Constellation software	EC declaration of conformity	—	—
Premaitha Health PLC[b] (London,UK)	IONA® test (including software) IONA® test HTA (including software)	EC full quality assurance system approval certificate: design, development and manufacture of IVD reagents and associated software for noninvasive assessment of genetic anomalies, including trisomy 21 (Annex IV)	UL International (UK)	2015
Ariosa Diagnostics Inc./ Roche Sequencing Solutions Inc.[b] (California,USA)	Harmony IVD kit AcfS software	EC full quality assurance system approval certificate; design and manufacture of reagents and associated software for NIPT of fetal chromosome aneuploidy, including trisomy 21 (Annex IV)	UL International (UK)	2017

Company	Products with CE certificate	Type of CE certificate (if available)	Organisation issuing approval	Year of approval
LifeCodexx AG[b] (Konstanz,Germany)	PrenaTest® DAP.plus software	EC full quality assurance system approved certificate for PrenaTest DAP.plus (limited to trisomy 21) (Annex IV) EC declaration of conformity for trisomy 13 and trisomy18 EC full quality assurance certification from certification body: design, development and provision of IVD software for the application in the field of prenatal diagnostics	TÜV Rheinland (Germany)	Renewed 2017
Erbios Futura S.r.l.[b] (Italy)	Prenataltest® kit	EC IVD certification	—	—
Multiplicon (Belgium)	Clarigo™ test Clarigo Reporter™ software	EC declaration of conformity (Annex III)	—	2015 2015
Genesupport (Switzerland)	Prendia software for the interpretation of sequencing data in the prenatal diagnosis of trisomy 21	EC certificate from notified body of full quality assurance: design and manufacture of the Prendia software for the interpretation of sequencing data in the prenatal diagnosis of trisomy 21 (Annex IV)	BSI, UK	2013

Abbreviations: EC=European Conformity; HTA=health technology assessment; IVD=in vitro diagnostic; NIPT=noninvasive prenatal testing.

[a] Source: company webpage.

b Source: submission file/information provided by the company.

[A0021] – What is the reimbursement status of the technology/comparator?

NIPT is delivered mainly through private providers, not yet being available in many publicly funded antenatal services outside the context of research studies in most European countries. According to a global survey chromosome aneuploidy a growing number of payers consider NIPT for all pregnancies and cover NIPT for all pregnancies in the USA (Anthem Blue Cross and Blue Shield, Blue Cross and Blue Shield and Cigna). The information provided by manufacturers on the reimbursement status/recommendations in Europe can be found in Tables A14–A15 in Appendix 2.

Table 5. Summary of reimbursement recommendations for noninvasive prenatal testing in European countries

Country and issuing organisation[a]	Status of recommendation	Level of reimbursement
UK, National Screening Committee	Positive for T21, T13 and T18 (contingent screening with high-risk women)	Implementation starting in 2018 for a 2-year study. Fully reimbursed on contingent with risk greater than 1:150 with FCT)
Belgium, Ministry of Health	Positive for T21	Full or near full reimbursement for all pregnant women (€8 out-of-pocket cost)

Country and issuing organisation[a]	Status of recommendation	Level of reimbursement
Denmark	Positive for T21, T13 and T18	Fully reimbursed on contingent for high-risk women (risk 1:300 with FCT)
France, French National authority for Health (HAS)	Positive for T21	Fully reimbursed on contingent with risk greater than 1:1000 with FCT
Germany, Federal Joint Committee (GBA)	Ongoing	No reimbursement at present Offered privately
Greece	No national programme	Private
Ireland	No national programme	Private
Italy, National Plan Genomics	Positive for T21, T13 and T18	No reimbursement at present Offered privately
Netherlands, Ministry of Health	Positive for T21, T13 and T18	Implementation of government Trident 2 research study started in 2017 for a 3-year study, choice between FCT and primary NIPT Fully reimbursed for women with risk greater than 1:200 with FCT, €170 out-of-pocket cost for risk less than 1:200
Norway, Health Directorate	Positive for T21, T13 and T18	Not yet released
Poland	No national programme	Private
Sweden, Swedish National Board of Health and Welfare	No national programme	No reimbursement Offered privately
Spain	Ongoing	Reimbursed in some regions Offered privately
Switzerland, Federal Office of Public Health (FOPH/BAG)	Positive for T21, T13 and T18	Full reimbursement on contingent screening with risk greater than 1:1000 with FCT with NGS-based technologies only (Analysenliste 1.7.2017 BAG)

Abbreviations: FCT=first-trimester combined testing; NGS=next-generation sequencing; NIPT=noninvasive prenatal testing.

[a] Sources: submission files/information provided by the manufacturers.

3.3 Discussion

NIPT seems to constitute an apparently simple approach to the screening of fetal chromosomal aneuploidies. However, it must be acknowledged that whilst the general method for trisomy assessment is common to all currently marketed noninvasive prenatal tests, these have potentially differentiating characteristics and modes of action. Existing noninvasive prenatal tests differ with regard to many features, including the actual platform used, the depth of read, the quality control measures, the statistical algorithms and risk score used for risk calculation and the measurement of the fetal fraction. Whilst the different companies claim that some of these innovative features could potentially contribute to improve the performance of these tests in relation to other assays on the market, there are important uncertainties regarding the real influence of these in practice since direct comparison trials have not been performed.

One of the main limitations of cfDNA resides in the fact that it is derived from trophoblasts and the fetal chromosomal constitution and the placental layers are not always correlated, leading to possible erroneous classification of some women [90]. Moreover, the fact that tests require a minimum fetal fraction for test interpretation constitutes a challenge, especially because low fetal fraction can

appear for different technical and biological reasons. Low fetal fractions have been associated with increased risk of T13, T18 and T21, and this should be further explored and discussed in the light of the current findings as they could be liable to have important implications for the accuracy of these tests. The resampling of low fetal fraction cases is another issue which should also be further evaluated and reflected on, as resampling is not always effective and could contribute to delayed diagnosis.

Especially in the context of assessing NIPT in comparison with FCT, it is important to take into account that the benefits of NIPT will differ depending on whether NIPT will be used as a first-line screening test (alone or in combination with FCT) or in a contingent model (add-on to FCT). As a primary test it is claimed to considerably increase the accuracy for the detection of T13, T18 and 21, whilst reducing unnecessary invasive testing given the lower FP rates. However, this benefit can be realised only in a healthcare setting where most women who participate in first-trimester screening undergo invasive testing. It this is not the case, NIPT could actually increase unnecessary invasive testing and loss of healthy fetuses. However, if used as a sole screening test, it could be liable to miss clinically relevant anomalies which the traditional screening approaches may bring to light when screening for T21 [91], on the one hand, because the information from NT assessment is essential to detect other major conditions such as cardiovascular defects, and on the other, because it could miss neural tube defects, ventral wall defects and other atypical chromosomal anomalies with phenotypic significance which are detected with serum analytes or ultrasound [92]. In this sense, some laboratories recommend the test only for high-risk groups and others highlight the importance of using NIPT in combination with other information and/or ultrasound examination. From the information provided it can be inferred that there are uncertainties regarding the best way to incorporate NIPT into existing screening pathways, as well as inconsistencies regarding the best follow-up approach for women with undeterminate or uninterpretable results.

The potential role of NIPT for screening for fetal anomalies in twin pregnancies or medically assisted pregnancies (IVF or egg donor) is another matter of concern. Although most assays are offered for these indications, it has been highlighted that these women could pose certain challenges for NIPT assessment as they have lower levels of cfDNA [6, 39]. The fact that most tests include a fetal sex chromosome complement could also pose certain challenges regarding the reporting of results when the screening is not offered for these anomalies. Challenges are also expected when noninvasive prenatal tests which have expanded their panels to cover microdeletions and other anomalies are used. These new additions are not part of this assessment as they currently lack sufficient supportive evidence, it being anticipated that in most cases the PPV would be low, undermining the benefits of NIPT to reduce the need for invasive testing [93].

4 HEALTH PROBLEM AND CURRENT USE OF THE TECHNOLOGY

4.1 Research questions

Element ID	Research question
A0002	What are the fetal chromosomal aneuploidies in the scope of this assessment?
A0003	What are the known risk factors for fetal chromosomal aneuploidies?
A0004	What is the natural course of fetal chromosomal aneuploidies?
A0005	What are the symptoms and the burden of disease of chromosomal aneuploidies?
A0006	What are the consequences of chromosomal aneuploidies for society?
A0007	Who is the target population for prenatal aneuploidy screening?
A0011	How much is NIPT used?
A0023	How many people belong to the target population for prenatal aneuploidy screening?
A0024	How are chromosomal aneuploidies currently screened and diagnosed according to published guidelines and practice?
A0025	How are chromosomal aneuploidy pregnancies currently managed according to published guidelines and in practice?

4.2 Results

Overview of the disease or health condition

[A0002] – What are the fetal chromosomal aneuploidies in the scope of this assessment?

Noninvasive prenatal tests are validated for use in pregnant women and are currently applied in Europe for the assessment of the fetal chromosomal anomalies T21 (DS), T18 (Edwards syndrome) and T13 (Patau syndrome), which are the aneuploidies in the scope of this assessment. The tests can also detect sex chromosome aneuploidies and select microdeletions, although the accuracy for these is much lower.

DS (International Classification of Diseases, 10th revision, code Q90) originates in most cases from a cell division error which leads to a full trisomy of chromosome 21 (95%) [94]. This type of DS, called *trisomy 21* (T21), is the most common chromosomal disorder among newborns. The remaining cases are due to either an inherited meiotic nondisjunction (4%) or a mosaicism-mitotic nondisjunction of chromosome 21 (1%). Like with other common autosomal trisomies, the number of pregnancies affected by T21 has experienced a rising trend in recent decades because of the increase in maternal age. However, studies are consistent in claiming that the increasingly widespread use of prenatal screening and termination of pregnancy have counteracted the effect and resulted in a relatively stable live birth prevalence for DS [95]. According to EUROCAT [12], which is the main source of information on the epidemiology of congenital anomalies in Europe, the total and live birth prevalence of DS is 24 and 9.62 per 10 000 births (2010–2014 registry data; covers 43 registries from 23 countries) [95]. Prevalence rates differ widely among countries, which has been explained by the maternal age distribution, except in France and Switzerland, which have higher estimates in comparison with other countries [95].

The T18 and T13 syndromes (International Classification of Diseases, 10th revision, code Q91), also known as *Edwards syndrome* and *Patau syndrome*, are the second and fourth most common autosomal chromosomal anomalies in Europe. Most T18 and T13 cases originate from maternal meiotic nondisjunctions, even though for T13 a minority of cases can be caused by unbalanced translocations with a high recurrence rate in parental carriership [97]. Less than 2%–5% are mosaicisms [96, 97]. According to EUROCAT data [12], the overall prevalence in 2010–2014 for T18 and T13 was 5.6 and 2.08 per 10,000 births, respectively. In a study conducted from a regional UK population-based register, the adjusted live birth prevalence of T18 was 6.8 per 10,000 in mothers aged 35 years or older and 1.06 per 10,000 in mothers aged less than 35 years [96].

[A0003] – What are the known risk factors for the disease or health condition?

The causes of the three trisomies are relatively unknown. Advanced age at conception is the strongest epidemiological risk factor for all three trisomies. For DS prevalence increases from 0.6 to 4.1 per 1000 between the age of 15 years and the age of 45 years.

[A0004] – What is the natural course of the fetal chromosomal aneuploidies?

The outcome of chromosomal anomaly cases has changed very much since the availability of prenatal screening. Since then, there has been a continuous increase in the proportion of cases diagnosed prenatally, although the uptake of screening programmes differs widely between countries. For example, the uptake is 90% or more in France [98] and Denmark [99] and less than 30% in Sweden [100].

Overall, it can be estimated, on the basis of EUROCAT registry data from 2010–2014 [12], that 68.7% of DS cases, 94.3% of Patau syndrome cases and 93.4% of Edwards syndrome cases are diagnosed prenatally. Prenatally diagnosed cases of fetal chromosomal aneuploidies could result in elective termination of pregnancy, miscarriage (20–23 weeks' gestation age), stillbirth (deaths of fetuses delivered at 24 weeks or more) or live birth.

In Europe, around 55% of DS pregnancies are terminated following prenatal diagnosis, and 3.5% result in spontaneous fetal death/stillbirths, although termination rates are also highly variable [12] (EUROCAT). More than 90% of the prenatally diagnosed cases recorded in the National Down Syndrome Cytogenetic Register (UK) between 1989 and 2000 ended in termination [101]. Overall, it is estimated that if DS pregnancies were not terminated, about 32%–40% of fetal losses would still occur between CVS and birth and about 23%–25% would still occur between amniocentesis and birth [101, 102].

Several population studies show that more than 90% of DS infants are expected to survive beyond the age of 20 years in developed countries [13-16]. In two of these studies, survival was shown to be significantly better for children with T21 mosaicism, in comparison with the other two chromosomal errors [15, 16]. Congenital heart defects and respiratory infections were found to be the main cause of death among persons with DS who die before the age of 20 years [13, 16, 18]. On the basis of existing data sets from Australia and Denmark the survival probabilities at 50 and 60 years would be expected to be around 70% and 60%, respectively [13, 16].

T13 and T18 are lethal conditions characterised by major structural malformations, which can be frequently detected by ultrasound examination, as more than 90% of the fetus present sonographic anomalies. The most common malformations for T18 are omphalocele, ventricular septal defects, abnormal posturing of the hands and megacystis [22]. The most common anomalies found in T13

fetuses are cleft lip and/or palate, holoprosencephaly and talipes or rocker bottom feet [103]. On the basis of EUROCAT data [12], more than 90% of cases in Europe are diagnosed prenatally. In Ireland, where there is no national policy on prenatal screening for fetal aneuploidy, it has been documented that only 33% of T13 cases and 65% of T18 cases are diagnosed before birth [103]. EUROCAT registries [12] show that overall 77% of affected pregnancies are electively terminated following prenatal diagnosis, and that of those T13 and T18 pregnancies which are allowed to continue, 6% and 9%, respectively end in spontaneous abortions or stillbirths. In the Irish study, almost half of the pregnancies resulted in live-born infants. A UK study shows that the uptake of termination can differ by ethnic group, being much lower in Pakistanis in relation to non-Pakistanis (58.6% vs. 86.6%) [96].

The published literature is consistent regarding the fact that few T18 and T13 live-born infants survive beyond the first year [22]. Although the medium survival time for children is less than 15 days, some recent studies [104, 105] have found longer survival than those previously reported for these trisomies (5%–10% at 1 year), consistent with some publications claiming increased survival following more aggressive medical interventions. A multistate population-based study from the National Birth Defects Prevention Network showed that 9.7% of patients with T13 and 12.3% of patients with T18 survived beyond 5 years [104]. A retrospective cohort study conducted in Ontario reported that the 1-year survival rate was 19.8% for T13 and 12.6% for T18. At 10 years, 12.9% of the T13 cohort and 6.4% of the T18 cohort was alive. Overall, 23% of the T13 children and 13.8% of the T18 children had undergone surgical procedures [105]. One study reported a 1-year survival rate of more than 40% but this was highly biased as it was questionnaire based. On the basis of the analysis of data recorded in the National Down Syndrome Cytogenetic Register it was established that survival was significantly greater for children with mosaicisms with regard to the full trisomies (70%–80% vs. 8%) [106].

Effects of the disease or health condition

[A0005] – What are the symptoms and the burden of disease of fetal chromosomal aneuploidies?

Down syndrome

Although the presentation can differ, the DS population is characterised by mental retardation associated with physical growth delay and certain physical traits. DS individuals have a variety of phenotypic clinical features, including mongoloid faces, protruding tongue, transverse single palmar, depressed nasal bridge, small low-set ears, upward-slanted eyes with epicanthic fold, short neck and hypotonia. Cognitive impairment frequently ranges from mild to moderate [17, 18], although in one study conducted in Italy, 65% of the participants had severe impairment [19]. DS will frequently require speech therapy, physical therapy and occupational therapy. Special education and development interventions also play an important role in the social integration of these children [21, 107].

Congenital heart disease is a common comorbidity recognised in these patients. About half of the children are born with congenital heart disease (44%–60%), and this anomaly is a major cause of morbidity and death in childhood [20, 108-110]. The most common lesions are atrioventricular septal defects (45%), ventricular defects (35%) and patent ductus arteriosus (7%), although many other lesions could arise [108, 111, 112]. In many cases, early corrective surgery might be required to prevent irreversible damage [108, 110]. In a community study conducted in New York, up to 60% of the children with heart disease required surgery [113].

DS individuals frequently have vision and hearing problems as well as thyroid dysfunction. Hearing problems have been reported in up to 38% and 78% of the DS population [18, 111], which can be conductive, sensorineural or mixed. Around 38% of children younger than 12 months and 80% of children aged 5–12 years have vision problems, with refractive errors, strabismus and nystagmus being the most frequent. Hypothyroidism frequency also increases with age, and it has been shown to be as high as 50%–75% in some studies [114].

In general, children with DS have more neurobehavioural and psychiatric problems than the general population. The most frequent problems are disruptive behaviour disorders, such as attention deficit hyperactivity disorder, conduct/oppositional disorder, aggressive behaviour and obsessive–compulsive disorders [18, 111]. One recent study showed that the prevalence of attention deficit hyperactivity disorder can be as high as 43.9% [115]. More than 20% of adults have been found to have a psychiatric disorders, most frequently aggressive behaviour or depression disorders [116]. Autism or autism spectrum disorders have been noted in DS children (7%) [18].

Disorders such as leukaemia and epilepsy also occur more frequently among DS children than in the general population. A systematic review found that the pooled estimate for epilepsy was 12.4% [117]. Whilst solid tumours are rare, the cumulative risk of leukaemia is 2% by the age of 5 years and 2.7% by the age of 30 years [118]. Other related comorbidities include skin disorders, airway anomalies, juvenile rheumatoid arthritis, diabetes mellitus and sleep apnoea.

DS predisposes to premature ageing, and adults are commonly affected with chronic conditions resembling those of the geriatric population. Common conditions encountered in adults with DS include Alzheimer disease, epilepsy, mood and behavioural disorders, osteoarthritis and autoimmune diseases, such as thyroiditis and coeliac disease [19]. Histological changes in the central nervous system resemble those of Alzheimer disease, and according to recent data, it is more than 80% may experience dementia by the age of 65 years [119].

Because of the different medical problems, assessment, monitoring, prevention and guidance is recommended from birth [18, 21]. The high prevalence of associated disorders requires lifetime care and individualised treatment plans for children and adults.

Edwards syndrome and Patau syndrome

These two syndromes are characterised by prenatal growth deficiency, special craniofacial features, major and minor anomalies and marked psychomotor and cognitive development delay. Most of the children have feeding difficulties and require nasogastric/nasojejunal feeding in the neonatal period or placement of a gastrostomy tube in older children. Most also have major malformations, which can affect different organs. Among 468 live borns with T18 registered in 16 European countries (2000–2011), 80% had cardiac anomalies, 21% had nervous system anomalies, 8% had oesophageal atresia and 10% had an orofacial cleft. Among 240 T13 live-born babies, 57% had a cardiac anomaly, 39% had a nervous system anomaly, 30% had an eye anomaly, 44% had polydactyly and 45% had an orofacial cleft [120].

Although little follow-up information is available, it has been reported that mental delay ranges from marked to profound. Most individuals do not achieve expressive language or walk independently. Whilst development age in older children is around 6–8 months, older children have some skills of older children, including sleeping independently, self-feeding, imitating, using a few words/signs, following simple commands and understanding a cause and effect [121].

[A0006] – What are the consequences of chromosomal aneuploidies for society?

Down syndrome

DS is an important health issue since those born with this condition have special medical, social and educational needs. Taking into account that around 5.1 million children are born in the EU-28 annually (EUROSTAT fertility statistics), it is be estimated that approximately 5000 DS infants would be born each year, assuming a live birth prevalence of 1 per 1000. These infants will require in many cases early medical support and surgical interventions during childhood.

The key role of the parents is recognised in children with special needs and can require a lot of parental time and resources. In a longitudinal cohort study performed in the UK, which included 138 adults with DS, only eight (4.3%) lived independently, 54.3% lived with a family carer and the rest had paid carer support [116]. Although information regarding abandonment of children with DS at birth is practically nonexistent, a follow-up study conducted in France in 1995 found that 12% of children born with DS were placed for adoption [122].

Edwards syndrome and Patau syndrome

The rarity and low survival of patients beyond 1 year have resulted in a very low number of affected patients.

Current clinical management of the disease or health condition

[A0024] – How are chromosomal aneuploidies currently screened and diagnosed according to published guidelines and in practice?

Whilst there is consensus that all pregnant women should be offered aneuploidy screening and diagnostic testing, recommendations regarding screening tests differ widely, and significant differences exist regarding the screening and diagnostic strategies adopted among the different European countries. A range of maternal serum biochemical and fetal ultrasound biomarkers are available for establishing aneuploidy risks, and these are being offered during the first, second and/or third trimester with different screening protocols [7, 9].

Published guidelines propose that NIPT could be offered as a primary method, secondary to a high-risk value calculated on the basis of combined screening, or contingently to women ascertained as having high or intermediate risk by conventional screening. Guidelines highlight the limitations of these tests for the detection of other relevant chromosomal aberrations which might be present in the fetus. Some guidelines recommend that women who have a no-call result with NIPT should also be offered comprehensive ultrasound evaluation and diagnostic testing because of the increased risk of aneuploidy [38]. For more explicit information regarding NIPT recommendations, see Table A1 in Appendix 1.

Most countries offer a second-trimester ultrasound examination for dating, growth and detection of severe structural defects. Ultrasonographic markers can identify other disorders, and various markers are also associated with T21, T18 and T13. Prenatal cytogenetic diagnosis (amniocentesis or CVS) is recommended for women who have high risk of DS\chromosomal defects, with independence of the screening tests used to determine this risk. Many countries also offer these tests for other indications, including maternal age (range 35–40 years), previous child or fetus with a chromosomal anomaly or a monogenetic disease, family history of DNA anomaly or metabolic disorders, fetal major structural anomalies or soft markers detected by prenatal ultrasound examination.

[A0025] – How are chromosomal aneuploidy pregnancies currently managed according to published guidelines and in practice?

In most European countries, combined first-trimester screening is offered to all pregnant women in the form of national or regional population screening programmes, although there are some countries, such as Ireland, Austria and Malta, which still have no official prenatal screening policies [23]. Screening is a voluntary informed patient choice, with shared decision making. Currently, women who test above a predetermined high-risk cut-off value receive genetic counselling and are given the choice of no further screening or invasive testing (amniocentesis or CVS). In Europe, the threshold frequently used to define high risk is 1 in 250 to 1 in 300, although this might differ between countries. Guidelines recommend no further testing for low-risk patients. If contingency screening is available, the moderate-risk group will be offered second-trimester screening, although this threshold is not standardised in most countries.

Women opting to undergo diagnostic invasive testing receive pretest counselling and information as there is a risk of miscarriage associated with these procedures. If a chromosomal aneuploidy is diagnosed, further counselling is offered and women can opt for termination. In some countries termination of pregnancy is illegal.

Target population

[A0007] – Who is the target population for prenatal aneuploidy screening?

[A0023] – How many people belong to the target population for prenatal aneuploidy screening?

As far as authorisation is concerned, the target population is all pregnant women who will accept prenatal screening for T21, T18 and T13. This would mean that around 5.1 million women would be possible candidates for NIPT in the EU-28 (EUROSTAT fertility statistics) [24]. However, the precise target population is difficult to estimate because it will vary substantially depending on different issues, including whether the pregnant women wants to undergo screening, if NIPT will be used as a first- or second-tier test or on the risk threshold used.

[A0011] – How much is NIPT used?

The uptake of NIPT-based prenatal screening is relatively unknown.

4.3 Discussion

The existing literature shows that the options for prenatal screening differ widely among European countries, highlighting that the degree of uptake and use can differ substantially depending on the country or even the region. Among the possible factors that could be responsible for these differences are the availability of resources, the degree of medicalisation, the termination of pregnancy laws and social and cultural factors [23, 100]. These factors are viewed to be determinants of how screening is offered, perceived and used and should be reflected on when one is analysing the potential application of NIPT in different contexts.

Current guidelines/position statements consider that NIPT is appropriate for T21, T18 and T13 screening, but there is no common agreement regarding the best clinical care pathway (first- or second-tier test), the complementary tests or the threshold of risk used to define which women

would undergo NIPT or the management of “no-call” results, among other issues. Most guidelines focus on appropriate use of NIPT, but fail to provide firm recommendations on the best alternative screening option, highlighting once again the great uncertainties that remain regarding the best choice. There is widespread agreement that NIPT is not yet ready to replace invasive diagnostic tests because of its limitations. It is also acknowledged that the value of NIPT can be substantially different for T21 than for T18 and T13 because the sensitivity and specificity can be lower and these last two trisomies can be detected by ultrasonographic findings in more than 90% of fetuses.

Whilst it is commonly accepted that women should have the opportunity to receive the best possible estimate of their personal risk for common chromosomal anomalies so to allow an informed choice to be made, there are many ongoing debates regarding the advantages and disadvantages of existing screening strategies. Currently, robust analyses are lacking regarding the performance and outcomes of the different existing approaches. Current reviews and meta-analyses commonly tend to focus only on the accuracy of the tests for detecting the specific trisomies, providing insufficient documentation of overall effectiveness of the screening, which would be important, especially from the point of view of deciding which strategy should be offered as a public health programme. It should be taken into account that since prenatal screening is a morally sensitive practice because of its connection with abortion [93], establishing the benefits and harms of the different alternatives can be highly challenging. It is important to note that countries might weight the aspects differently, and that even if most agree with the choices made by the experts of what could constitute potentially relevant outcomes not all have the same healthcare priorities. For example, some countries might wish to prioritise the whole detection rate, whilst others might only want to prioritise the minimisation of the risk of miscarriage [91].

5 CLINICAL EFFECTIVENESS

5.1 Research questions

Element ID	Research question
D0024	What is the accuracy of NIPT versus the reference standard?
D1002	How does NIPT screening compare with other optional screening approaches in terms of accuracy measures?
D1006	Does the test reliably rule in or rule out chromosomal aneuploidies?
D1007	How does the accuracy of NIPT differ in different settings?
D0001	What is the expected beneficial effect of prenatal screening with NIPT on neonatal mortality?
D0005	How does prenatal screening with NIPT affect the frequency of newborns with aneuploidies?
D0006	How does prenatal screening with NIPT affect the progression of pregnancy?
D0012	What is the effect of NIPT on mothers' health-related quality of life?
D0030	Does the knowledge of the NIPT results affect the population's non-health-related quality of life?
D0017	Was the population satisfied with NIPT?
D0020	Does the use of NIPT lead to improved detection of chromosomal aneuploidies?
D0021	How does NIPT change physicians' management decisions?
D0022	Does NIPT detect other potential health conditions that can impact the subsequent management decisions?
D0010	How does the technology modify the need for hospitalisation?
D0029	What are the overall benefits and harms of NIPT in terms of health outcomes?

5.2 Included studies

5.2.1 NIPT as a primary screening test for the general singleton pregnancy population (total replacement of FCT)

Study characteristics

The systematic review retrieved eight studies which assessed prenatal screening based on NIPT as a primary testing method in the general singleton population (five comparative studies, i.e., NIPT vs. combined serum screening, and four DTA studies). One study considered only women whose health records were available [30], another included only women with known results [43] and another restricted NIPT to samples verified by invasive testing [44]. Zhang et al. [43] included cases where NIPT had been offered either as primary or as secondary screening. Comas et al. [40] also included a small percentage of patients who had already been defined as high risk by FCT (16.5%). Song et al. [45] included only women younger than 35 years. The sample size of the eligible studies ranged from 324 to 146,958 women.

The mean gestational age of the women ranged from around 12 weeks to 20.3 weeks. Five of the studies included patients screened during the first or second trimester. Only one enrolled women exclusively undergoing FCT. All but two exclusively assessed singleton pregnancies. In these studies

[43, 44] only 0.2%–0.36% of the included women had twin pregnancies. The studies included naturally conceived pregnancies and pregnancies conceived by assisted reproductive technology (ART), with these ranging from 0.8% to 17% in the studies included. Norton et al. [31] and Pergament et al. [44] excluded IVF with user donor oocytes.

Of the nine studies included, four used the Harmony® prenatal test (Ariosa Diagnostics Inc., San Jose, CA, USA) (SNP), one used the Panorama test (Natera, San Carlos, CA, USA), one used the Harmony® test and the Panorama test, one used Verifi™ (Illumina Inc. San Diego, CA, USA), one used the NIFTY™ test (BGI Diagnostics, China) and one used BambniTest (Berry Genomics, China). Three of the studies indicated that they performed a blinded analysis of the index test and reference standard [30, 31, 44]. Confirmation of outcomes was done by invasive testing (amniocentesis or CVS), telephone contact, clinical history or neonatal examination. Only one of the studies verified miscarriages, terminations and stillbirths [44]. Three offered verification data for test failures, although this was not taken into account for diagnostic accuracy assessment.

The comparators in the five general population studies differed widely, as did the cut-off points used to consider patients at high risk. The comparator in three studies was FCT [31, 41, 42], in one study it was first and second serum biochemical assays with or without NT assessment [30] and in the final study it was triple serum screening in the second trimester [45]. The cut-off point used by Norton et al. [31] to define high risk was 1 in 270 for T21 and 1 in 150 for T18 and T13. In the other two studies, patients were classified as high risk if the estimated risk was 1 in 100 [41, 42].

In one of the studies the comparator was FCT, quadruple screening or combined first- and second-trimester screening (serum integrated, sequential combined screening) [30].

Risk of bias and QoE

All of the studies which assessed NIPT as a primary testing method were judged “at risk of bias” and/or as having “concerns regarding applicability”. Eight of the nine the studies (88.9%) were judged to have a high risk of bias for the flow and timing domain because of the great number of women excluded from the analysis (no-call results or lack of reference standard results). The reference standard used for verification of negative cases was also considered to have an unclear or high risk of bias in eight of the nine studies (i.e., databases, telephone contact). The index test risk of bias was unclear because of nonblinded interpretation of NIPT and/or nonreporting of NIPT analysis (33%; three of the nine studies).

High or unclear concern regarding the applicability in the patient selection was identified in seven of the nine studies (77.8%) because of the restricted enrolment of patients. Almost all studies had a low concern regarding applicability for the index test and reference standard domain (Figure 2 and Figure 3).

Evidence assessment of individual studies can be reviewed in Appendix 1 (Table A8).

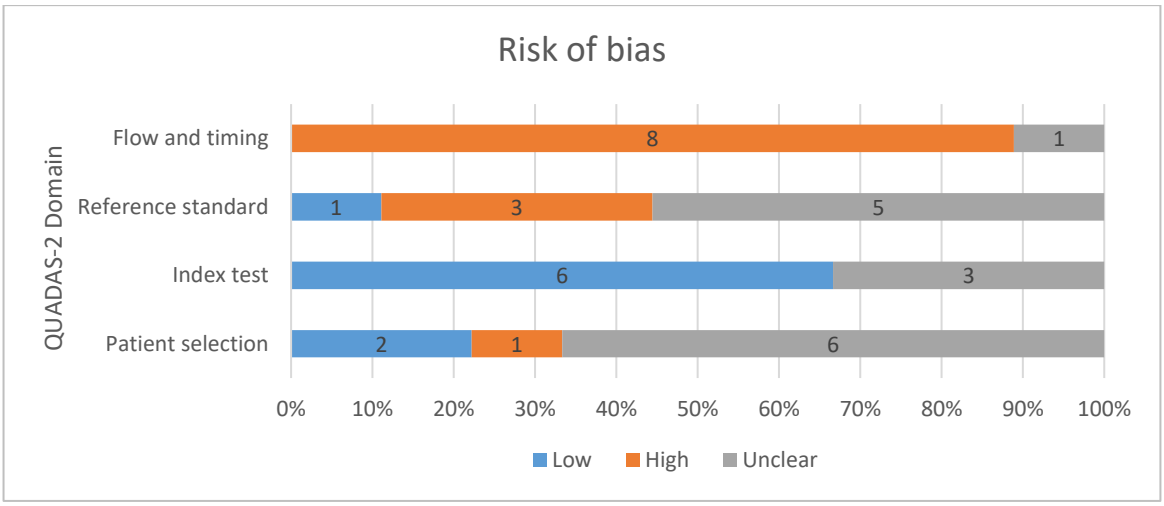


Figure 2: Risk of bias assessed by the QUADAS-2 tool for noninvasive prenatal testing as a primary testing method in singleton pregnancies

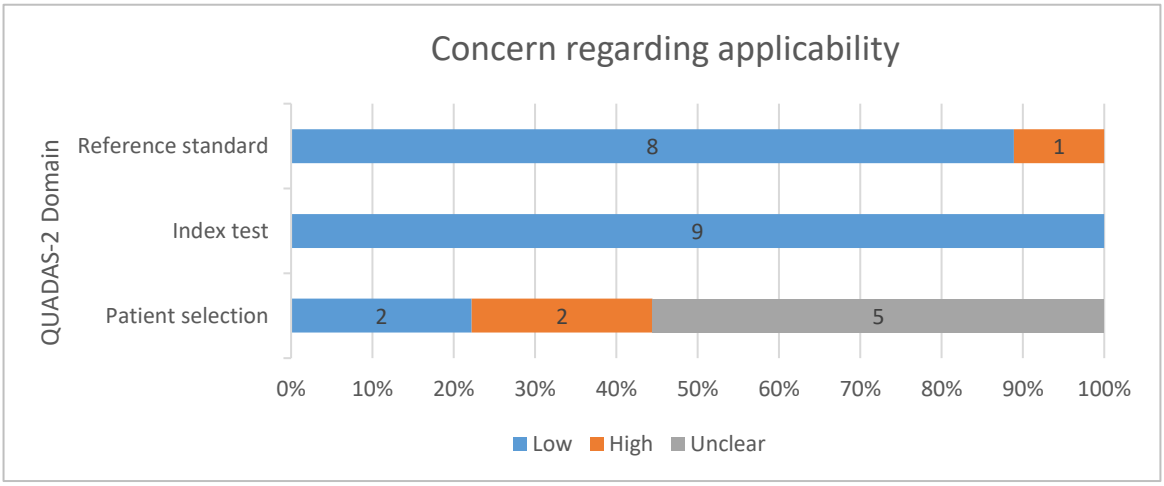


Figure 3: Concern regarding applicability of the use of the QUADAS-2 tool for noninvasive prenatal testing as a primary testing method in singleton pregnancies

According to the GRADE approach, the DTA studies provide moderate QoE for T21 sensitivity and low-quality evidence for specificity because of the serious risk of bias in the reference standard and flow and timing domain of most studies. The QoE for T18 and T13 was low/very low for sensitivity and specificity because of the sparse cases, high risk of bias and/or imprecision of the estimates. Tables of GRADE assessment can be found in Appendix 1 (Tables A9–A12).

5.2.2 NIPT as an add-on to FCT for the high risk singleton pregnancy population

Study characteristics

None of the studies included fulfilled the inclusion criteria but 26 studies which assessed the performance of NIPT in the high-risk singleton pregnancy population were retrieved for indirect assessment of outcomes. The population in these 26 DTA studies (cross-sectional design) ranged between 41 and 7740 patients. Two studies included singleton and twin pregnancies but twin pregnancies accounted for less than 2% of the patients included in these studies [54, 58]. All studies

included women who had been screened during the first and second trimesters. The mean gestational age of the enrolled women ranged from around 11 to 21 weeks. The mean maternal age ranged from 31 to 37 years.

The high-risk classification was done on the basis of first- and second-trimester screening and/or other individual risk factors, including abnormal serum screening findings, advanced maternal age, family history of chromosomal anomaly, previous aneuploid pregnancy, sonographic markers or ultrasound anomalies. Advanced maternal age was the main or only indication for NIPT in seven studies [46, 52, 57, 61-63, 70]. NIPT was predominantly indicated on the basis of FCT results in five studies [48, 49, 60, 66]. The threshold for high-risk classification in these studies ranged from 1 in 200 to 1 in 300. Several studies included women with anxiety, not suitable for invasive tests, or who just wished to have NIPT for other reasons [50, 52, 60-63, 67, 68, 70]. The proportion of women with no risk indication was 15% or less in all studies.

Four of the 25 studies used the Harmony® prenatal test (Ariosa Diagnostics Inc., San Jose, CA, USA) [52, 63, 67, 69]. The rest of studies used the WGS method. Four specified using the Panorama test or sending samples to Natera (San Carlos, CA, USA) [49, 56, 60, 66], one offered Panorama and Prendia (Genesupport, Switzerland) [123], two reported usuing MateriniT 21 or sending samples to Sequenom Laboratories [55, 70], two used the Verifi™ test (Illumina Inc., San Diego, CA, USA) [50, 68], one sent samples to LifeCodexx [62], three sent samplers to Berry Genomics [57, 58, 65] and two sent samples to BGI Laboratories in China [47, 64]. Eight other studies performed in-house testing using WGS methods; five used Illumina sequencing platforms [48, 50, 61] and two used the Ion PGM and the Torrent™ Personal Genome Machine™ (PGM) System (Life Technologies, CA, USA) [46, 59]. Three other NIPT-based studies reported that NIPT was performed by Berry Genomics, China [57, 58, 65], and one study sent samples to LabGenomics Clinical Laboratory (Korea) [54]. Three studies failed to report the testing method [53, 64, 67]. Twelve studies reported that NIPT was blinded to reference standard results [46, 50, 54, 57, 61, 62, 64, 65, 68-70].

The reference standard (full karyotyping results obtained with CVS or amniocentesis) was performed in all pregnancies in 18 of the 27 studies [46-49, 54, 57, 59, 61, 62, 65-70, 123]. In the rest, invasive testing was used only for verification of positive cases; negative cases were verified by follow-up.

Risk of bias and QoE

All but two of the studies [68, 70] on the high-risk pregnant population were judged “at risk of bias” and/or as having “concerns regarding applicability” (one or more domains were judged as “high” or “unclear”). Twenty of the 25 studies (80%) had a high or uncertain risk of bias in the flow and timing domain. With respect to the reference standard, 11 of the 25 studies (36%) had an unclear or high risk of bias. The proportion of studies with uncertain/high risk of bias in the index domain rose to approximately 16% (4 of 25). Twenty-one studies had an unclear or high risk of bias with respect to the patient selection domain (Figure 4).

With regard to applicability, almost all studies had a low concern for the index test and reference standard domains. However, 19 of the 25 studies (76%) had an unclear/high concern regarding applicability for the patient selection domain because of of the uncertainties regarding patient enrolment (Figure 5).

Evidence assessment of individual studies can be reviewed in Appendix 1 (Table A8).

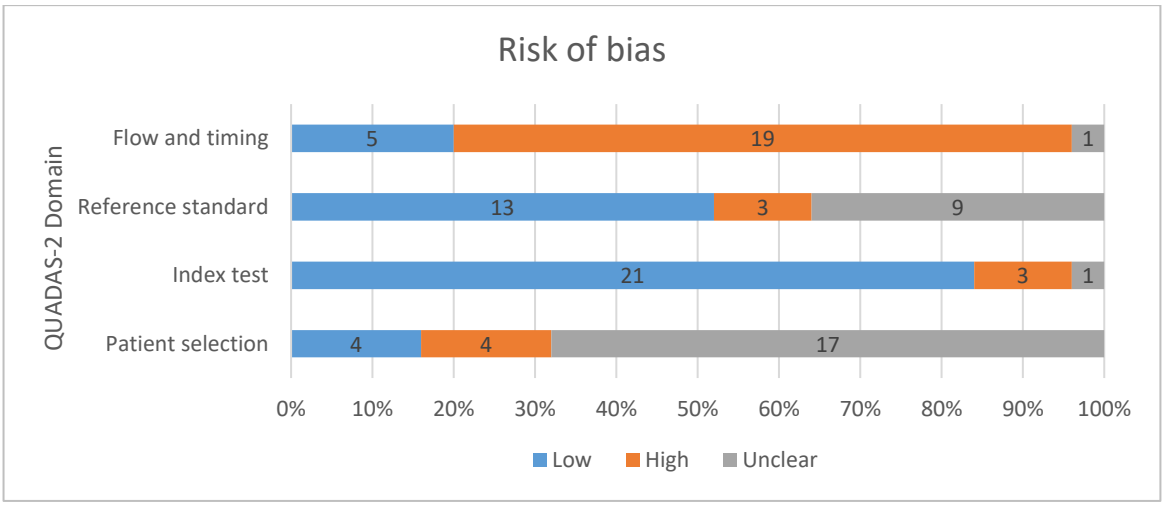


Figure 4: Risk of bias assessed by the QUADAS-2 tool for noninvasive prenatal testing in women with high-risk singleton pregnancies

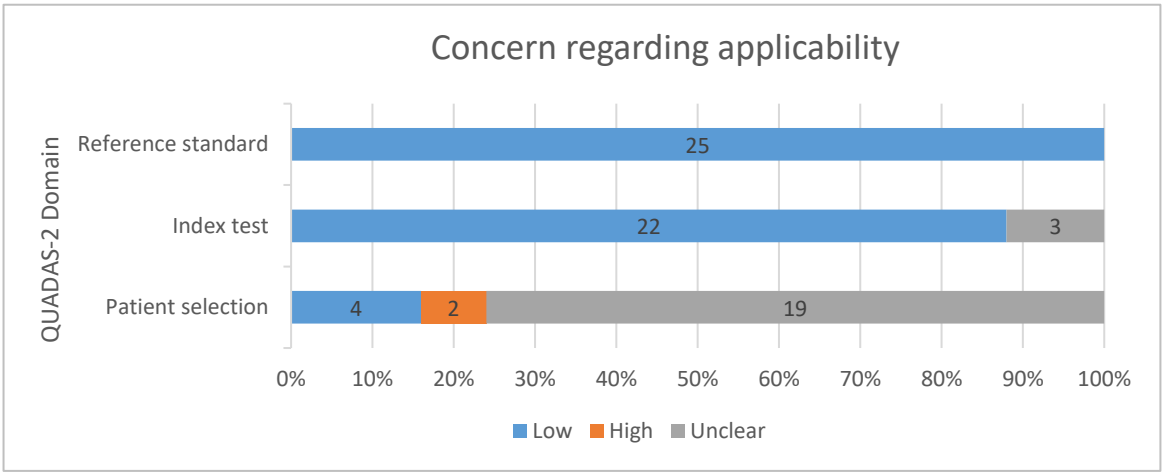


Figure 5: Concern regarding applicability of the use of the QUADAS-2 tool for noninvasive prenatal testing in women with high-risk singleton pregnancies

According to the GRADE approach, the QoE of NIPT accuracy for T21 was moderate for sensitivity and low for specificity as all studies were judged "at risk of bias" or as having "concerns regarding of applicability". However, for T18 and T13, the QoE was low or even very low because of the presence of risk of bias, publication bias and/or imprecision of sensitivity estimations. Tables of GRADE assessment can be found in Appendix 1 (Tables A9–A12).

5.2.3 NIPT as an add-on to FCT for the high-and intermediate-risk singleton pregnancy population

The only DTA study [71] which focused on high-and intermediate-risk population (risk of 1 in 2500) reported on 4012 women with singleton pregnancies who had undergone contingent NIPT (combined serum screening during 11–13 weeks' gestation followed by NIPT). The risk of 1 in 100 or greater was selected to define the high-risk group. This group was offered the options of CVS, NIPT or no testing. The risk of 1 in 2500 was chosen to select patients who would be offered the options

of NIPT or no testing. Following combined testing, 3552 cases were classified as high and intermediate risk and 460 as high risk. NIPT was done with the Harmony® prenatal test (Ariosa Diagnostics Inc., San Jose, CA, USA) in 3698 cases (i.e., 3246 high risk and 276 intermediate risk). Invasive testing was performed in 2.7% of the study population. The median maternal age of high- and intermediate-risk women was 36.1 and 34.8 years, respectively.

Risk of bias and QoE

This study had a high risk of bias for the flow and timing domain. Regarding applicability, low concern was identified in the index test and reference standard domains. Evidence assessment of individual studies can be found in Appendix 1 (Table A8).

According to the GRADE approach, the only DTA study performed in the high- or intermediate-risk pregnant population showed a moderate QoE of NIPT sensitivity and specificity for T21. The QoE of NIPT for T18 and T13 sensitivity was low because of high imprecision of pooled sensitivity, whilst NIPT specificity was moderate for these trisomies. Tables of GRADE assessment can be found in Appendix 1 (Tables A9–A12).

5.2.4 NIPT as an add-on to FCT for the high risk population twin pregnancy population

The six DTA studies (cross-sectional design) which reported on twin pregnancies had samples size which ranged from 12 to 565 patients. Sarno et al. [39] included singleton and twin pregnancies, but only twin pregnancies are taken into account in this section. One of the studies enrolled pregnant women from the general population, with or without prior screening results, who were prospectively tested by NIPT after ART treatment [73] and the other four assessed women for whom NIPT was indicated because of high risk. The indication for screening in this last group was based on advanced age, abnormal ultrasound findings, previous affected pregnancy or positive serum screening (n=1) [72]; abnormal serum screening, sonographic markers or anxiety (n=1) [75]; prior first- and second-trimester combined screening or ultrasound examination (n=1) [74]; and positive traditional screening results and decision not to have invasive testing (n=1) [76]. In two of the studies [74, 75] 53%–58% of the patients had also undergone ART treatment. In the four studies which reported on chorionicity, more than 80% of twins were dichorionic diamniotic.

The median maternal age of enrolled women ranged from 29.8 to 36.8 years. Although all of the studies included first- and second-trimester pregnancies, the median gestational age was around 12–13 weeks in all but one of the studies [75], indicating that most of the patients were analysed during the first trimester.

One of the studies used the Harmony® prenatal test (Ariosa Diagnostics Inc., San Jose, CA, USA) [74]. The rest were based on WGS; three used BGI technology [73, 75, 76] and one used the Verifi™ test (Illumina Inc., San Diego, CA, USA) [72].

Huang et al. [75] confirmed all results with karyotyping. Tan et al. [73] used karyotyping to confirm positive cases and obtained clinical outcomes of negative cases from telephone interview 1 month after delivery. The rest confirmed cases by invasive diagnostic procedures and newborn testing/physical examinations. Fosler et al. [72] also considered ultrasound evaluations for verification purposes.

Risk of bias and QoE

All studies but one performed in the twin pregnancy population were judged “at risk of bias” and/or as having “concerns regarding applicability” (one or more domains were judged “high” or “unclear”) [75]. As can be observed in Figure 6, five of the six studies (83%) had a low risk of bias for the index test. Four studies (66.7%) showed a high or unclear risk of bias due to the reference standard domain as neonatal examination was used as the reference standard in negative NIPT cases and ultrasound findings were used as the reference standard for all NIPT cases. Most of the studies included had high risk of bias due to flow and timing, mainly due to cases excluded from the analysis.

All studies had a low concern regarding applicability for the reference standard and index test. With respect to the patient selection domain, three studies had high concern for different reasons; that is, 100% of pregnant women included had a pregnancy by ART, opted to have invasive testing included or NIPT indications do not match the review question (Figure 7).

Evidence assessment of individual studies can be found in Appendix 1 (Table A8).

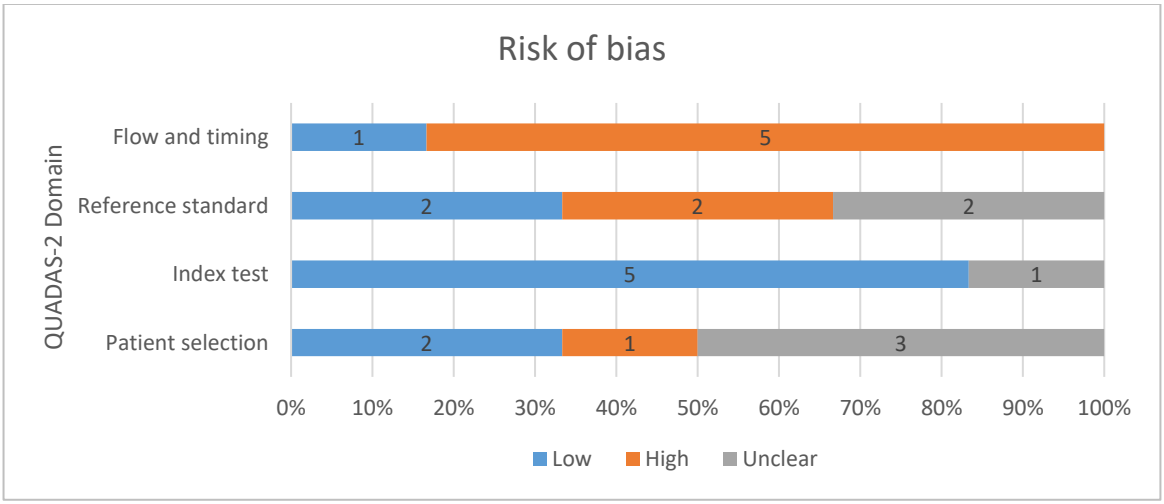


Figure 6: Risk of bias assessed by the QUADAS-2 tool for noninvasive prenatal testing in women with high-risk twin pregnancies

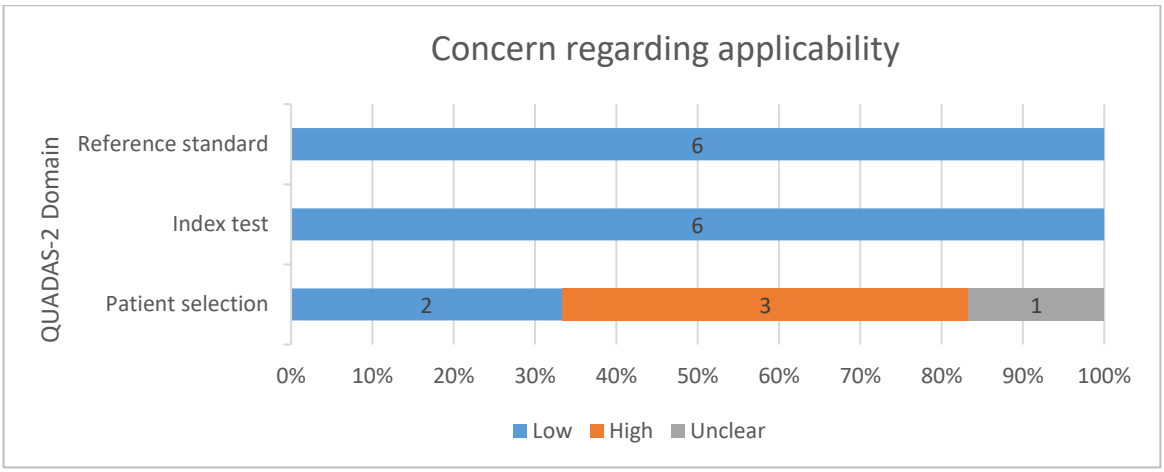


Figure 7: Concern regarding applicability of the use of the QUADAS-2 tool for twin pregnancy population studies

According to the GRADE approach, the DTA studies performed in the twin pregnancy population showed a low QoE of NIPT specificity for T21 because of scarce data and the presence of the risk of bias. The QoE of NIPT sensitivity was very low because of high imprecision of pooled sensitivity. Tables of GRADE assessment can be found in Appendix 1 (Tables A9–A12).

5.3 Test accuracy

[D0024] – What is the accuracy of NIPT versus the reference standard?

As described already, the accuracy of NIPT was evaluated through sensitivity, specificity, PPV and NPV of the test for each trisomy. NIPT accuracy was analysed separately for each type of intervention considered in the assessment. Whenever possible, a meta-analysis was performed by risk group for each type of aneuploidy and for all three types of aneuploidy together.

5.3.1 NIPT as a primary screening test for the general singleton pregnancy population

5.3.1.1 NIPT accuracy for T21

Sensitivity and specificity values

The DTA for NIPT as a primary testing method for T21 was based on eight studies (five comparative studies, i.e., NIPT vs. combined serum screening, and three DTA studies) involving 136,544 pregnant women (885 aneuploidy and 135,659 euploidy cases). The sensitivity and specificity provided, excluding miscarriages, fetal losses and no-call results, was more than 99.9% in all studies included (Table 6). Three of the studies [31, 42, 44] provided data on no-call results, showing eight missed cases (among 85 cases), two missed cases (among 51 cases) and three missed cases (among 488 cases) among these women. The sensitivity and specificity were recalculated in these three studies on the basis of two hypotheses: (1) considering no result cases as positive cases or (2) considering no result cases as negative cases. Under the first hypothesis, sensitivity did not change and thus remained at 100% but the specificity decreased from 99.9%–100% to around 92%–97%. Under the second scenario, specificity was unchanged but sensitivity decrease from 100% to around 89%–94%.

The meta-analysis of DTA, excluding miscarriages, fetal losses and no-call results, computed with a bivariate random-effect model yielded a pooled estimate of sensitivity of 99.3% (95% CI 97.8%–99.8%) and specificity of 99.9% (95% CI 99.8%–99.9%) (Figure 8). Because the model was unstable because of the low variability in sensitivity and specificity (studies reporting 100% sensitivity and specificity) caused by the great number of zeros in the contingency tables, two independent univariate random-effects meta-analyses were applied to support the results obtained with the bivariate model, showing similar results. The sensitivity and specificity found with the univariate model were 99.3% (95% CI 98.4%–99.7%) and 99.9% (95% CI 99.8%–99.9%), respectively. The prediction ellipses of the HSROC curves provided no information because all studies generated estimates in the upper left corner. The HSROC curves and other endpoints of interest obtained with bivariate meta-analysis (i.e., DOR, LR+, and LR–) can be found in Appendix 4 (Table A16 and Figure A1).

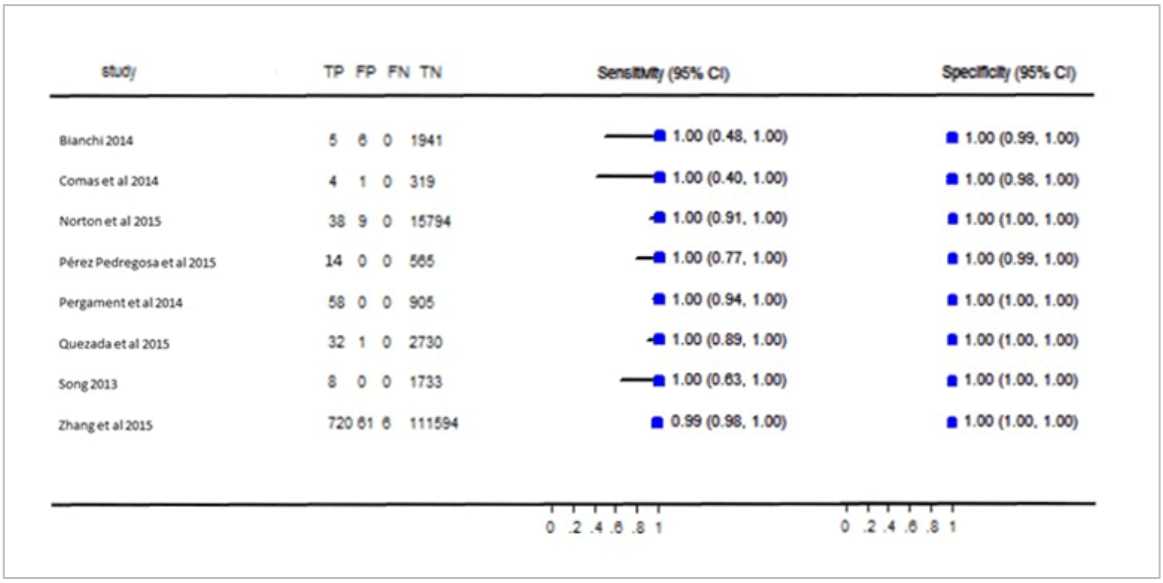


Figure 8: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 21

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

PPV and NPV

The PPV in the studies included ranged between 80% to 100%, except in the study of Bianchi et al. [30], which reported a PPV of 45.5%. The NPV was more than 99% in all studies included in the assessment when no-call results were excluded. When no result cases were considered as positive, the NPV remained unchanged but the PPV decreased from 80.9% to 7.66% in the study of Norton et al. In the studies of Quezada et al. [42] and Pergament et al. [44] the PPV would be lowered from 96.9% to 40.7% and from 100% to 46%, respectively. If no results were classified as low risk, the PPV would not be altered in the study of Pergament et al [44] but would vary from 100% to 99.2% in the second case.

5.3.1.2 NIPT accuracy for T18

Sensitivity and specificity values

Seven of the studies included (five comparative studies, i.e., NIPT vs. combined screening, and two DTA studies) which assessed the general population evaluated T18 (234 T18 cases and 135,405 euploidy cases). The sensitivity found in these individual studies, excluding miscarriages, follow-up losses and no-call results, ranged from 89.1% to 100% (Table 6). Specificity ranged from 99.8% to 100%. Two [31, 44] of the three [31, 42, 44] studies which performed a confirmation of aneuploidy states for pregnancies with no-call results found missed cases in 5.9% (5/85) and 0.2% (1/488) of the no-recall samples, respectively. The rate of missed cases in these no-call result samples was 5.6% (5/8) and 0.2% (1/488), respectively. When sensitivity and specificity were recalculated in these two studies taking into account these missed cases and assuming that no-call results would be treated as negative cases, sensitivity would decrease from 90% to 81.8% and from 96% to 75.7%, respectively. Specificity would not be influenced under this scenario. If no-call results were considered as positive cases, sensitivity would remain unchanged but specificity would decrease from 100% to 97% and from 99.9% to 92.2%, respectively.

The meta-analysis pooled estimate of sensitivity, excluding miscarriages, fetal losses and no-call results, calculated on the basis of the bivariate random-effects model was 97.4% (95% CI 94.4%–98.8%) and the specificity was 99.90% (95% CI 99.87%–99.97%) (Figure 9). However, the model gave unreliable parameter estimates because of sparse data and the low number of trisomy cases in comparison with nontrisomy cases. The correlation parameter between sensitivity and specificity logits was 0.009 (standard error 9.45, 95% CI –1 to 1). Similar results were observed when the univariate random-effects model was applied. Other endpoints of interest obtained with bivariate meta-analysis (i.e., DOR, LR+, LR– and HSROC curves) can be found in Appendix 4 (Table A16 and Figure A1).

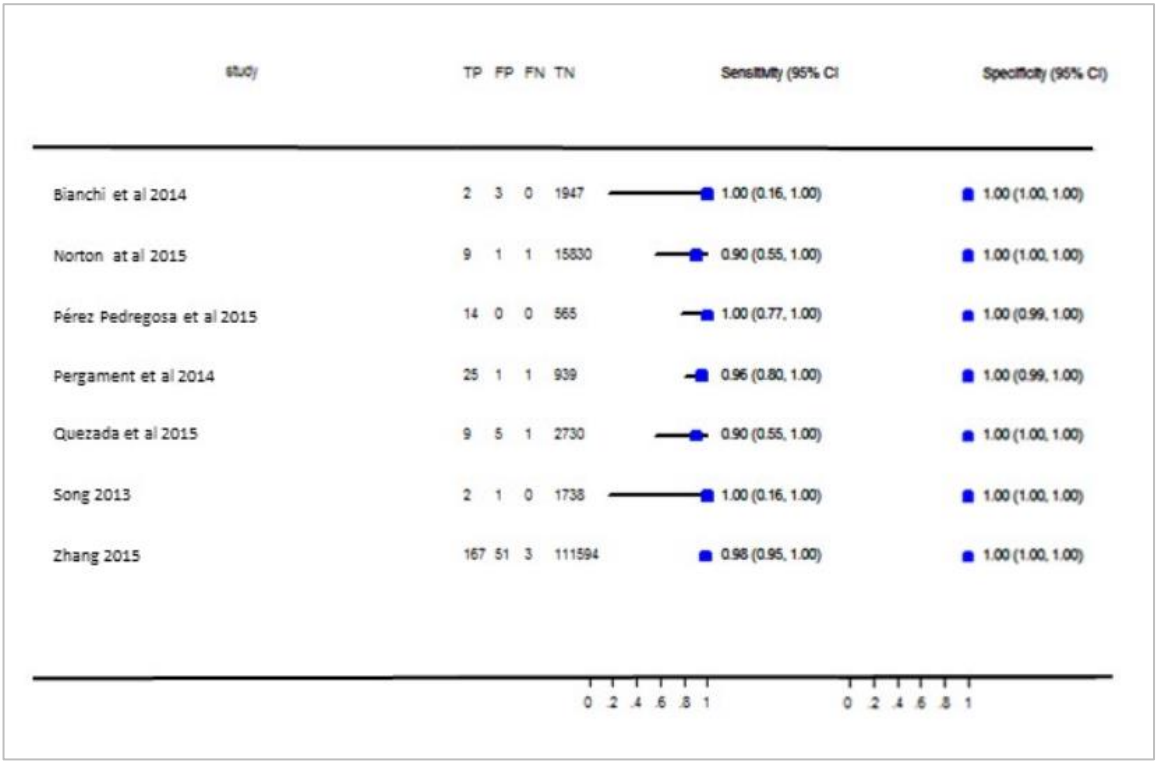


Figure 9: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 18

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

PPV and NPV

The PPV reported in individual studies ranged from 40% to 100%. The NPV of NIPT for T18 was more than 99.9% in all of the studies. The PPV in the study of Norton et al. [31] would decrease from 90% to 2% if it was recalculated taking into account no-call results as positive cases. In the study of Pergament et al. [44] it would decrease from 96.1% to 28.8%. The NPV would remain more than 99% in all studies.

5.3.1.3 NIPT accuracy for T13

Sensitivity and specificity

Six studies (four comparative studies, i.e., NIPT vs. combined screening, and two DTA studies) which reported on 43 cases of T13 and 130,160 euploidy cases were included for assessment purposes. The sensitivity and specificity in these individual studies, calculated excluding no-call results and patients with no follow-up data, ranged form 40% to 100% and from 99.9% to 100%,

respectively. Two studies [31, 44] found missed cases of T13 among no-call results (2.3% and 0.4%, respectively). Specificity would decrease in these studies from 100% to 95.8% and from 100% to 91.9% if test accuracy was recalculated assuming that no-call results were treated as positive cases. If no-call results were to be considered as negative cases, sensitivity would decrease from 100% to 50% and 85%, respectively.

When the bivariate random-effects model was applied, pooled sensitivity was estimated to be 98.8% (95% CI 1.41%–100%) and specificity was 99.9% (95% CI 99.94%–99.97%) (Figure 10). The model was unstable because of the lack of variability between sensitivity and specificity and the great number of zeros in the accuracy contingency tables. The correlation parameter between logits of sensitivity and specificity approximated 1 and standard error CIs estimates could not be estimated. A univariate random-effects meta-analysis, performed to support the results obtained, equally showed great imprecision (sensitivity of 99.8%, 95% CI 3.49%–100%, and a pooled specificity of 99.9%, 95% CI 99.94%–99.97%). Other endpoints obtained with bivariate meta-analysis (i.e., DOR, LR+, LR– and HSROC curves) can be found in Appendix 4 (Table A16 and Figure A1).

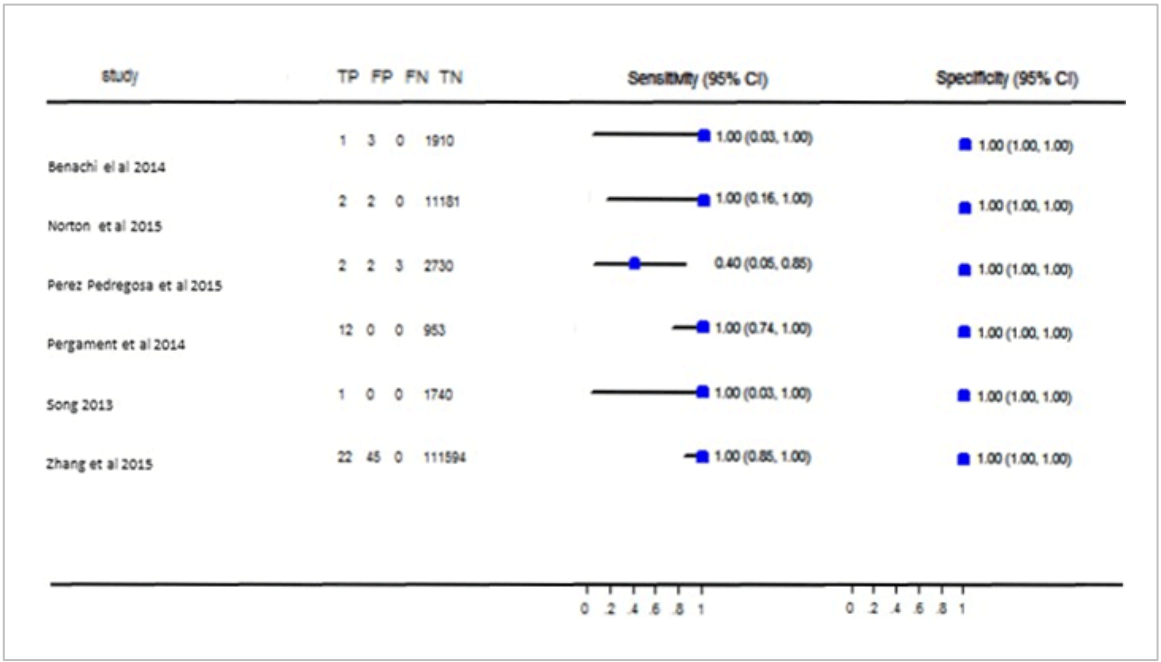


Figure 10: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 13

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

PPV and NPV

The PPV calculated from the study results ranged between 32.8% and 50%, except in two studies [44, 45], which reported a rate of 100%. The NPV was more than 99% in all studies. The recalculated PPV considering no results as positive samples was 0.8% in the study of Norton et al. [31] and 14.4% in the study of Pergament et al. [44].The NPV was more than 99.8% in all cases.

Table 6: Characteristics and noninvasive prenatal testing accuracy results of individual studies retrieved for noninvasive prenatal testing as a primary testing method for the general singleton pregnancy population

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Sarno et al. [39], 2016, UK Prospective DTA study Oct 2012–Aug 2015	Singleton or twin pregnancy women at 11 weeks' gestation to 13 weeks 6 days who received NIPT as an option following FCT or as part of routine screening	10,530	Median maternal age (years): 36.3 (33.2–39.3) Median GA (weeks): 11.9 (10.6–12.9)	Index test: Harmony prenatal test Cut-off point: >99% or <0.01%	Overall: 100% (fetal karyotype)	S=98.7	S=89.1	S=53.3
Comas et al. [40], 2015, Spain Prospective DTA study Jan–Dec 2013	All singleton pregnancy women were offered NIPT in addition to FCT Exclusion criteria: cases with US anomalies or those at high risk for other conditions	324	Median age (years): 37 (21–46) Mean GA (weeks): 14.6 (9.5–23.5) Mean BMI: 22.9 (17.1–42.5)	Index test: Panorama prenatal test Cut-off point: not reported	Fetal karyotyping (n=5) and follow-up (n=310)	S=100 (51–100) Sp=99.6 (98.3–99) PPV=80 (37.6–96.4) NPV=100 (98.8–100)	—	—
Norton et al. [31], 2015, USA, Canada, Sweden, Belgium, Netherlands and Italy Multicentre comparative prospective blinded DTA study	Women aged>18 years. with a singleton pregnancy (10–14 weeks) Exclusion criteria: outside GA window, no standard screening result, maternal aneuploidy or cancer, donor oocyte conception, twin pregnancies or an empty gestational sac	15,841	Median maternal age (years): 31 (18–48) Mean GA (weeks): 12.5 (10.0–14.3) Pregnancy by ART: 3.0%	Index test: Harmony prenatal test Cut-off point: >1/100	Genetic testing (amniocentesis, CVS or products of conception or newborn) (3.9% or 625/15,481)	S=100 (90.7–100) Sp=99.9 (99.9–100) PPV=80.9 (66.7–90.9) NPV=100 (99.9–100)	S=90.0 (55.5–99.7) Sp=100 (99.9–100) PPV=90.0 (55.5–99.7) NPV=100 (99.9–100)	S=100 (15.8–100) Sp=100 (99.9–100) PPV=50 (6.8–93.2) NPV=100 (99.9–100)
Pérez-Pedregosa et al. [41], 2015, Spain Comparative prospective DTA study Data collection period not reported	Women with a singleton pregnancy of at least 10 weeks' gestation	579	Median maternal age (years): 36.5 (22–47) Median GA (weeks): not reported	Index test: Harmony prenatal test Cut-off point: >99% or <0.01%	Overall: 100% Fetal karyotype (amniocentesis, CVS) in population with high-risk Telephone contact in the rest of women	S=100 (73.2–100) Sp=100 (99.1–100) PPV=100 (73.2–100) NPV=100 (99.1–100)	S=100 (30.9–100) Sp=100 (99.1–100) PPV=100 (30.9–100) NPV=100 (99.1–100)	—

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Quezada et al. [42], 2015, UK Comparative prospective DTA study Oct 2012–Jan 2014	Women with a singleton pregnancy and a live fetus	2851	Median maternal age (years): 36.9 (20.4–51.9) Median GA: 10 weeks 4 days (10 weeks to 11 weeks 6 days)	Index test: Harmony prenatal test Cut-off point: >99% or <1/10,000	Overall: 100% (n=2857) Fetal karyotype (amniocentesis, CVS or neonate blood) or neonate phenotype examination	S=100 (89.3–100) Sp=99.9 (99.8–100) PPV=96.9 (84.7–99.5) NPV=100 (99.9–100)	S=90.0 (59.6–98.2) Sp=99.8 (99.6–99.91) PPV=64.3 (38.3–83.7) NPV=99.9 (99.8–100)	S=40.0 (11.8–76.9) Sp=99.9 (99.7–100) PPV=50.0 (15.0–85) NPV=99.9 (99.7–100)
Zhang et al. [43], 2015, China Multicentre prospective DTA study Jan 2012–Aug 2013	Women aged>18 years with a singleton or twin pregnancy of at least 9 weeks' gestation	112669	Median age (years): 30.9 (18–46) Mean GA (weeks): 18.7 (9–36)	Index test: HiSeq2000 (Illumina) Cut-off point: not reported	Fetal karyotyping (68%, or 1055) and clinical follow-up (111,605)	S=99.17 (98.52–99.83) Sp=99.95 (99.93–99.95) PPV=92.19 (90.31–94.07) NPV=99.9 (99.9–100)	S=98.24 (94.93–99.63) Sp=99.95 (99.94–99.97) PPV=76.61 (70.99–82.23) NPV=100 (99.99–100)	S=100 (84.56–100) Sp=99.96 (99.95–99.97) PPV=32.84 (21.59–44.08) NPV=100 (99.99–100)
Bianchi et al. [30], 2014, USA Multicentre comparative prospective blinded DTA study Jul 2012–Jan 2013	Women aged>18 years with a singleton pregnancy of at least 8 weeks' gestation who had planned to undergo or completed standard serum screening for fetal aneuploidies	1952	Mean age (years): 29.6±5.54 Mean GA (weeks): 20.3±8.6	Index test: Verifi™ prenatal test Cut-off point: ≥4.0 high risk; ≤3.0 low risk	Fetal karyotyping (amniocentesis, CVS, testing of products of conception and postnatal evaluation) (n=57) Newborn physical examination (n=1857)	S=100 (47.8–100) Sp=99.7 (99.3–99.9) PPV=45.5 (16.7–76.6) NPV=100 (99.8–100)	S=100 (15.8–100) Sp=99.8 (99.6–100) PPV=40.0 (5.3–85.3) NPV=100 (99.8–100)	S=100 (20.7–100) Sp=99.8 (99.5–99.9) PPV=25 (4.6–69.9) NPV=100 (99.8–100)
Pergament et al. [44], 2014, USA Prospective DTA study Data collection period not reported	Women aged>18 years with a singleton pregnancy of ≤7weeks' gestation and who provided signed consent Exclusion criteria: sex chromosome anomaly, triploid or fetal mosaicism	966	Median age (years): 30.0 (18–47) Mean GA (weeks): 14.3 (7.6–40.6)	Index test: NS (SNPs) Cut-off point: not reported	Fetal karyotyping (amniocentesis, CVS or products of conception) or genetic testing of umbilical cord blood, buccal sample, saliva	S=100 (93.8–100) Sp=100 (99.6–100) PPV=100 (93.8–100) NPV=100 (99.6–100)	S=96 (79.7–99.9) Sp=99.9 (99.4–100) PPV=96.1 (79.7–99.9) NPV=99.9 (99.4–100)	S=100 (75.7–100) Sp=100 (99.6–100) PPV=100 (75.7–100) NPV=100 (99.6–100)
Song et al. [45], 2013, China Comparative prospective DTA study Apr 2011–Dec 2011	Pregnant women aged<35 years	1741	Mean age (years): 29.03±2.70 Mean GA (weeks): 16.57±1.56 Pregnancy by ART: 0.8%	Index test: HiSeq2000 (Illumina) Cut-off point: Z score≥3	Fetal karyotyping (amniocentesis, CVS or cordocentesis(n=2017) Neonatal follow-up (n=1805)	S=100 (59.8–100) Sp=100 (99.7–100) PPV=100 (59.8–100) NPV=100 (99.7–100)	S=100 (19.79–100) Sp=99.9 (99.6–99.9) PPV=66.7 (20.8–93.1) NPV=100 (99.8–100)	S=100 (5.5–100) Sp=100 (99.7–100) PPV=100 (5.5–100) NPV=100 (99.7–100)

Abbreviations: ART=assisted reproductive technology; BMI=body mass index; CI=confidence interval; CVS=chorionic villus sampling; DTA=diagnostic test accuracy; FCT=first-trimester combined testing; GA=gestational age; NIPT=noninvasive prenatal testing; NPV=negative predictive value; NS=not specified; PPV=positive predictive value; S=sensitivity; SNP=single nucleotide polymorphism; Sp=specificity; US=ultrasound.

[a] Number of samples with NIPT and reference standard results.

[b] Calculations provided/based on study results (exclusion of low fetal fraction, uncertain results, test failures and miscarriages).

5.3.2 NIPT as an add-on to FCT for the high risk singleton pregnancy population

There is lack of direct data regarding the accuracy of the prenatal strategy which includes NIPT as an add-on to the FCT. However, 27 DTA studies which assessed the performance of NIPT as a second-tier test in singleton women who had been classified as high risk of aneuploidy were included to assess NIPT accuracy for indirect inferences and modelling. The individual characteristics and results of these individual studies are displayed in Table 7.

5.3.2.1 Accuracy of NIPT for T21 as a second-tier test

Sensitivity and specificity values

The 24 studies which provided data on sensitivity and specificity involved 1408 aneuploidy cases and 99,818 euploidy cases. The reported sensitivity in these individual studies, excluding miscarriages, follow-up losses and no-call results, was 100% in all but five of the studies (≥94.2% in all cases). Specificity ranged from 99.7% to 100% (Table 7). One of the studies [49] which reported the trisomy status of cases among no-call results (n=10) found two missed cases (20%). If these cases were included in the analysis and no-call results were treated as positive samples, sensitivity would remain unchanged but specificity would decrease from 100% to 95.9%. If no results were considered as negative samples, specificity would be maintained but sensitivity would be reduced from 97.2% to 94.6%.

The pooled sensitivity obtained when the bivariate random-effects model was applied was 99.2% (95% CI 98.59%–99.56%) and specificity reached 99.95% (95% CI 99.93%–99.96%). The model failed to converge because of the lack of variation between studies, and therefore two independent univariate random-effects meta-analyses were applied. The meta-analysis results showed a pooled sensitivity of 99.24% (95% CI 98.64%–99.58%) and a pooled specificity of 99.95% (95% CI 99.93%–99.96%) (Figure 11).

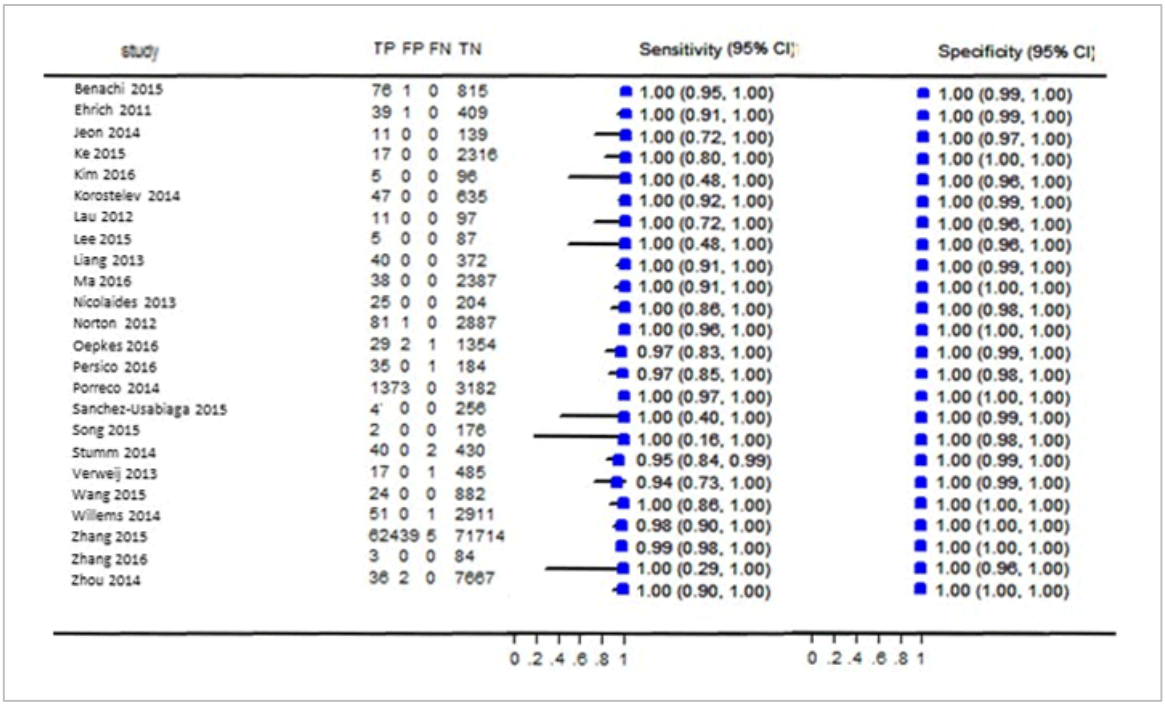


Figure 11: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 21

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

The HSROC curves did not provide any information because once more all studies generated estimates in the upper left corner. HSROC curves and other endpoints of interest obtained with bivariate meta-analysis (i.e., DOR, LR+ and LR–) can be found in Appendix 4 (Table A16 and Figure A2).

PPV and NPV

Similarly to the results reported for the general population, in all studies in the population at high risk of aneuploidies, NIPT showed an NPV of more than 99%. When no-result studies were included and classified as negative cases, the calculated NPV in the study of Persico et al. [49] was 99.1%. The PPV was 100%, except in six studies, where it ranged from 93.5% to 98.8% [48, 51, 55, 61, 64, 69]. The value recalculated in the study of Persico et al. [49] was 80%.

5.3.2.2 NIPT accuracy for T18

Sensitivity and specificity values

The evidence for T18 was derived from 21 DTA studies on high-risk women which reported on 211 T18 cases and 26,636 euploidy cases. The sensitivity calculated, excluding women with no-call results and follow-up losses ranged from 80% to 100% in all but one of the studies (40%). Specificity was 99.8% or greater in all studies (Table 7). Only one study [49] reported the trisomy status of cases with no test results, and the recalculation of diagnostic accuracy showed a reduction of specificity from 100% to 96.7% when these no-call results were considered positive; sensitivity did not change in this scenario. Specificity remained unchanged but sensitivity was 86.7% if the no-call results were considered negative.

When the bivariate random-effects model was applied, pooled sensitivity was estimated to be 96.86% (95% CI 88.35%–99.21%) and pooled specificity was 99.97% (95% CI 99.93%–99.98%) (Figure 12).

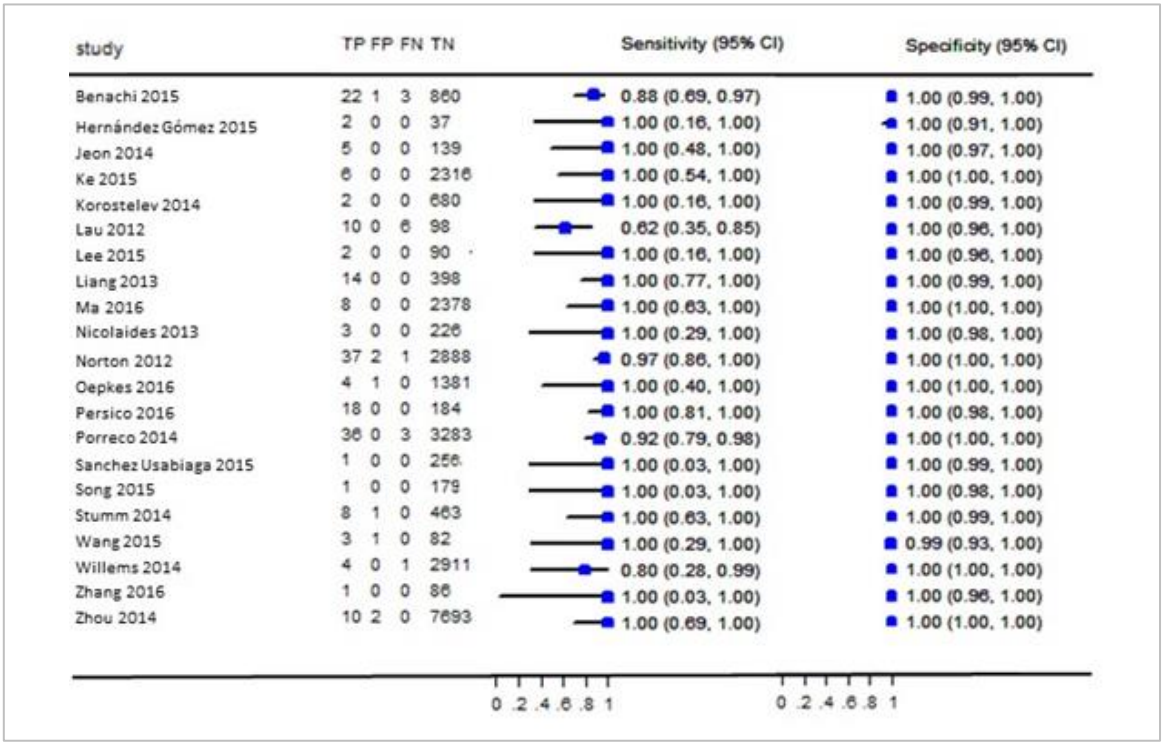


Figure 12: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 18

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

The model was unreliable because of the lack of variability in the specificity and the great number of cells with zero values. Although the model did converge, the correlation parameter between sensitivity and specificity logits was –1, and standard error and CIs could not be estimated. Therefore two independent univariate random-effects meta-analyses were performed to support the results obtained with the bivariate model. The pooled sensitivity was 96.6% (95% CI 88.35%–99.24%) and the pooled specificity was 99.97% (95% CI 99.94%–99.98%). Other endpoints of interest obtained with bivariate meta-analysis (i.e., DOR, LR+, LR– and HSROC curves) can be found in Appendix 4 (Table A16 and Figure A2).

PPV and NPV

NIPT showed an NPV for women with high risk of aneuploidies of more than 99%, except in the study of Lau et al. [68], where it was reported to be 94.2%. The PPV was 100% in most studies, although in seven studies it ranged between 66.7% and 95.6% [48, 51, 55, 57, 58, 62, 64, 69]. In the study of Persico et al. [49], the PPV decreased from 100% to 65.2% when no results were included as positive cases. Assuming the contrary, the NPV decreased from 100% to 99.2%.

5.3.2.3 NIPT accuracy for T13

Sensitivity and specificity values

Sixteen DTA studies (63 T13 cases and 23,468 euploidy cases) provided sensitivity and specificity data. The sensitivity calculated, excluding women with no-call results, miscarriages and follow-up losses, ranged from 75% to 100%; 13 studies showed a sensitivity of 100%. In one study sensitivity was 0% because of the lack of positive cases [68] (Table 7). Specificity was 99.8% or greater in all studies (Table 7). Only one study [49] reported the trisomy status of cases with no test results, and the recalculation of diagnostic accuracy showed a reduction of specificity from 100% to 96.4% when these two missed cases were considered positive; sensitivity did not change under this assumption. The sensitivity reached 83.3% if the two missed cases were considered negative; there were no changes in specificity under this scenario.

The bivariate random-effects model showed a pooled sensitivity of 97.67% (95% CI 59.68%–99.91%) and a pooled specificity of 99.98% (95% CI 99.92%–99.99%) (Figure 13). The model was again relatively unstable, showing a correlation parameter between logits of sensitivity and specificity of –1. The pooled sensitivity estimated with the univariate random-effects model was 95.81% (95% CI 47.59%–99.82%) and the pooled specificity was 99.98% (95% CI 99.91%–99.99%). Other endpoints of interest obtained with bivariate meta-analysis (i.e., DOR, LR+, LR– and HSROC curves) can be found in Appendix 4 (Table A16 and Figure A2).

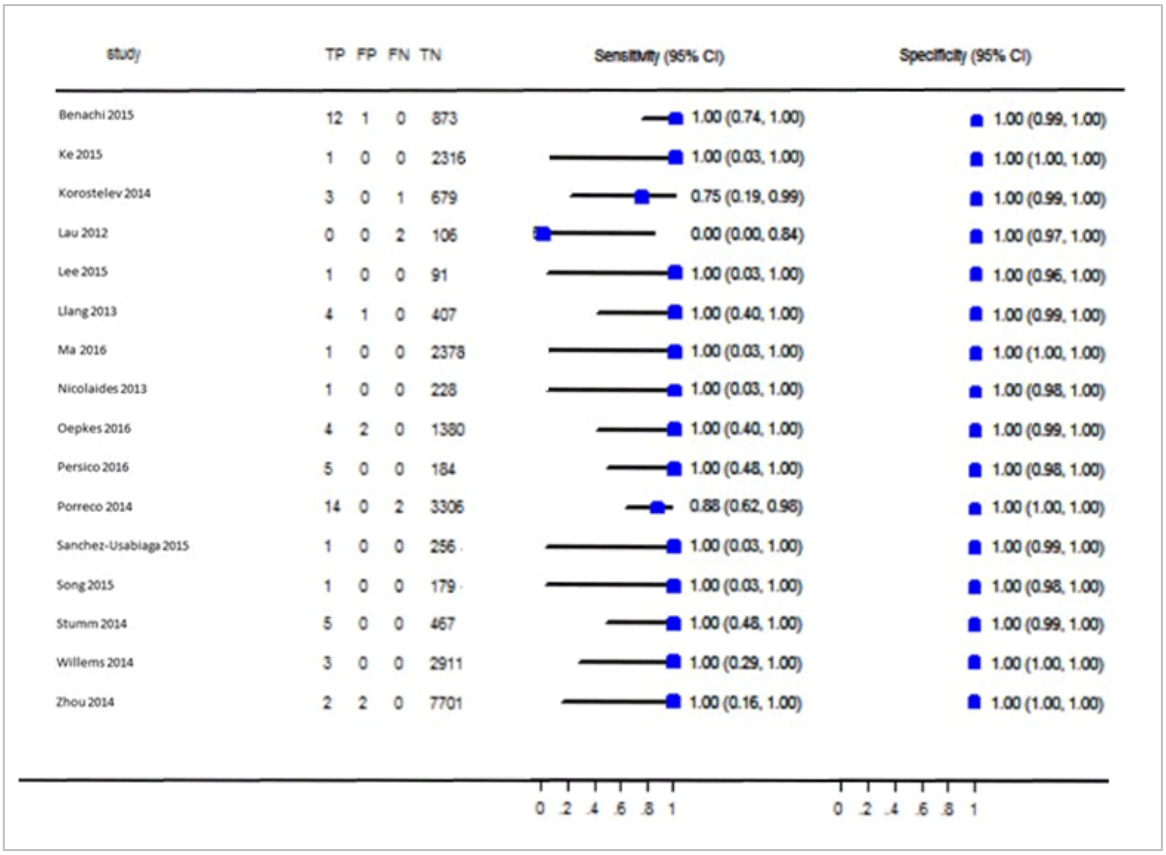


Figure 13: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 13

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

PPV and NPV

The NPV of NIPT for T13 screening was more than 99% in all studies. The PPV differed substantially between studies (50%–100%) (Table 7). The PPV was 0% in one study because of the lack of positive cases [68]. In the study of Persico et al. [49], the PPV would be reduced from 100% to 40% if no-call results were included in the analysis as positive cases; the NPV would remain the same under this assumption. The NPV would decrease from 100% to 99.6% if no-call results were classified as negative cases; the PPV would not change.

Table 7: Characteristics and noninvasive prenatal testing accuracy of individual studies performed in the high-risk singleton pregnancy population

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Kim et al. [46], 2016, Korea Prospective DTA study Dec 2014–Apr 2015	High-risk pregnant women	101	Mean maternal age (years.): 35.5±3.63 GA (weeks): 11–13 (69.3%), 14–18 (30.7%) Indication: high risk by first-trimester combined FTS tests or second-trimester quadruple screening	Ion Torrent PGM *Z* score>2.07 Proton platform (PGM) *Z* score>2.10	Overall: 100% (IT)	S=100 (47.8–100) Sp=100 (96.2–100) PPV=100 (47.8–100) NPV=100 (96.2–100)	—	—
Ma et al. [47], 2016, China Prospective DTA study (18 centres) Feb–May 2014	Prospective samples of high-risk pregnant women Exclusion criteria: organ donation history or maternal chromosome anomaly	2425	Median maternal age (years): 32 Median GA (weeks): 19 (10–35) Indication: positive serum screening (49%); family history (4.74%), US markers (5.5%), other risk factors (29%), without risk (12%)	BGI *Z* score>3	Overall: 100% (karyotyping results)	S=100 (90.8–100) Sp=99.8 (99.4–100) PPV=100 (90.8–100) NPV=100 (99.4–100)	S=100 (60.3–100) Sp=100 (99.9–100) PPV=100 (60.3–100) NPV=100 (99.9–100)	S=100 (2.5–100) Sp=100 (99.9–100) PPV=100 (2.5–100) NPV=100 (99.9–100)
Oepkes et al. [48], 2016, Netherlands Prospective DTA study, multicentre (8 centres) TRIDENT Apr–Sep 2014	All high-risk pregnant women (FCT or medical history) Exclusion criteria: multiple pregnancies, vanishing twins, NT≥3.5mm, other structural or chromosome anomalies, GA<10 weeks, age<18 years	1390	Mean maternal age (years): >36 (43%); >36 (51%); unknown (6%) GA (weeks): NS Indication: high risk by first-trimester combined FTS risk (≥1:200) (87.1%); medical history (19.9%)	Test: NS WGS (Illumina platform) *Z* score>3 or WISECONDOR algorithm	Overall: 100% IT (80.9% positive cases); questionnaire after pregnancy/ birth (rest)	S=96.7 (83.3–99.4) Sp=99.9 (99.5–100) PPV=93.5 (79.3–98.2) NPV=99.9 (99.6–100)	S=100 (51–100) Sp=99.9 (99.6–100) PPV=80 (37.6–96.4) NPV=100 (99.7–100)	S=100 (51–100) Sp=99.9 (99.5–100) PPV=66.7 (30–90.3) NPV=100 (99.7–100)
Persico et al. [49], 2016, Italy Prospective DTA study, multicentre (4 centres), blinded to IT results	Consecutive pregnancies after first-trimester screening (11–13 weeks) with risk≥1:250	249	Median maternal age (years): 36 (20–46) GA (weeks): NS Indication: women defined as high risk by first trimester combined FTS tests (risk≥1:250)	Panorama NATERA Cut-off: >100 (genotypic information included)	Overall: 100% (IT and US examination with additional genetic testing if required)	S=97.2 (65.8–99.5) Sp=100 (98.2–100) PPV=100 (90.1–100) NPV=99.5 (97.4–99.9)	S=100 (77.2–100) Sp=100 (98.4–100) PPV=100 (77.2–100) NPV=100 (98.4–100)	S=100 (56.6–100) Sp=100 (98.4–100) PPV=100 (56.6–100) NPV=100 (98.4–100)

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Zhang et al. [50], 2016, China Prospective DTA study Jan 2012–Dec 2013	Inclusion criteria: age≥35 years, singleton pregnancy, high risk of DS, elevated NT; structural anomalies in second-trimester screening, not suitable for IT, GA<12 weeks	87	Mean maternal age (years): 37.5±2.17 Median GA (weeks): 19.0 (12.4–32.5) Indication: serological positive screening (45.2%); sonography positive screening (16%)	Test: NS WGS (Illumina platform) *Z* score>3	Amniocentesis (24.1%), neonatal blood karyotyping (42.3%); follow-up examination of newborn (33.6%)	S=100 (43.8–100) Sp=100 (95.6–100) PPV=100 (43.8–100) NPV=100 (95.6–100)	S=100 (20.7–100) Sp=100 (95.7–100) PPV=100 (20.7–100) NPV=100 (95.7–100)	—
Benachi et al. [51], 2015, France Multicentre prospective DTA study Dec 2012–Oct 2013	Singleton or twin pregnancy women aged>38 years, ≥10 weeks' gestation, family history of trisomy or positive FCT	892	Mean maternal age (years): 35 (30–39) Mean GA (weeks): 15.1 (10.2–34.5)	HiSeq1500 Cut-off point: T21 3; T18 and T13 3.95	Overall: 100% (amniocentesis or CVS)	S=100 (95.3–100) Sp=99.9 (99.3–100) PPV=98.7 (93.0–99.8) NPV=100 (99.5–100)	S=88.0 (68.8–97.5) Sp=99.9 (99.4–100) PPV=95.6 (79.0–99.2) NPV=99.6 (99.0–99.9)	S=100 (73.5–100) Sp=99.9 (99.4–100) PPV=92.3 (66.7–98.6) NPV=100 (99.6–100)
Hernandez-Gomez et al. [52] 2015, Mexico Prospective, DTA study Aug 2013–Jan 2015	Women who requested testing Exclusion of women with US markers of chromosomepathy	42	Mean age (years): 37.1 (23–46) Mean GA (weeks): 13.3 (10–18.6) Indications: advanced maternal age, positive serum screening (71.4%), clinician's decision (14%), positive FCT (7%), anxiety due to fetal loss or chromosome X (7%)	Harmony prenatal test (DANSR® and FORTE) >99% or <0.01%	IT (50% of positive cases Results confirmed at birth (100% of negative cases)	S=100 (34.2–100) Sp=100 (90.6–100) PPV=100 (34.2–100) NPV=100 (90.6–100)	—	—
Ke et al. [53], 2015, China Prospective DTA study Mar 2012–May 2013	Singleton pregnancy women with high risk of aneuploidies, i.e., aged ≥35 years, aneuploidy history or abnormal serum screening or abnormal US findings	2340	Maternal age (years): 2061 women 35 and 279 women ≥35 GA (weeks): 80 women at 12–14, 2239 women at 15–20 and 21 women at 24 Indications: advanced maternal age (n=147), abnormal screening results or history of aneuploidies (n=1189) and abnormal US findings (n=74)	Test: NS Cut-off point: not reported	Karyotyping (24 positive NIPT cases) Neonatal follow-up (2316 negative NIPT cases)	S=100 (81.6–100) Sp=100 (99.8–100) PPV=100 (81.6–100) NPV=100 (99.8–100)	S=100 (61–100) Sp=100 (99.8–100) PPV=100 (61.0–100) NPV=100 (99.8–100)	S=100 (20.7–100) Sp=100 (99.8–100) PPV=100 (20.7–100) NPV=100 (99.8–100)

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Lee et al. [54], 2015, Korea Prospective DTA study Aug 2014–Feb 2015	Singleton or twin pregnancy women of at least 8 weeks' gestation, aged≥35 years, positive serum screening/ US findings or history of aneuploidies	92	Median maternal age (years): 32 (21–43) Median GA (weeks): 21.2 (8.2–31.1)	Test: NS (LabGenomics Clinical Laboratory)	Overall: 100% (amniocentesis, CVS, cordocentesis, neonatal peripheral blood or products of conception)	S=100 (47.95–100) Sp=100 (95.8–100) PPV=100 NPV=100	S=100 (19.29–100) Sp=100 (95.94–100) PPV=100 NPV=100	S=100 Sp=100 PPV=100 NPV=100
Sago et al. [55], 2015, Japan Prospective, multicentre DTA study (23 centres) Apr 2013–Mar 2014	Women who requested NIPT, including those aged>35 years, US or positive serum screening findings, family history or parent Robertsonian translocation	7740	Mean age (years): 38.3 (21–48) Mean GA (weeks): 13.3 (10.0–19.9)	MaterniT PLUS	Karyotyping (88.7% of positive NIPT cases) Follow up (21.5% of negative cases)	PPV=95.9 (70/73) for patients with IT	PPV=81 (34/42) for patients with IT	PPV=81.8 (9/11) for patients with IT
Sánchez-Usabiaga et al. [56], 2015, Mexico Prospective DTA study Mar 2013–Feb 2015	Singleton pregnancy woman aged≥35 years with maternal anxiety or positive FCT Exclusion criteria: multiple pregnancies by donor oocytes or surrogate mother, previous bone transplant or did not give consent informed	266	Mean maternal age (years): 35 (21–45) Mean GA (weeks): 11.85 (9–26.3) Indications: advanced maternal age (n=114), maternal anxiety (n=84) and positive FCT (n=72)	Test: NS SNPs Cut-off point: not reported	Karyotyping (CVS or amniocentesis, 6 positive NIPT cases) or neonatal examination at birth (78% of NIPT negative cases)	S=100 (51.0–100) Sp=100 (98.5–100) PPV=100 (51.0–100) NPV=100 (98.5–100)	S=100 (20.7–100) Sp=100 (98.5–100) PPV=100 (20.7–100) NPV=100 (98.5–100)	S=100 (20.7–100) Sp=100 (98.5–100) PPV=100 (20.7–100) NPV=100 (98.5–100)
Song et al. [57], 2015, China Prospective DTA study, double blind May 2012–Aug 2013	Women arbitrarily presenting for NIPT Inclusion criteria: Age ≥35 years, singleton pregnancies, GA 8 weeks to 12 weeks 6 days	212	Median maternal age (years): 37.25 (35–45) Median GA: 9 weeks 6 days (8 weeks to 12 weeks 6 days) Pregnancy by ART: 6%	Berry Genomics *Z* score>3	Overall: 100% (karyotyping 84%; clinical assessment 26%)	S=100 (19.79–100) Sp=100 (97.35–100) PPV=100 (19.79–100) NPV=100 (97.35–100)	S=100 (5.46–100) Sp=100 (97.35–100) PPV=100 (5.46–100) NPV=100 (97.35–100)	S=100 (5.46–100) Sp=100 (97.35–100) PPV=100 (5.46–100) NPV=100 (97.35–100)
Wang et al. [58], 2015, China Prospective DTA study Jan 2013–Dec 2013	Women with abnormal results on FCT (56.8%), aged≥35 years (32.7%), adverse pregnancy history/ abnormal amniotic fluid volume (9.8%) or abnormal US findings (0.65%)	917	Maternal age range (years): 18–46 GA range (weeks): 14–26	Illumina HiSeq2000 Cut-off point: not reported	Overall: 100% (conventional karyotyping analysis or FISH and neonatal follow-up)	S=100 (86.2–100) Sp=100 (99.6–100) PPV=100 (86.2–100) NPV=100 (99.6–100)	S=100 (43.8–100) Sp=99.8 (99.4–100) PPV=75 (30.1–95.4) NPV=100 (99.6–100)	—

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Jeon et al. [59], 2014, China Prospective DTA study Mar 2012–Oct 2013	High-risk pregnant women scheduled for IT (aged≥19 years, singleton pregnancy, ≥12 weeks' gestation)	155	Mean age (years): 30.7±4.99 GA range (weeks): 12–16 (18%), 17–21 (55.5%), ≥22 (26.5) Indications: high risk by first-trimester combined FTS tests or second-trimester serum values alone or in combination with FTS	Ion Proton™ system Cut-off point: T21 2.459; T18 2.566	Overall: 100% (IT)	S=100 (71.5–100) Sp=100 (97.5–100) PPV=100 (71.5–100) NPV=100 (97.5–100)	S=100 (47.8–100) Sp=100 (97.6–100) PPV=100 (47.8–100) NPV=100 (97.6–100	—
Korostelev et al. [60], 2014, Russia Prospective, DTA study 2012–2014	Sample of women from private clinics who underwent NIPT. All were subjected to first-trimester screening (n=1728)	682	Mean age (years): 34.4 (26–45) Mean GA (weeks): 14 (9–33) Indications: FCT risk<1:250 (53.8%), advanced maternal age (27.9%), personal wish (11%), bad reproductive history (3.6%), or IVF procedure (2.4%)	Natera (NATUS algorithm) Cut-off point: NS	IT (241; 100% of positive cases) Birth follow-up (negative cases)	S=100 (92.4–100) Sp=100 (99.4–100) PPV=100 (92.4–100) NPV=100 (99.4–100)	S=100 (34.2–100) Sp=100 (99.4–100) PPV=100 (34.2–100) NPV=100 (99.4–100)	S=75 (30.1–95.4) Sp=100 (99.4–100) PPV=100 (43.8–100) NPV=99.9 (99.2–100)
Porreco et al. [61], 2014, USA Prospective multicentre blinded DTA study (31 centres) Sep 2009–Apr 2011	Women who were judged to be at risk and made the decision to undergo IT Inclusion criteria: age≥35 years, high risk at first- or second-trimester screening; US anomalies or family history Exclusion criteria: no consent, multiple or fetal demise	3322	Mean age (years)): 35.1 (SD 5.6) Mean GA (weeks): 16.3 (SD 3.5)	Test: NS WGS (HiSeq 2000, Illumina Inc.) *Z* score≥3 (T21); ≥3.95 (T18 and 13)	IT (100% of samples)	S=100 (97.34–100) Sp=99.9 (99.72–99.98) PPV=97.9 (93.8–99.56) NPV=100 (99.98–100)	S=92.3 (79.13–98.38) Sp=100 (99.89–100) PPV=100 (90.26–100) NPV=99.9 (99.73–99.9)	S=87.5 (61.65–98.45) Sp=100 (99.89–100) PPV=100 (76.84–100) NPV=99.9 (99.78–99.9)
Stumm et al. [62], 2014, Germany and Switzerland Prospective, multicentre blinded DTA study (5 centres)	Consecutive enrolled women Inclusion criteria: signed informed consent, age≥18 years, high-risk pregnancy, performance of IT, blood drawn before IT	472	Mean age (years): 36.0 (19–47) Mean GA (weeks): 15.6 Indications: age≥35 years, (n=363), positive serum markers (n=58), US anomaly (n=205), family history (n=11), parental chromosomal aberration (n=2) or other risk factors (n=78)	LifeCodexx Cut off point: *Z* score≥3 (T21); 3.9 (T18); 3.2 (T13)	IT (100% of samples)	S=95.2 (84.2–95.7) Sp=100 (99.1–100) PPV=100 (91.2–100) NPV=99.5 (98.3–99.9)	S=100 (67.6–100) Sp=99.8 (98.8–100) PPV=88.9 (56.5–98) NPV=100 (99.2–100)	S=100 (56.6–100) Sp=100 (99.2–100) PPV=100 (56.6–100) NPV=100 (99.2–100)

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Willems et al. [63], 2014, Belgium Prospective DTA study Mar–Dec 2013	Women with NIPT indication Inclusion criteria: positive FCT (>1:200 in Netherlands and >1:300 in Belgium), maternal age>37 years, family history of aneuploidies or none of the previous indications	2968	Mean maternal age (years): 36±3 Mean GA (weeks): 13±2 Indications: advanced maternal age (40.06%), none of the fixed indications (34.73%), positive FCT (22%) and family history of aneuploidies (3.27%)	Harmony prenatal test Cut-off point: not reported	Karyotyping (amniocentesis or CVS) (n=47)	S=98 (89.9–99.7) Sp=100 (99.9–100) PPV=100 (93.0–100) NPV=99.9 (99.8–100)	S=80 (37.6–96.4) Sp=100 (99.9–100) PPV=100 (51.0–100) NPV=99.9 (99.8–100)	S=100 (43.8–100) Sp=100 (99.9–100) PPV=100 (51.0–100) NPV=100 (99.8–100)
Zhou et al. [64], 2014, China Prospective DTA study Sep–Jun 2013	Pregnant women of 12–14 weeks' gestation Inclusion criteria: advanced maternal age, DS risk based on serum or abnormal US findings or women without previous screening	7701	Maternal age (years): 40.4% of women 35 and 59.6% of women 35 GA (weeks): NS Indications: high risk of DS based on serum or US findings (32.1%) and without previous screening (56.6%)	Test: NS (BGI Laboratory) Cut-off point: not reported	Karyotyping (54 samples) and neonatal follow-up (n=3894)	S=100 (72.2–100) Sp=99.9 (99.8–100) PPV=94.7 (82.7–98.5) NPV=100 (99.9–100)	S=100 (72.1–100) Sp=99.9 (99.8–100) PPV=83.3 (55.2–95.3) NPV=100 (99.9–100)	S=100 (34.2–100) Sp=99.9 (99.8–100) PPV=50.0 (15.0–100) NPV=100 (99.9–100)
Liang et al. [65], 2013, China Prospective DTA study Mar 2009–Jun 2011	Women with indication for IT Inclusion criteria: positive serum screening, advanced maternal age, US anomaly or more than one indication	412	Mean maternal age (years): 31±5.9 Median GA: 21 weeks 3 days (11 weeks 3 days to 39 weeks 3 days)	HiSeq200 (Illumina) Cut-off point: T21 3; T18 5.91; T13 5.72; T9 7.45; SCA: –2.91 to 2.91	Overall: 100% (amniocentesis)	S=100 (91.2–100) Sp=100 (99.0–100) PPV=100 (91.2–100) NPV=100 (99.9–100)	S=100 (78.5–100) Sp=100 (99.0–100) PPV=100 (78.5–100) NPV=100 (99.0–100)	S=100 (51.0–100) Sp=99.75 (98.6–100) PPV=80 (37.6–96.4) NPV=100 (99.1–100)
Nicolaides et al. [66], 2013, UK Prospective DTA study Data collection period not reported	Women at 11–13 weeks' gestation who underwent IT Inclusion criteria: positive FCT, previous aneuploidy pregnancy, advanced maternal age or presence of sickle cell disease	229	Median maternal age (years): 35.7 (18.5–46.5) Median GA (weeks): 13.1 (11.3–13.9) Indications: positive FCT (n=227), previous aneuploidy (n=6) and advanced maternal age (n=5)	Test: NS (SNPs, NATUS algorithm)	Overall: 100% (CVS)	S=100 (86.7–100) Sp=100 (98.2–100) PPV=100 (86.7–100) NPV=100 (98.2–100)	S=100 (43.8–100) Sp=100 (98.3–100) PPV=100 (43.8–100) NPV=100 (98.3–100)	S=100 (20.7–100) Sp=100 (98.3–100) PPV=100 (20.7–100) NPV=100 (98.3–100)
Verweij et al. [67], 2013, Norway, Sweden and Netherlands Prospective DTA study	High-risk women (based on FCT) scheduled for IT because of US anomalies or anxiety	504	Median maternal age (years): 36.4 (20–47) Median GA (weeks): 14.0 (10–28)	Harmony® prenatal test (samples were analysed by Ariosa Diagnostic)	Overall: 100% of population included (n=520)	S=94.4 (72.7–99.9) Sp=100 (99.4–100) PPV=100 (81.6–100) NPV=99.8 (99.8–100)	—	—

Authors, year, countries, study characteristics	Eligibility criteria	N[a]	Population characteristics	Index test/ cut-off point	Reference test(s)	Performance results[b], % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
May 2011–Mar 2012	Exclusion criteria: >1 fetus, IT prior to blood sampling, history or active malignancy or language restriction to understand study information			Cut-off point: 1:100 (1%)	(amniocentesis in 240 samples and CVS in 280 samples)			
Lau et al. [68], 2012, Japan Prospective blinded DTA study Data collection period not reported	Women with indication for IT Inclusion criteria: positive FCT or first-trimester US markers, other structural anomalies, maternal anxiety or previous trisomy	108	Mean maternal age (years): 37±4.3 Median GA: 12 weeks 5 days (11 weeks 4 days to 28 weeks)	Verifi™ prenatal test (Illumina) Cut-off point: *Z* score>3	Overall: 100% (94.4% CVS and 5.6% amniocentesis)	S=100 (20.7–100) Sp=100 (96.2–100) PPV=100 (20.7–100) NPV=100 (96.2–100)	S=62.5 (38.6–81.5) Sp=100 (96.2–100) PPV=100 (72.2–100) NPV=94.2 (88.0–97.3)	S=0 Sp=100 PPV=0 NPV=98.1
Norton et al. [69], 2012, USA, Netherlands and Sweden Prospective blinded DTA study Aug 2010–Nov 2011	Women with a singleton pregnancy who planned to undergo IT for any indication Exclusion criteria: >fetus, presence of known aneuploidy, history or active malignancy or women who received IT	3080	Median maternal age (years): 34.3 (18–50) Median GA (weeks): 16.9 (10.0–38.7)	Harmony® prenatal test (DANRS and FORTE analysis algorithm) Cut-off point: 1:100 (1%)	Overall: 100% (n=3080) (amniocentesis in 74.7% and CVS in 25.3%)	S=100 (95.5–100) Sp=99.97 (99.8–99.99) PPV=98.8 (93.4–99.8) NPV=100 (99.9–100) For other cut-off point (1:1000 (0.1%), 1:300 (0.33%) and 1:10 (10%)) Sp=99.90 S not changed	S=97.4 (86.5–99.9) Sp=99.9 (99.7–99.9) PPV=94.9 (83.1–96.6) NPV=99.9 (99.8–100) For cut-off point 1:1000 (0.1%) Sp=99.79 For cut-off point 1:300 (0.33%) Sp=99.86 For cut-off point 1:10 (10%) S=94.7	—
Ehrich et al. [70], 2011, USA Prospective DTA study, double blinded From May 2009	Women classified as having high risk for DS scheduled for IT (singleton pregnancies)	449	Median maternal age (years): 37 (8–36) Mean GA (weeks): 16 (8–36) Indications: high serum levels (30.1%), age≥35 years (68.3%), US anomalies (13.9%), family history (5.2%), other (10.2%)	Sequenom (Qiagen kit) Cut off-point: *Z* score 2.5	Overall: 100% (fetal karyotyping)	S=100 (89–100) Sp=99.7 (98.5–100) PPV=100 (87.1–99.6) NPV=100 (99.1–100)	—	—

Abbreviations: ART=assisted reproductive technology; CI=confidence interval; CVS=chorionic villus sampling; DS=Down syndrome; DTA=diagnostic test accuracy; FCT=first-trimester combined testing; FISH=fluorescence in situ hybridisation; FTS=; GA=gestational age; IT=invasive testing; IVF=in vitro fertilisation; NIPT=noninvasive prenatal testing; NPV=negative predictive value; NS=not specified; NT=nuchal translucency; PPV=positive predictive value; S=sensitivity; SCA=sex chromosome aneuploidy; SD=standard deviation; SNP=single nucleotide polymorphism; Sp=specificity; T9=trisomy 9; T13=trisomy 13; T18=trisomy 18; T21=trisomy 21; US=ultrasound; WGS=whole genome sequencing.

[a] Number of samples with NIPT and follow-up results.

[b] Calculations provided/based on study results (exclusion of low fetal fraction, uncertain results, test failures and miscarriages).

5.3.3 NIPT as an add-on to FCT for the high- and intermediate-risk singleton pregnancy population

The only study included which assessed this screening strategy showed a sensitivity for T21, T18 and T13, excluding no results, of 97.7%, 87.5% and 50%, respectively. The specificity was 99.9% for all three trisomies. The PPV was 97.7% for T21, 84% for T18 and 33.3% for T13. The NPV was 99.9%, 100% and 99.9%, respectively. Three T18 cases (12.5%) were not detected because the test did not provide a result [71] (Table 8).

Table 8: Characteristics and noninvasive prenatal testing accuracy of individual studies performed in the high- and intermediate-risk singleton pregnancy population

Authors, year, country, study characteristics	Eligibility criteria	Population characteristics	N[a]	Index test, cut-off point, reference test(s)	Performance results[b], % (95% CI, %)		
					Trisomy 21	Trisomy 18	Trisomy 13
Gil et al. [71], 2016, UK Prospective DTA study Oct 2013–Feb 2015	Women with a singleton pregnancy between 11 and 13 weeks' gestation classified as of high (≥1:100) or intermediate (1:2500) risk of aneuploidies by FCT	Median maternal age (years): high risk 36.1 (32.1–39.5); intermediate risk 34.8 (30.8–38.4); low risk 29.9 (25.8–33.2) Median GA: not reported Pregnancy by ART (n): high risk 16 (3.5%); Intermediate risk 135 (3.8%); low risk 172 (2.2%) Indications: high or intermediate risk, 3633 samples; low risk, 7680 samples	3633	Harmony prenatal test Cut-off point not reported Overall: 100% (CVS)	S=97.7 (88.2–99.6) Sp=99.9 (99.8–100) PPV=97.7 (88.2–99.6) NPV=99.9 (99.8–100)	S=87.5 (69.0–95.7) Sp=99.8 (99.7–100) PPV=84 84.0 (65.3–93.6) NPV=100 (99.9–100)	S=50 (15.0–85.0) Sp=99.8 (99.7–100) PPV=33.3 (9.7–70.0) NPV=99.9 (99.8–100)

Abbreviations: ART=assisted reproductive technology; CI=confidence interval; CVS=chorionic villus sampling; DTA=diagnostic test accuracy; FCT=first-trimester combined testing; GA=gestational age; NPV=negative predictive value; PPV=positive predictive value; S=sensitivity; Sp=specificity.

[a] Number of samples with noninvasive prenatal testing and reference standard results.

[b] Calculations provided/based on study results (exclusion of low fetal fraction, uncertain results, test failures and miscarriages).

5.3.4 NIPT as an add-on to FCT for the high-risk twin pregnancy population

5.3.4.1 NIPT accuracy for T21

Sensitivity and specificity

Five DTA studies reported results on high-risk twin pregnancy populations. These included 33 T21 cases and 1547 euploidy cases), which were included in the analysis. When the bivariate random-effects model was applied, pooled sensitivity was 99.2% (95% CI 27.1%–99.9%) and pooled specificity was 99.8% (95% CI 98.5%–99.9%) (Figure 14). The correlation parameter between sensitivity and specificity was 1, showing instability in the model. Therefore, two independent univariate random-effects meta-analyses were performed to support the results obtained with the bivariate model. The results obtained with the univariate random-effects model were similar for specificity (99.8%, 95% CI 98.5%–99.9%) but sensitivity was lower (96.9%, 95% CI 81.3%–99.5%). Only Sarno et al. [39] provided data on NIPT sensitivity, which reached 100%. Other endpoints of interest obtained with bivariate meta-analysis (i.e., DOR, LR+, LR– and HSROC curves) can be found in Appendix 4 (Table A16 and Figure A3).

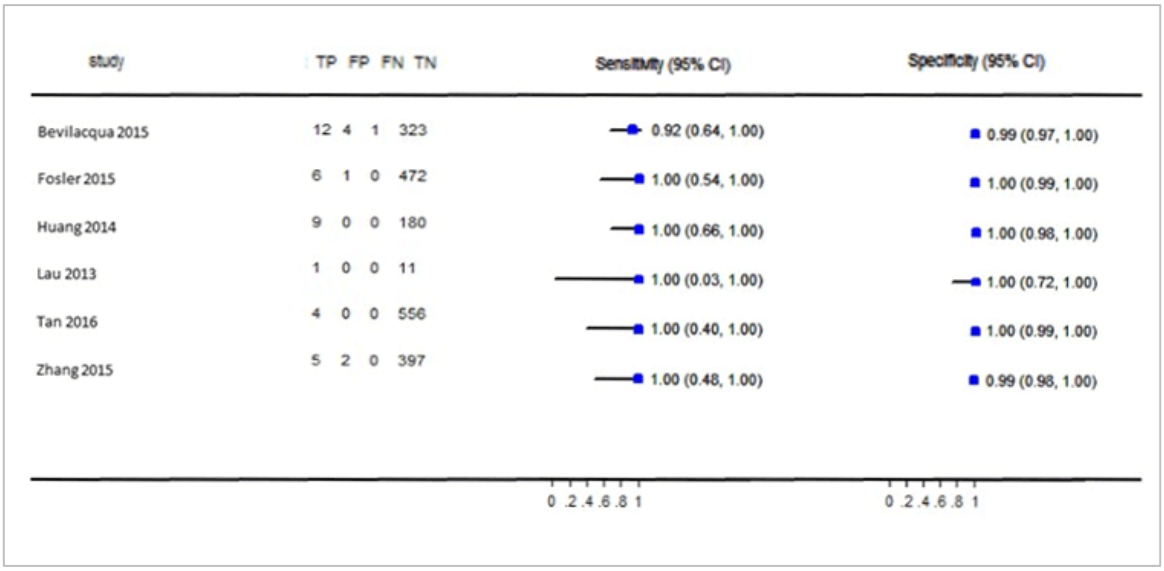


Figure 14: Paired forest plot of sensitivity and specificity of noninvasive prenatal testing for trisomy 21

Abbreviations: CI=confidence interval; FN=false negatives; FP=false positives; TN=true negatives; TP=true positives.

PPV and NPV

According to the retrieved studies, the NPV of NIPT was more than 99% and PPV was 100% in three studies. Fosler et al. [72] and Bevilacqua et al. [74] found PPVs of 75% and 85%, respectively. In the study of Tan et al. [73] the PPV would decrease from 100% to 44.4% if test failures are considered to be cases. The NPV and PPV would remain unaltered if they are treated as negative.

5.3.4.2 NIPT accuracy for T18

Sensitivity and specificity

Only three DTA studies assessed T18. The results of these studies are discordant. Whereas Bevilacqua et al. [74] found a sensitivity of 100%, it reached 75% in the study of Sarno et al. [39], and Huang et al. [75] reported a value of 50%. The specificity observed in the retrieved studies [74, 75] was 96%–100%. None of the studies provided data on no-call results.

PPV and NPV

Similarly to the evidence mentioned previously, the NPV of NIPT for T18 was more than 99%. For the PPV, Huang et al. [75] found a value of 100% and it reached 29.4% in the study of Bevilacqua et al. [74]. None of the studies provided data on no-call results.

NIPT accuracy data in the twin pregnancy population reported in individual studies can be found in Table 9.

Table 9: Characteristics and noninvasive prenatal testing accuracy of individual studies performed in the high risk twin pregnancy population

Authors, year, countries, study characteristics	Eligibility criteria	Population characteristics	N[a]	Index test/comparator cut-off point	Reference test(s)	Performance results, % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Fosler et al. [72], 2017, USA Prospective DTA study (only cohort B included) Data collection period not reported	Women aged>18 years of at least 8 weeks' gestation who had advanced maternal age, abnormal US findings, previous affected pregnancy or positive serum screening	Mean maternal age (years): 35.5±4.9 Mean GA (weeks): 13.7±3.9 Pregnancy by ART: not reported Chorionicity: not reported	479	Verifi™ prenatal test Cut-off point: not reported	Karyotyping in 171 samples (CVS or amniocentesis)	S=100 (61.0–100) Sp=99.7 (99.8–100) PPV=85.7 (48.7–97.4) NPV=100 (99.2–100)	—	—
Sarno et al. [39], 2016, UK Prospective DTA study (only reported twin pregnancy results) Oct 2012–Aug 2015	Singleton or twin pregnancies at 11 weeks' gestation to 13 weeks 6 days who received NIPT following FCT or as part of routine screening	Median maternal age (years): 37.3 (34.6–40.0) Median GA (weeks): 11.7 (10.4–12.9) Pregnancy by ART (*n*): 246 (56.2%) Chorionicity: 373 (85.2%) were dichorionic and 65 (14.8%) were monochorionic Median BMI (kg/m^2): 23.5 (21.0–26.9)	417	Harmony prenatal test Cut-off point: >99% or <0.01%	Overall: 100% (fetal karyotyping)	All trisomies S=84.6 (57.8–95.7) Sp=99.7 (98.6–100) PPV=91.7 (64.6–98.5) NPV=99.5 (98.2–99.9)		
Tan et al. [73], 2016, China Prospective DTA study Jan 2012–Dec 2013	Women with a twin pregnancy after use of ART, aged>18 years, >10 weeks' gestation, with or without prior DS screening result, for one- to two-embryo transfer, confirmation of live twin pregnancy by US examination and for three-embryo transfer confirmation of live twin pregnancy and no demise fetus by US examination	Median maternal age (years): 31 (20–43) Median GA (weeks): 12 (11–28) Pregnancy by ART: 100% Chorionicity: 96.3% dichorionic diamniotic, 1.9% monochorionic diamniotic and 1.2% monochorionic monoamniotic	560	Index test: not reported (MPS) Cut-off point: *t* score>2.5 and *L* score>1 were considered as high risk; *t* score>2.5 or *L* score>1 was considered as in the "warning zone"; *t* score<2.5 and *L* score<1 were considered as low risk	Overall: 100% Fetal karyotyping (3.1%, positive NIPT result) Follow-up (96.9%, negative NIPT result)	S=100 (51.0–100) Sp=100 (99.3–100) PPV=100 (51–100) NPV=100 (99.3–100)	—	—

Authors, year, countries, study characteristics	Eligibility criteria	Population characteristics	N[a]	Index test/comparator cut-off point	Reference test(s)	Performance results, % (95% CI, %)		
						Trisomy 21	Trisomy 18	Trisomy 13
Bevilacqua et al. [74], 2015, Belgium, UK and Spain Prospective DTA study May 2013–Sep 2014	Women with twin pregnancies at 10–28 weeks' gestation who underwent cfDNA testing because of prior high risk of aneuploidies (first- or second-trimester serum screening or US examination)	Median maternal age (years): 36.8 (19.0–50.3) Median GA (weeks.): 13.6 (10.0–34.7) Pregnancy by ART: 52.8% Chorionicity: not reported	340	Harmony prenatal test Cut-off point: >99% or <0.01%	Overall: 351 samples Fetal karyotyping (amniocentesis, CVS) Neonatal blood Neonatal examination	S=92.3 (66.7–98.6) Sp=98.7 (96.9–99.5) PPV=75.0 (50.5–89.8) NPV=99.6 (98.3–99.9)	S=100 (56.6–100) Sp=96.4 (93.8–97.9) PPV=29.4 (13.3–53.1) NPV=100 (98.8–100)	—
Huang et al.[75], 2014, China Prospective DTA study Data collection period not reported	Women with twin pregnancies who required IT because of abnormal serum screening or US findings or maternal anxiety Exclusion criteria: women with intrauterine fetal demise at the time of sampling or without fetal karyotype results	Median maternal age (years): 31 (22–44) Median GA (weeks): 19 (11–36) Pregnancy by ART: 59.8% Chorionicity: 80.4% dichorionic diamniotic, 16.4% mono-chorionic diamniotic, 1.1% monochorionic monoamniotic and 2.1% unknown	189	Index test: not reported Cut-off point: *t* score>2.5 and *L* score>1 were considered as high risk; *t* score>2.5 or *L* score>1 was considered as in the "warning zone"; *t* score<2.5 and *L* score<1 were considered as low risk	Overall: 100% Fetal karyotyping: amniocentesis (94.2%), CVS (2.1%) or cordocentesis (3.7%)	S=100 (70.1–100) Sp=100 (97.9–100) PPV=100 (70.1–100) NPV=100 (97.9–100)	S=50 (9.5–90.5) Sp=100 (98.0–100) PPV=100 (20.7–100) NPV=99.4 (97.0–99.9)	—
Lau et al. [76], 2013, China Comparative prospective DTA study Aug 2011–Apr 2012	Women with a twin pregnancy who have rejected IT on the basis of standard screening results	Mean maternal age (years): 36.5 (28–41) Median GA: 13 weeks 1 day (11 weeks 6 days to 20 weeks 1 day) Pregnancy by ART: 66.7% Chorionicity: 83.3% dichorionic and 16.7% monochorionic	12	Index test: NIFTY test Cut-off point: *t* score>2.5 and *L* score>1 were considered as high risk; *t* score>2.5 or *L* score>1 were considered as in the "warning zone"; *t* score<2.5 and *L* score<1 was considered as low risk Comparator: first- or second-trimester serum screening or US examination (NT, fetal nasal bone or Doppler assessment of the tricuspid valve or ductus venosus)	Overall: 100% Fetal karyotyping or clinical examination of the newborn	S=100 (20.7–100) Sp=100 (52.3–94.9) PPV=33.3 (6.1–79.2) NPV=100 (70.1–100)	—	—

Abbreviations: ART=assisted reproductive technology; cfDNA=cell-free DNA; CI=confidence interval; CVS=chorionic villus sampling; DS=Down syndrome; DTA=diagnostic test accuracy; FCT=first-trimester combined testing; GA=gestational age: IT=invasive testing; NIPT=noninvasive prenatal testing; NPV=negative predictive value; NT=nuchal translucency; PPV=positive predictive value; S=sensitivity; Sp=specificity; US=ultrasound.

[a] Number of samples with NIPT and reference standard results.

5.4 Comparative performance

[D1002] – How does NIPT screening compare with other optional screening approaches in terms of accuracy measures?

5.4.1 NIPT as a primary screening test for the singleton pregnancy population in comparison with FCT

Sensitivity and specificity for T21, T18 and T13

Information available for T21 and T13 comes from four included studies. In one of these studies, NIPT accuracy was compaired against first- or second-trimester serum screening combined or not combined with ultrasonographic findings. T13 was assessed in only three of these studies.

Stratified analysis for each trisomy was performed by independent univariate random-effects meta-analysis because the bivariate random-effects model failed to converge. According to these studies, NIPT achieved a significantly higher specificity than combined screening for T21, T18 and T13. The sensitivity was similar between both screening methods for T13 and T18; however, NIPT showed a higher sensitivity for T21 compared with standard screening (100% vs. 94%, p<0.001 respectively; χ^2) (Table 10).

PPV and NPV for T21, T18 and T13

With respect to PPV and NPV, only two studies provided statistical analysis between both screening tests. Norton et al. [31] found the PPV and NPV for T21 were higher with NIPT than with combined screening (PPV of 80.9% vs. 3.4% and NPV of 100% vs. 99.9%), Bianchi et al. [30] did not find any difference. Regarding the other two trisomies assessed, only Norton et al. [31] reported the PPV for T18 was higher with NIPT than with combined screening (90.0% vs. 14.0%) and there were no differences in the NPV for T18 or the PPV or NPV for T13.

Table 10: Noninvasive prenatal testing versus combined screening test accuracy for each trisomy

	No. of studies	Nº TP/Nº diseased Nº TN/Nº nondiseased	Combined screening % (95% CI, %)	TP/diseased TN/nondiseased	NIPT % (95% CI, %)	*P* value
Trisomy 13	3					
Sensitivity		7/8	0.88 (0.46, 0.98)	5/8	0.62 (0.28, 0.87)	0.20
Specificity		14,710/14,912	0.99 (0.98, 0.99)	15,821/15,828	1.00 (1.00, 1.00)	<0.0001
Trisomy 18	4					
Sensitivity		22/24	0.92 (0.72, 0.98)	23/25	0.92 (0.73, 0.98)	0.97
Specificity		20,913/21,141	0.99 (0.99, 0.99)	21,083/21,092	1.00 (1.00, 1.00)	<0.0001
Trisomy 21	4					
Sensitivity		79/89	0.94 (0.87, 0.99)	89/89	1.00 (0.99, 1.00)	<0.001
Specificity		19,981/21,081	0.95 (0.94, 0.95)	21,050/21,046	1.00 (1.00, 1.00)	<0.0001

Abbreviations: NIPT=noninvasive prenatal testing; TN=true negatives; TP=true positives.

5.4.2 NIPT as an add-on test to FCT for the high-risk singleton pregnancy population in comparison with FCT

There is lack of data regarding the comparison of these two screening strategies. On the basis of the 2×2 test accuracy data of the Cochrane review [127] and with the use of a bivariate meta-analytic model, the estimated pooled sensitivity of FCT for the risk level of 1 in 300 is estimated to be 87.26% (95% CI 85.18%–89.09%). The estimated pooled specificity is 95.50% (95% CI 94.86%–96.05%). Assuming a prevalence of T21 of 24 in 10,000 (EUROCAT data) [12] and assuming that all women testing positive with FCT would undergo NIPT (pooled sensitivity estimate of 99.24%, 95% CI 98.64%–99.58%, and pooled specificity estimate of 99.95%, 95% CI 99.93%–99.96%), the estimated sensitivity of the add-on strategy (FCT plus NIPT) for a hypothetical cohort of 10,000 estimated on the basis of the pooled high-risk data would be 86.8% (95% CI 82.2%–90.4%). Estimated specificity would be 100% (95% CI 99.9%–100%). The PPV would be 99.1% (95% CI 96.7%–99.7%%) and the NPV would be 100% (95% CI 99.9%–100%). The hypotherical scenario used to estimate sensitivity and specificity of NIPT as an add-on test is shown in Figure 15.

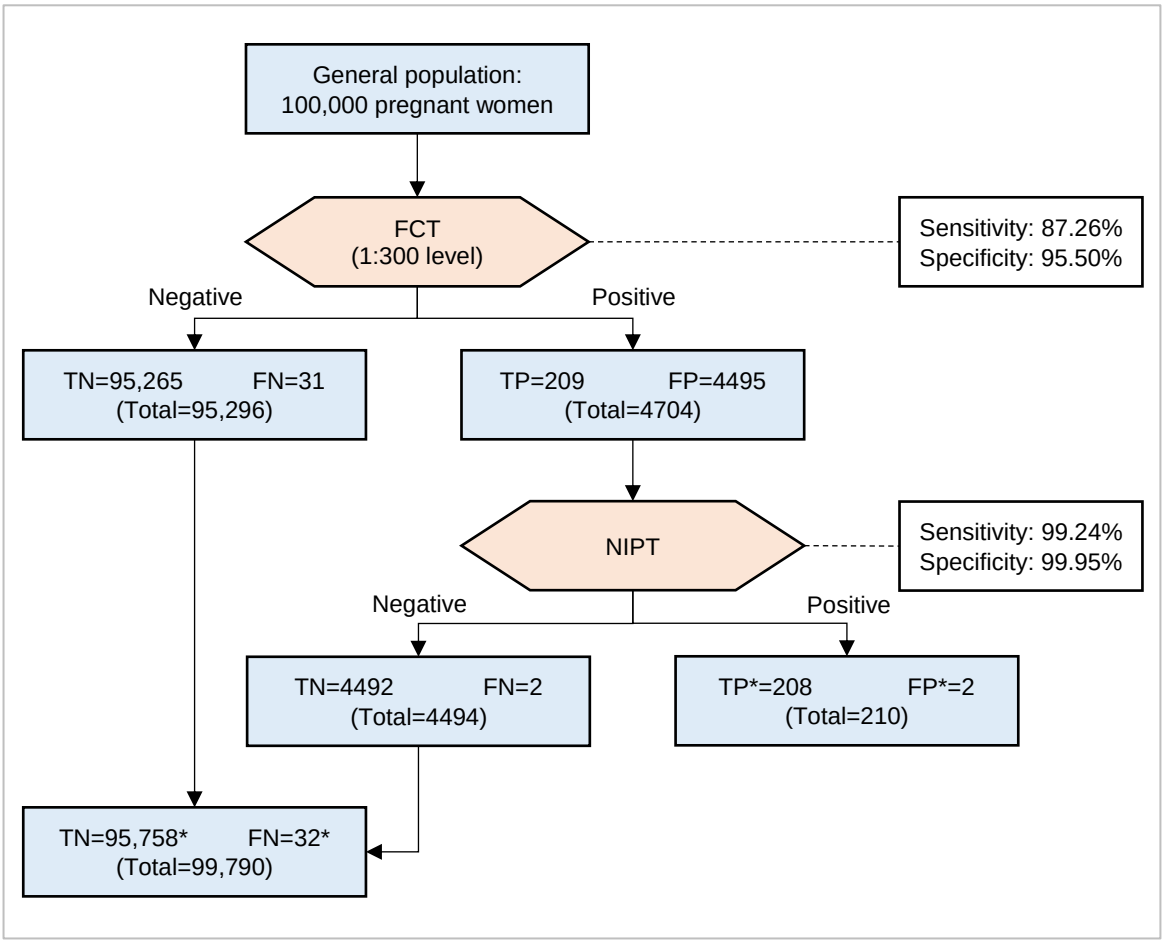


Figure 15: Diagnostic test results in a hypothetical scenario of NIPT as an add-on test

Abbreviations: FCT=first-trimester combined testing; FN=false negatives; FP=false positives; NIPT=noninvasive prenatal testing; TN=true negatives; TP=true positives.

* The results displayed appear incorrect because the calculation of the postive and negative values for the add-on strategy (FCT and NIPT) was done using all decimal places in order to avoid round-off errors).

5.4.3 NIPT as an add-on to FCT for the high- and intermediate-risk singleton pregnancy population in comparison with FCT

The available data are inappropriate for estimations.

5.4.4 NIPT as part of FCT for singleton pregnancy population

The available data are inappropriate for estimations.

[D1006] – Does the test reliably rule in or rule out chromosomal aneuploidies?

NIPT is not indicated as a diagnostic test as its sensitivity and specificity does not reach 100%. Currently, it is considered a prenatal screening method aimed at determining the risk of fetal aneuploidy.

[D1007] – How does the accuracy of NIPT differ in different settings?

Existing data do not allow stratified analysis to be performed.

5.5 Relative effectiveness

5.5.1 Mortality

[D0001] – What is the expected beneficial effect of prenatal screening with NIPT on neonatal mortality?

Two outcomes were considered relevant to assess the expected beneficial effect of NIPT on neonatal mortality (i.e., reduction of miscarriages or stillbirths of individuals affected by T13, T18 and T21 and miscarriages) related to invasive testing with NIPT compared with standard screening.

None of the studies included provided data on these outcomes.

5.5.2 Morbidity

[D0005] – How does prenatal screening with NIPT affect the frequency of newborns with aneuploidies?

Given the sensitive nature of prenatal screening, because of its association with abortion, the frequency of newborns with T13, T18 and T21 was not considered a relevant endpoint. The outcome considered relevant to this end was the influence of NIPT on the reduction in the number of children born with undiagnosed T13, T18 and T21, but none of the studies provided appropriate data to assess this outcome.

[D0006] – How does prenatal screening with NIPT affect the progression of pregnancy?

The studies included did not provide data regarding the reduction in invasive testing (amniocentesis or CVS). Estimations for T21 in singleton pregnancies were made on the basis of simulation models. Modelling was not considered for the other two trisomies (T18 and T13) given the low QoE.

5.5.3 Simulation modelling for NIPT as an add-on test to combined screening in the high- and intermediate-risk singleton population in comparison with FCT

On the basis of the simulation model shown in Figure 15, the combined test strategy would result in a total of 32 FN test results and a total of two FP test results. Assuming that all women who tested positive in NIPT would undergo invasive testing, 210 women would have to undergo invasive testing so as to confirm the T21 status of their unborn child after NIPT (0.002%) in comparison with 4704 after FCT (4.7%). This would result in a 95.5% reduction of invasive tests in comparison with combined testing.

5.5.4 Simulation modelling for NIPT without FCT (NIPT-only strategy)

The second scenario was developed to estimate test results for a strategy of using only NIPT. This model is based on the same prevalence estimate and the same test accuracy data for NIPT as the previous model for the combined strategy.

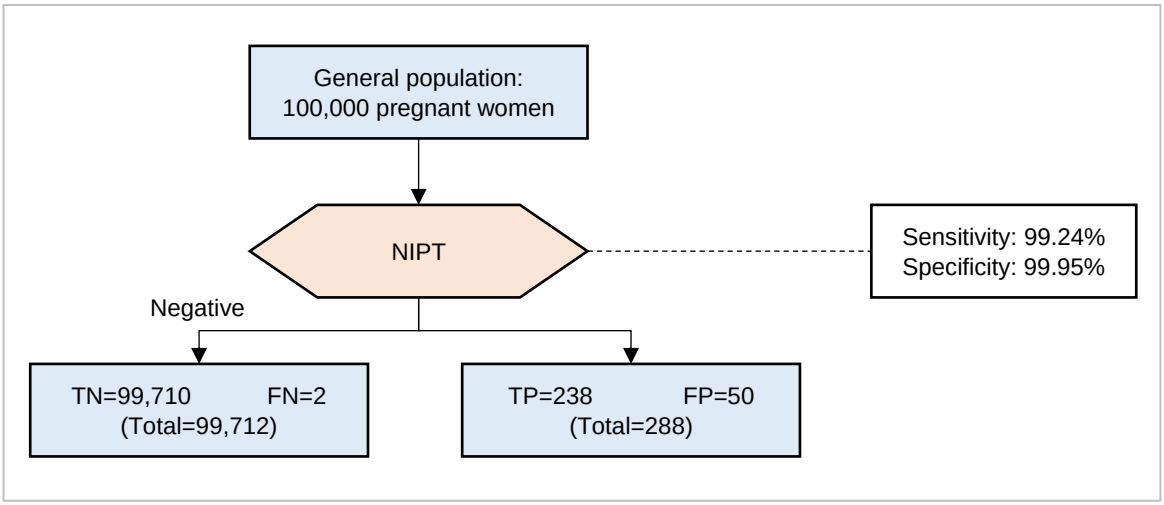


Figure 16: Diagnostic test results in a hypothetical scenario of NIPT only

Abbreviations: FN=false negatives; FP=false positives; NIPT=noninvasive prenatal testing; TN=true negatives; TP=true positives.

1. Comparison of simulation models

From the simulation models, it is not possible to directly estimate how the possible implementation of NIPT would change key outcomes, such as T21 detection and invasive testing. This is because there are no data available for Europe to estimate which results are being achieved with the currently available tests (i.e., only FCT without NIPT). Specifically, the proportion and the risk spectrum of women undergoing FCT and subsequent invasive testing are largely unknown.

In an extreme scenario of using only NIPT versus FCT with the 1 in 300 threshold, it could be expected that the detection rate would increase slightly (29 excess cases detected in 100,000 pregnant woman) and that NIPT would reduce invasive testing, because the specificity of FCT (95.5%) is much lower than that of NIPT. Although it is unrealistic to assume that all 4704 women would accept invasive testing, this scenario is able to show that the introduction of NIPT would most likely reduce the number of invasive tests. Even if only a small proportion of women tested positive by FCT would receive NIPT, the number of invasive tests in both scenarios using NIPT is much smaller.

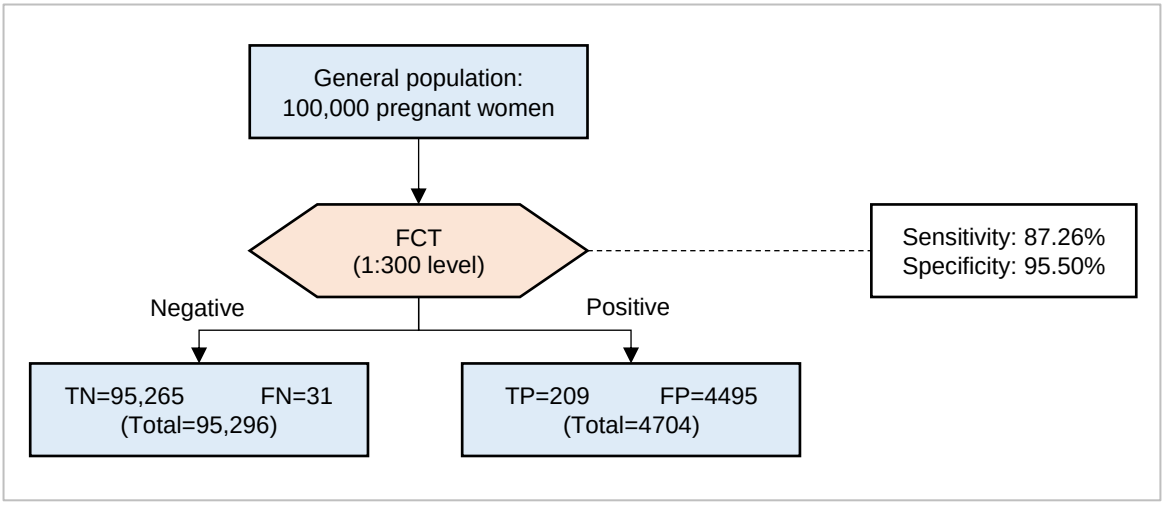


Figure 17: Diagnostic test results in a hypothetical scenario of first-trimester combined testing (first-trimester combined testing only)

Abbreviations: FCT=first-trimester combined test; FN=false negatives; FP=false positives; NIPT=noninvasive prenatal testing; TN=true negatives; TP=true positives.

In the comparison of the two strategies for using NIPT (i.e., NIPT only vs. FCT and NIPT), both models show advantages and disadvantages. Using only NIPT reduces the number of undetected T21 cases to two (Table 11) but requires a larger number of invasive tests (288). Combined testing fails to increase the T21 detection rate (with 32 undetected cases) but allows – as opposed to the NIPT-only strategy – the number of invasive tests offered to pregnant women to be reduced to a larger degree. In essence, the aim of detecting 30 extra cases of T21 (i.e., 32 vs. 2) has to be weighed against an increase in invasive testing offered (i.e., 288 vs. 210). These estimations can change if no-call results are included in the analysis. Given the lack of data on the rate of missed cases among no-call results, this modelling could not be done.

Table 11: Comparison of simulation modelling results (all based on a general population of 100,000 pregnant women)

	FCT only	NIPT only	Combined testing (FCT and NIPT)
Total no. of FCTs applied (% of all women)	100,000 (100)	—	100,000 (100)
Total no. of NIPTs applied (% of all women)	—	100,000 (100%)	4704 (4.7%)
No. of detected T21 cases (% of all T21 cases)	209 of 240 (87.3)	238 of 240 (99.2)	208 of 240 (86.6)
No. of undetected T21 cases (% of all T21 cases)	31 (12.7)	2 (0.8)	32 (13.4)

	FCT only	NIPT only	Combined testing (FCT and NIPT)
No. of women undergoing invasive testing to confirm T21 (% of all women)	4704[a] (4.7)	288 (0.3)	210 (0.2)

Abbreviations: FCT=first-trimester combined test; NIPT=noninvasive prenatal test; T21=trisomy 21.

[a] It is highly unlikely that all 4704 will women undergo invasive testing because the pretest probability of a fetus with T21 is very low (i.e., <5%).

[D0012] – What is the effect of NIPT on mothers' health-related quality of life?

Studies retrieved in the bibliographic search did not provide information for us to analyse how NIPT influenced health-related quality of life.

[D0030] – Does the knowledge of the NIPT results affect the population's non-health-related quality of life?

None of the studies included reported on the population's non-health-related quality of life.

[D0017] – Was the population satisfied with NIPT?

None of the studies included which met the eligibility criteria provided information on satisfaction.

[D0020] – Does the use of NIPT lead to improved detection of chromosomal aneuploidies?

Randomised controlled trials are required to be able to determine if NIPT screening pathways will improve the detection of chromosomal aneuploidies in comparison with conventional approaches [128, 129]. Currently, there is no information regarding comparison of the different screening approaches.

[D0021] – How does NIPT change physicians' management decisions?

The available evidence is inappropriate to assess how NIPT changes physicians' management decisions.

[D0022] – Does NIPT detect other potential health conditions that can impact the subsequent management decisions?

The information provided in the studies was insufficient to establish the impact of NIPT on other potential health conditions identified by NIPT.

[D0010] – How does the technology modify the need for hospitalisation?

The retrieved evidence did not provide information for us to analyse the effect of prenatal screening with NIPT in management decisions or modify the need for hospitalisation.

[D0029] – What are the overall benefits and harms of NIPT in terms of health outcomes?

The overall benefits and harms of NIPT cannot be assessed with the available evidence.

5.5.5 Discussion

Existing evidence comes from prospective cohort studies which are aimed at evaluating the performance of NIPT for the detection of the three targeted aneuploidies (T21, T18 and T13) in the general population and the high-risk population. Four of the eight studies included for the general population are paired designs which provide a comparison of the accuracy of NIPT versus that of combined testing. No randomised comparative trials comparing NIPT pathways and conventional pathways were identified.

Given that the performance of NIPT can differ substantially between aneuploidies, results have been provided for each of these aneuploidies and an aggregate analysis was performed to estimate the overall detection of these three chromosomal anomalies in the different clinical settings/ subgroups. With regard to the use of NIPT as a replacement for FCT for screening of the general population, the meta-analysis for T21 shows a pooled sensitivity of 99.4% (95% CI 96.1%–99.9%) and a pooled specificity of 99.9% (95% CI 99.8%–99.9%), suggesting that NIPT could be highly sensitive and specific for the detection of T21 as a primary test compared with combined serum screening. The pooled sensitivity for T18 and T13 was also high (97.4%, 95% CI 94.4%–98.8%; 98.8%, 95% CI 4.2%–100%), although the models were judged to be unreliable because of the sparse data and the low number of trisomy cases in comparison with nontrisomy cases. The few existing paired trials which compare NIPT with combined testing found significantly higher sensitivity with NIPT than with FCT. Furthermore, the PPV for T21 seems to be also markedly higher (≥80 in all studies), which could suggest that these tests might have the potential to reduce unnecessary invasive testing in comparison with combined approaches, which PPV below 24% although with a wide range (3.4%–24%). On the basis of the simulation model of using only NIPT versus FCT with the 1 in 300 threshold, it could be expected that the detection rate for T21 would increase slightly and that NIPT would lead to a substantial reduction in invasive testing, although these results should be analysed with care given the uncertainties that exist regarding the number of women who would undergo invasive testing subsequent to FCT or how NIPT adoption might change current screening adoption rates.

In general terms, drawing solid conclusions regarding the accuracy of NIPT as a primary test for general population screening of T21 is not possible because of the lack of evidence on primary outcomes and the low QoE for the specificity outcome as assessed by GRADE. Follow-up was incomplete in most studies, and two of the studies which contribute most to the results, given the sample size (Norton et al. [31] and Zhang et al. [43]), present losses as high as 16.4% and 23%, respectively. The verification of negative NIPT cases was done in most studies by review of medical records, general practitioner databases and telephone interviews, raising also important concerns regarding the completeness and reliability of this information. Information is also lacking in most of the paired comparative studies regarding the verification of standard screening positive cases.

Similarly to what was found in the general population, the meta-analysis for high-risk singleton pregnancy women showed a very high pooled sensitivity and specificity for T21 (sensitivity 99.2%, 95% CI 98.6%–99.5%; specificity 99.9% 95% CI 99.9%–99.9%), with NPVs and PPVs close to or equal to 100%. The pooled estimates were also similar for T18 (98.01%, 95% CI 89.38%–99.65%), although the PPV was significantly higher (66.7%–100%). Likewise, we observed great imprecision in the T13 estimates. The fact that most of the studies were insufficiently powered because of the small sample size could have greatly contributed to this imprecision and could also explain why many of the studies failed to show FN or FP cases for these aneuploidies. FN cases could have been missed because of the lack of verification of all negative cases with invasive testing. On the other hand, excluding cases of miscarriages, stillbirths and cases with no or uncertain results could have led to an overestimation of the PPV in both the general population and the high-risk population.

No valid statement can be made regarding the sensitivity for estimates for twin pregnancies because of the small size of the populations and the imprecision of the estimates.

The proportion of samples not returning a result because of a low fetal fraction or other quality issues ranged from 0.09% to 8.1% in the general population and from 0.02% to 6.3% in the high-risk population. The ACOG and the Society for Maternal-Fetal Medicine recommend that women whose cfDNA screening results are not reported, are undeterminate, or uninterpretable should receive counselling and be offered comprehensive ultrasound evaluation and diagnostic testing because of increased risk of aneuploidy. The few existing studies which provide information regarding no results (three studies in the general population, one study in the high-risk pregnant population and one study in in twin pregnancy population) support this increased risk of aneuploidy. In the study of Norton et al. [31], the prevalence of aneuploidies was 2.7% in the group with no results versus 0.4% in the overall general population cohort. Pergament et al. [44] found that no-call samples were six times more likely to be abnormal than samples with fetal fractions greater than 3.4%. Likewise, Persico et al. [49] showed a significantly higher risk of aneuploidies in high-risk patients with no result rates in comparison with patients with NIPT results (8.5% vs. 2.1%). When we reassessed NIPT performance including these no results as positive cases, we observed substantial reductions in the specificity for T21, T18 and T13 for several studies. On the other hand, reductions in sensitivity were found if these no results were classified as negative, highlighting the great uncertainties that remain regarding the best approach for these cases. Pergament et al. [44] propose that these patients cannot be automatically classified as either high or low risk, and that a modified risk score should be determined in light of other factors (gestational age, high-resolution ultrasound findings, modified risk if available and other indications). Some authors [2, 31] propose that obesity and gestational age could be associated with lower fetal fractions but further research is required to verify these findings and optimise the screening approach.

The generalisability of the studies included is another matter of concern. Even though most of the studies which assess NIPT in the general population include the average-risk population, the prevalence of aneuploidies is above that estimated in most European countries. In three of the studies [41, 42, 44] the prevalence of T21 was similar to that found in high-risk studies, suggesting that these populations might not be representative of the use of NIPT as a primary test in routine settings given the influence of the prevalence on the PPV and NPV. These studies also differ substantially regarding factors such as maternal age, gestational age and maternal weight. The influence of these factors on accuracy measures could not be analysed in the current analysis given the low number of general population studies and the wide variability of the spectrum of pregnant women included in the high-risk population.

We observed that the prevalence of aneuploidies also varied widely in the studies of high-risk singleton pregnancies, ranging from 0.4% to 50% for T21. Likewise, studies differed substantially regarding the criteria used for the classification of high-risk patients. We found that the vast majority of studies included patients screened during the first and second trimester and did not provide an FCT cut-off point for high risk, offering these tests for multiple indications, including elevated levels of serum markers, advanced maternal age (range 35–40 years), abnormal ultrasound findings, sonographic anomalies, previous child or fetus with a chromosomal anomaly and family history of DNA anomaly and anxiety. It would be important to have unbiased information regarding how these different indications would influence test accuracy and patient outcomes, particularly in the case of T18 and T13, since these trisomies are commonly characterised by malformations which can easily be detected by first-trimester ultrasound examination. Although envisioned, the existing data did not allow a stratified analysis to be performed.

The lack of information concerning key outcomes such as the reduction in invasive testing and miscarriages constitutes an important limitation for ascertaining the consequences of NIPT implementation. Assuming that all high-risk patients would opt for invasive testing, we could estimate that offering NIPT as a primary test would increase the detection rate slightly and would contribute to reduce invasive testing although this reduction would be increased with combined testing (FCT and NIPT). However, these results could be highly misleading because the percentage of women who will take up invasive screening for confirmation of positive results can vary widely depending on many different factors. The two routine care studies performed in the UK highlight very well this phenomenon. In the UK RAPID study, the number of women who opted for invasive testing confirmation because of a high-risk result increased with the availability of NIPT (54%–80%), and this led to an only modest decrease in the rate of invasive testing [130]. In the other NHS study, which assessed the implementation of contingent NIPT screening for intermediate-risk to high-risk women, invasive testing of high-risk patients was reduced by 43% in comparison with the previous 1-year rate but the overall rate was similar (2.6%) [71]. These figures could differ substantially if invasive testing were done in patients with no results from NIPT. For example, in the study by Norton et al. [31] we could hypothesise that invasive testing for T21 would still be reduced by 2.2% but could increase for T18 (2.75%) and T13 (4%) if no test results were considered as high risk. Large population studies assessing these and other patient-relevant outcomes are necessary to determine the real clinical implications derived from NIPT implementation.

It is important to highlight that whilst paired study designs have advantages over randomised controlled trials to establish diagnostic accuracy, given that they may be more feasible and would require fewer patients, they are inappropriate for assessing trade-offs between different approaches. To determine if NIPT would serve as a replacement, triage or add-on requires more information than the accuracy of the test. It needs assessment of the performance of the different test strategies, taking into account detection of all anomalies, abortions, miscarriages and other patient-related outcomes. To date, important uncertainties remain regarding the best screening pathway. As illustrated by the modelling results, however, it is important for decision makers to find the right balance between the different aims of using NIPT: the aim of detecting all T21 cases might be achieved only with a slightly higher rate of invasive testing; the aim of reducing invasive testing, on the other hand, comes with the disadvantage of not detecting all cases of T21. Nevertheless, the model alone is an insufficient basis for any decision, as it is based on several assumptions and simplifications.

6 SAFETY

6.1 Research questions

Element ID	Research question
C0008	How safe is NIPT in relation to the comparators?
C0006	What are the consequences of false positives, false negatives and incidental findings generated by use of the technology from the point of view of patient safety?
B0010	What kind of data/records and/or registry is needed to monitor the use of NIPT and the comparators?

6.2 Included studies

Study characteristics and QoE

For the assessment of safety, all 41 primary studies were considered. The main characteristics of individual studies as well as the risk of bias and the QoE of the studies retrieved can be found in the clinical effectiveness domain.

Detailed information on the studies retrieved can be found in the evidence tables in Appendix 1 (Tables A2–A5).

6.3 Safety outcomes

[C0008] – How safe is NIPT in relation to the comparators?

The only existing comparative information relates to safety measures (FP and FN rates) and no-call results (test failures, low-quality samples, undeterminate results). These outcomes were analysed separately for each of the three types of aneuploidy, providing individual results for general, high and high and intermediate risk of aneuploidies (see the definition earlier) given the different disease prevalence. Twin pregnancies were also assessed individually given that NIPT can have a different performance in these patients.

6.3.1 NIPT as a primary screening test for the general singleton pregnancy population

Nine studies reported on safety outcomes for NIPT as a primary testing method: seven provided data on T21 (n=135,957 women), six on T21, T18 and T13 individually (n=135,378) and one on the three trisomies combined (n=106,898) (Table 12). Overall, the studies showed 78 FPs for T21 (0.06%), 62 FPs for T18 (0.04%) and 52 FPs for T13 (0.05%). The total number of FNs found was six for T21 (0.004%), six for T18 (0.004%) and three for T13 (0.02%).

Four of the paired accuracy studies included provided a comparative analysis of NIPT versus combined screening [30, 31, 41, 42] for T21 (Table 11). All showed higher FP rates for combined testing in comparison with NIPT. The FP rate ranged from 3.6% to 6.7% for combined testing and was below 0.3% for NIPT in all retrieved studies. Three of these studies [31, 41, 42] showed a zero or near zero FP rate. The FN rate obtained with combined testing was higher than that for NIPT in

two studies [31, 41]: 14.3% and 21% versus 0%. In the other two studies [30, 42], the FN rate was zero with both testing methods.

The proportion of no-call results in the comparative studies ranged from 0.5% to 3%. Two of these studies provided the incidence of aneuploidies in these samples [31, 42], and we estimated the FP and FN rates which would result from considering these pregnancies as either high risk or low risk. Under the first assumption, the FP rate would increase to 1.79% and 3% in the corresponding studies. Under the second assumption, the FN rate would increase to 5.88% and 7.3%, respectively. In the study of Quezada et al. [42] the estimated FN rate for NIPT would exceed that of combined screening (5.88% vs. 0%).

The four comparative DTA studies reported higher FP rates with standard screening (0.3%–5.8%) than with NIPT for T18 (0.006%–0.2%). The FN rate was zero in two studies for both approaches [30, 41]. In the study of Norton et al. [31] the FN rate was higher for standard screening than for NIPT (20% vs. 10%). In contrast, Quezada et al. [42] reported a higher FN rate with NIPT (10% vs. 0%).

When we reanalysed the results of Norton et al. [31] considering the no NIPT results as high-risk cases, the FP rate increased to 2.19% versus the 0.3% obtained with combined screening. The FN rate rose to 18.2% when these cases were considered as low risk, the FN rate being 20% with combined testing. Bianchi et al. [30] did not observe any T18 among the no-result cases.

The FP rate obtained for T13 was also lower with NIPT than with combined testing in the three paired comparative studies [30, 31, 42], although in two studies it was less than 1% with both approaches [30, 31]. In the study of Quezada et al. [42] the FP rate was 5.9% for combined testing versus 0.07% for NIPT. The FN rate achieved with NIPT was lower in the study of Norton et al. (0% vs. 50%) and higher in the study of Quezada et al. [42] (60% vs. 0%). The FN rate was 0% for both testing options in the study of Bianchi et al. [30].

When we recalculated these values for the study of Norton et al. [31] including no-result cases, we observed an FP rate of 4%. The FN rate was 50% if we considered these results as negative. No T13 cases were found among no-result samples.

Mainly, the four remaining noncomparative studies showed rates of NIPT FP, FN and test failures similar to those reported by comparative studies (Table 11). Only Sarno et al. [39] found a higher FN rate for T18 and T13 (10.8% and 46.7%, respectively), and Pergament et al. [44] reported an NIPT failure rate of around 8%.

Table 12: NIPT safety results of individual studies performed in the general singleton pregnancy population

Authors, year, countries	Safety, no. or rate			Final test failure rate	Failure rate at first attempt
	Trisomy 21	Trisomy 18	Trisomy 13		
Sarno et al. [39], 2016, UK	FN rate 1.26%	FN rate 10.8%	FN rate 46.7%	1.5% (n=168)	2.9% (n=316)
Comas et al. [40], 2015, Spain	FP=1 (0.3%) FN=0	—	—	1.2% (n=4)	2.8% (n=9)

Authors, year, countries	Safety, no. or rate			Final test failure rate	Failure rate at first attempt
	Trisomy 21	Trisomy 18	Trisomy 13		
Norton et al. [31], 2015, USA, Canada, Sweden, Belgium, Netherlands and Italy	cfDNA FP=9 (0.06%) FN=0 Standard screening FP=854 (5.4%) FN=8 (21%)	cfDNA FP=1 (0.01%) FN=1 (10%) Standard screening FP=49 (0.3%) FN=2 (20%)	cfDNA FP=2 (0.018%) FN=0 Standard screening FP=28 (0.25%) FN=1 (50%)	3% (n=488) (absence of cfDNA result)	3% (n=488)
Pérez-Pedregosa et al. [41], 2015, Spain	cfDNA FP=0 FN=0 Standard screening FP=38 (6.7%) FN=2 (14.3%)	cfDNA FP=0 FN=0 Standard screening FP=5 (0.86%) FN=0	—	0.5% (n=3)	0.5% (n=3)
Quezada et al. [42], 2015, UK	cfDNA FP=1 (0.04%) FN=0 Standard screening FP=139 (4.96%) FN=0	cfDNA FP=5 (0.18%) FN=1 (10%) Standard screening FP=163 (5.8%) FN=0	cfDNA FP=2 (0.07%) FN=3 (60%) Standard screening FP=168 (5.9%) FN=0	1.9% (n=54) (low FF or assay failure)	4.25 (n=122)
Zhang et al. [43], 2015, China	FP=61 (0.05%) FN=6 (0.83%)	FP=51 (0.05%) FN=3 (1.77%)	FP=45 (0.04%) FN=0	0.098% (n=145) (inappropriate samples: 0.14% or n=211; uncertain results: 0.2% or n=326)	0.098% (n=145)
Bianchi et al. [30], 2014, USA	cfDNA FP=6 (0.3%) FN=0 Standard screening FP=69 (3.6%) FN=0	cfDNA FP=3 (0.2%) FN=0 Standard screening FP=11 (0.6%) FN=0	cfDNA FP=3 (0.16%) FN=0 Standard screening FP=6 (0.67%) FN=0	0.9% (n=18)	0.9% (n=18)
Pergament et al. [44], 2014, USA **Prospective DTA study** **Data collection period not reported**	FP=0 FN=0	FP=1 (0.1%) FN=1 (3.84%)	FP=0 FN=0	8.1% (n=85/1051)	8.1% (n=85)
Song et al. [45], 2013, China **Comparative prospective DTA study** **Apr 2011–Dec 2011**	FP=0 FN=0	FP=1 (0.06%) FN=0	FP=0 FN=0	3.8% (n=73)	3.8% (n=73)

Abbreviations: cfDNA=cell-free DNA; DTA=diagnostic test accuracy; FF=fetal fraction; FN=false negative(s); FP=false positive(s).

* Number of samples with noninvasive prenatal testing and reference standard results.

6.3.2 NIPT as an add-on to FCT for the high-risk singleton pregnancy population

There is lack of direct data regarding the safety of the prenatal strategy which includes NIPT as an add-on to the combined test. However, 26 DTA studies which assessed the performance of NIPT in

singleton women who had been classified as having high risk of aneuploidy were included for indirect inferences and modelling. The individual characteristics and results of these individual studies are displayed in Table 13.

Only six of the 24 studies included which provided T21 accuracy data showed FP cases when verified against invasive testing and/or pregnancy follow-up [48, 49, 51, 61, 64, 69, 70]. The FP rate was less than 0.5% in all these studies. The overall mean rate of FPs in the whole population was 0.03% (10/28,591). The proportion of reported no-call/test failures/undeterminate results ranged from 0.002% to 6.3%. Only one of the studies provided aneuploidy findings for these no-result samples. The recalculated FP considering these no-call samples as positive would be 4% [49]. FN cases were reported in only five studies [48, 49, 62, 63, 67]. The FN rate in these studies ranged from 1.92% to 5.6%. The mean rate was 0.017% (2/28,591). When recalculated in the study of Persico et al. [49], with no results classified as negative, it would increase to 5.4%.

A total of 21 studies analysed NIPT in T18 (n=27,999 women). Six reported FP cases, showing FP rates between 0.05% and 0.21% [48, 51, 58, 62, 64, 69]. Overall, the mean rate of FP cases in the studied population was 0.028% (8/2799). The FP rate including no-call data in the study of Persico et al. [49] was 3.28%. Five studies reported FN cases, with FN rates from 0.33% to 37.5% [51, 61, 63, 68, 69] (overall mean rate 0.05%). The recalculated FN rate in the study of Persico et al. [49] was 13.3%.

Seventeen studies reported on NIPT T13 detection (n=23,760 participants). Of these, only five (29%) reported on FP cases. The FP rate observed in the studies ranged between 0.02% and 0.24% [48, 51, 64, 65] (overall mean rate 0.02%). When recalculated, the FP rate in the study of Persico et al. [49] rose to 3.55%. Three studies [60, 61, 68] identified FN cases, and the FN rate ranged in these studies from 12.5% to 100%. The mean value was 0.021% (5/23,760)

Table 13: Noninvasive prenatal testing safety results of individual studies performed in the high-risk singleton pregnancy population

Authors, year, countries	Safety, no. or rate			Final test failure rate	Failure rate at first attempt
	Trisomy 21	Trisomy 18	Trisomy 13		
Kim et al. [46], 2016, Korea	FP=0 FN=0	—	—	NS Low FF: 0%	NS
Ma et al. [47], 2016, China	FP=0 FN=0	FP=0 FN=0	FP=0 FN=0	0.06%	0.06%
Oepkes et al. [48], 2016, Netherlands	FP=2 (0.15%) FN=1 (3.33%)	FP=1 (0.07%) FN=0	FP=2 (0.14%) FN=0	0.3% (low FF 0.2%)	0.3% (n=3)
Persico et al. [49], 2016, Italy	FP=0 FN=1 (2.8%)	FP=0 FN=0	FP=0 FN=0	3.8% (Low FF: 3%)	3.8%
Zhang et al. [50], 2016, China	FP=0 FN=0	FP=0 FN=0	—	NS	NS
Benachi et al. [51], 2015, France	FP=1 (0.11%) FN=0	FP=1 (0.11%) FN=3 (0.33%)	FP=1 FN=0	0.7% (n=7)	0.7% (n=7)
Hernandez-Gomez et al. [52], 2015, Mexico	FP=0 FN=0	—	—	2.8% (n=1)	2.8% (n=1)
Ke et al. [53], 2015, China	FP=0 FN=0	FP=0 FN=0	FP=0 FN=0	0%	0%
Lee et al. [54], 2015, Korea	FP=0 FN=0	FP=0 FN=0	FP=0 FN=0	1.07% (n=1)	1.07% (n=1)

Authors, year, countries	Safety, no. or rate			Final test failure rate	Failure rate at first attempt
	Trisomy 21	Trisomy 18	Trisomy 13		
Sago et al. [55], 2015, Japan	—	—	—	0.05%	0.05%
Sánchez-Usabiaga et al. [56], 2015, Mexico	FP=0 FN=0	FP=0 FN=0	FP=0 FN=0	1% (n=4) Low FF: 0.5% (n=2)	1% (n=4)
Song et al. [57], 2015, China	FP=0 FN=0	FP=0 FN=0	FP=0 FN=0	0.5% (n=1)	0.5% (n=1)
Wang et al. [58], 2015, China	FP=0 FN=0	FP=1 (0.11%) FN=0	—	NS	NS
Jeon et al. [59], 2014, China	FP=0 FN=0	FP=0 FN=0	—	0%	0%
Korostelev et al. [60], 2014, Russia	FP=0 FN=0	FP=0 FN=0	FP=0 FN=1 (25%)	0%	0%
Porreco et al. [61], 2014, USA	FP=3 (0.09%) FN=0	FP=0 FN=3 (7.7%)	FP=0 FN=2 (12.5%)	1.3% (n=54)	1.3% (n=54)
Stumm et al. [62], 2014, Germany and Switzerland	FP=0 FN=2 (4.8%)	FP=1 (0.21%) FN=0	FP=0 FN=0	6.3% (n=32)	6.3% (n=32)
Willems et al. [63], 2014, Belgium	FP=0 FN=1 (1.92%)	FP=0 FN=1 (20%)	FP=0 FN=0	1.06% (32 samples)	1.8% (n=55)
Zhou et al [64], 2014, China	FP=2 (0.05% (0.02%–0.10%)) FN=0	FP=2 (0.05% (0.02%–0.10%)) FN=0	FP=2 (0.05% (0.02%–0.10%)) FN=0	0.05% (n=4) (absence of cfDNA result)	0.05% (n=4)
Liang et al. [65], 2013, China	FP=0 FN=0	FP=0 FN=0	FP=1 (0.24%) FN=0	2.76% (n=12) (failed sequencing quality control)	2.76% (n=12)
Nicolaides et al. [66], 2013, UK	FP=0 FN=NS	FP=0 FN=0	FP=0 FN=0	5.4% (n=13)	5.4% (n=13)
Verweij et al. [67], 2013, Norway, Sweden and Netherlands	FP=0 FN=1 (5.6%)	—	—	3.07% (n=16)	9.8% (n=51)
Lau et al. [68], 2012, Japan	FP=0 FN=0	FP=0 FN=6 (37.5%)	FP=0 FN=2 (100%)	0%	0%
Norton et al. [69], 2012, USA, Netherlands and Sweden	FP=1 (0.03% (0.002%–0.20%)) FN=0	FP=2 (0.07% (0.02%–0.25%)) FN=1 (2.6%)	—	4.8% (n=148)	4.8% (n=148)
Ehrich et al. [70], 2011, USA	FP=1 (0.24%) FN=0	—	—	4% (n=18) (processing error or quality control)	4.3% (n=20)

Abbreviations: cfDNA=cell-free DNA; FF=fetal fraction; FN=false negatives; FP=false positives; NS=not specified.

6.3.3 NIPT as an add-on to FCT for the high- and intermediate-risk singleton pregnancy population

Only one study retrieved assessed NIPT safety in the high- and intermediate-risk population. The FP rate for T21, T18 and T13 excluding no-result cases was 0.027%, 0.11% and 0.11%, respectively. The corresponding FN rates were 2.3%, 0% and 50% [71] (Table 14).

Table 14: Characteristics and noninvasive prenatal testing safety results of individual studies performed in the high- and intermediate-risk pregnant population

Author, year, country	Safety, no. or rate			Final test failure rate	Failure rate at first attempt
	Trisomy 21	Trisomy 18	Trisomy 13		
Gil et al. [71], 2016, UK	FP=1 (0.03%) FN=1 (2.3%)	FP=4 (0.11%) FN=3 (12.5%)	FP=4 (0.11%) FN=2 (50%)	1.75% (n=65)	2.8% (n=99)

Abbreviations: FN=false negatives; FP=false positives.

6.3.4 NIPT as an add-on to FCT for the high-risk twin pregnancy population

Taking to account the lack of comparative studies for assessing NIPT safety, assumptions on safety outcomes are based on the results reported by noncomparative DTA studies performed in the twin pregnancy population (Table 15). Two of the six studies on twin pregnancies reported FP rates for T21 ranging from 0.20% to 1.22% [72, 74]. Only one study observed FNs (FN rate 7.7%) [74]. This last study reported an FP rate for 3.58% for T18. The rate of FN for T18 ranged from 25% to 50% [39, 75]. Only one study assessed T13 in twin pregnancies, and found an FN rate of 100% (FP rate was not specified) [39].

Sarno et al. [39] reported a test failure rate of 4.8%. The failure rate in the study performed by Tan et al. [73] was 0.9%. Two of the studies did not indicate the number of no-call results [75, 76].

Table 15: Characteristics and noninvasive prenatal testing safety results of individual studies performed in the twin pregnancy population

Authors, year, countries	Safety, no. or rate			Final test failure rare	Failure rate at first attempt
	Trisomy 21	Trisomy 18	Trisomy 13		
Fosler et al. [72], 2017, USA Only cohort B included	FP=1 (0.20%) FN=0	—	—	0%	0%
Sarno et al. [39], 2016, UK Reported only twin pregnancy results	FP=NS FN=0	FP=NS FN=25%	FP=NS FN=100%	4.8% (n=21)	9.4% (n=41)
Tan et al. [73], 2016, China	FP=0 (0-.70) FN=0	—	—	0.9% (n=5)	0.9% (n=5)
Bevilacqua et al. [74], 2015, Belgium, UK and Spain	FP=4 (1.22%) FN=1 (7.7%)	FP=12 (3.58%) FN=0	—	3.1% (n=16) (low FF)	3.1% (n=16)
Huang et al. [75], 2014, China	FP=0 FN=0	FP=0 FN=1 (50%)	—	Not reported	Not reported
Lau et al. [76], 2013, China	cfDNA FP=0 FN=0 Comparator FP=2 (18.2%) FN=0	—	—	Not reported	Not reported

Abbreviations: cfDNA=cell-free DNA; FF=fetal fraction; FN=false negatives; FP=false positives; NS=not specified.

6.4 Additional safety outcomes

No evidence was found to answer the question regarding the comparison of NIPT and combined testing in relation to the number of children born with other major unconfirmed chromosomal conditions/anomalies (not targeted by prenatal aneuploidy screening) or the increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance (not targeted by prenatal aneuploidy screening).

The only comparative information available relating to missed chromosomal conditions comes from the findings of the study of Norton et al. [31] on no-call samples and the study of Persico et al. [49], which reports on the results of karyotyping for cases which were not targeted by NIPT. The study of Norton et al. [31] established that apart from T21, T18 and T13, NIPT missed four cases of triploidy, one of case trisomy 16 cases of mosaicism, one case of 11p deletion and one case of a structurally abnormal chromosome which may have been detected by standard combined screening. Persico et al. [49] reported on eight cases of chromosomal defects identified by fetal karyotype which are not targeted by NIPT (one case of 47XX4, one case of 47XX22, four cases of 46XY, one case of 46XX and one case of Mos45 X0/46XY). Pergament et al. [44] found three T21 cases, four T18 cases, two T13 cases and one monosomy X case in samples with no NIPT results, although this study, where NIPT was performed as a primary test, did not provide results on combined testing.

Several of the high-risk studies provided data on sex chromosome anomalies (monosomy X, trisomy X, Turner syndrome (45,X) or Klinefelter syndrome (47,XXY)). The FN rates for these ranged from 0% to 100% in the studies included.

[C0006] – What are the consequences of false positives, false negatives and incidental findings generated by use of the technology from the point of view of patient safety?

Since the studies were not outcome based, there is no information regarding the consequence of FPs, FNs or incidental findings generated by the technology. Incidental findings were not reported in any of the studies.

6.5 Safety risk management

[B0010] – What kind of data/records and/or registry is needed to monitor the use of NIPT and the comparators?

Important uncertainties exist regarding the performance of the different NIPT screening pathways in real-life scenarios. Data records and/or registries could provide information regarding the real uptake of NIPT screening when implemented in routine practice, as well as information on the rate of invasive testing, detection of all major anomalies, abortions, miscarriages, and other patient-relevant outcomes. This information is critical to determine the real implications of implementing NIPT as a public health screening programme.

6.6 Discussion

No evidence exists regarding the adverse consequences derived from NIPT screening in terms of patient outcomes. The only information available, derived from 41 prospective diagnostic accuracy studies, relates to the reporting of FPs and FNs and no-recall cases.

In this respect, the few comparative studies on the general population which were found are consistent with the finding that NIPT would lead to a reduction in the FP rate for all three trisomies in comparison with combined testing if used as a primary test. The studies also support that the FN rate could also be lower for T21, with only minor differences being observed for T18 and inconsistent results being observed for T13. However, as previously mentioned, all studies included are limited by important methodological shortcomings. General population studies frequently have important follow-up losses and lack in most cases verification of negative cases, miscarriages, abortions and no-recall cases. When we reanalysed the data including no-recall cases we observed that depending on how we classified cases, the FP rate and FN rate could be higher than those observed with combined testing. No-call results constitute an important challenge and should be assessed further.

The studies included were not explicitly designed for the comparison of FP and FN rates between FCT and NIPT screening, contingent on the high or high and intermediate results of FCT. On the basis of the 27 high-risk studies and the one study on intermediate and high risk, which assessed NIPT performance, it is estimated that NIPT would contribute to reduce the FP rate for all three types of aneuploidy (<1%), highlighting the potential contribution of these tests to reduce anxiety and invasive testing complications. However, given the sample size, the number of trisomic pregnancies was too small for valid conclusions to be drawn. Although it has not been not investigated in current studies, positive NIPT results could result from different biological and nonbiological causes, including confined maternal mosaicisms, maternal aneuploidy, maternal copy number variants, maternal malignancy or a co-twin demise. Therefore confirmation of positive cases with diagnostic testing is always required.

The eligible studies also seem to support that NIPT would lead to few missing cases, but these results should also be analysed with caution, particularly with regard to T18 and T13, since the FN rate for these trisomies was very high in some of the studies included [61, 63, 71, 76]. In the study of Gil et al. [71], NIPT would have missed 12.5% of T18 cases (3/24) and 50% of T13 cases (2/4) but these cases were all detected with the contingent screening approach, highlighting the possible role of invasive testing for very high risk patients with ultrasound anomalies. As previously pointed out, the limited sample size of the studies, considering the number of pregnancies available in clinical practice, could have greatly contributed to the heterogeneity observed. Only one of the high-risk studies reported on individuals with no results, and this is an important drawback given the possible implications of these no-call results in real-life practice.

Another important limitation of the studies is the lack of information regarding the detection of neural tube defects and other major chromosomal anomalies in relation to combined screening [91, 131]. Whilst it is acknowledged that NIPT will miss a large number of major anomalies that are incidentally diagnosed by invasive testing [132], the extent of these losses is relatively unknown. According to two retrospective population-based analyses conducted in California and Denmark, up to 17%–23% of clinically significant anomalies could be missed by NIPT [91, 133]. A retrospective cohort study established that NIPT as the sole method might miss 95% of the fetal findings detected with ultrasound examination [134]. The role of NT and biochemical markers needs to be evaluated in appropriately designed studies so as to establish the best option for T21, T18 and T13 screening. Uncertainty also exists regarding the implications of incidental findings on sex chromosome aneuploidies and other conditions which are not being targeted [135, 136].

7 POTENTIAL ETHICAL, ORGANISATIONAL, PATIENT AND SOCIAL, AND LEGAL ASPECTS

7.1 Research questions

Element ID	Research question
F0010	What are the known and estimated benefits and harms for pregnant women when NIPT is implemented or not implemented?
F0011	What are the benefits and harms of NIPT for relatives, other patients, organisations, commercial entities, societies, etc.?
F003	Are there any other hidden or unintended consequences of NIPT and its applications for pregnant women, relatives, other patients, organisations, commercial entities, society, etc.?
F004	Does the implementation or use of NIPT affect the pregnant woman's capability and possibility to exercise autonomy?
F006	Is there a need for any specific interventions or supportive actions concerning information so as to respect the pregnant woman's autonomy when the technology is used?
F0101	Does NIPT invade the sphere of the pregnant woman/user?
F0012	How does implementation or withdrawal of the technology affect the distribution of resources?
F0017	What are the ethical consequences of the choice of endpoints, cut-off values and comparators/controls in the assessment?
G0001	How does NIPT affect the current work processes?
G0100	What kind of pregnant woman/participation flow is associated with the new technology?
G0002	What kind of involvement has to be mobilised for pregnant women/participants and important others and/or carers?
G0003	What kind of process ensures proper education and training of staff?
G0004	What kind of cooperation and communication of activities has to mobilised?
G0012	In what way is the quality assurance and monitoring system of NIPT organised?
G0005	How do decentralisation or centralisation requirements influence the implementation of NIPT?
G0006	What are the costs of processes related to acquisition and setting up of NIPT?
G0023	How does NIPT modify the need for other technologies and use of other resources?
G0007	What is the likely budget impact of implementing the technologies being compared?
G0008	What management problems and opportunities are attached to NIPT?
G0009	Who decides which pregnant women are eligible for NIPT and on what basis?
G0010	How is NIPT accepted?
H0100	What expectations and wishes do pregnant women have with regard to NIPT and what do they expect to gain from the technology?
H0006	How do pregnant women perceive NIPT?
H0002	What is the burden on carers?

H0012	Are there factors that could prevent a group or person from gaining access to NIPT?
H0202	How are screening options explained to pregnant women?
H0203	What specific issues may need to be communicated to pregnant women to increase acceptance of NIPT?

7.2 Included studies

The systematic review retrieved 34 studies which assessed ethical, legal, organisational or social issues related to NIPT implementation in a routine prenatal care (Table 16). The studies were mainly performed in the USA and the UK (10 in each country), and the remaining studies were from Belgium, Spain, Sweden, the Netherlands, Australia, and Canada; only two studies were multicentre studies. Most of documents were questionnaire-based or interview-based surveys focusing on analysing attitudes or preferences towards to NIPT of pregnant women/parents or health professionals (n=13) or studies aimed at assessing the costs and benefits or cost-effectiveness of NIPT used in different screening strategies (n=10). The questionnaire-based survey were administered in writing, by telephone, or online mainly. Six full cost-effectiveness analysis determined the cost of NIPT as a contingent or primary strategy compared with the conventional screening pathway in a hypothetical cohort of pregnant women or a sample of those recruited from a routine prenatal care setting. Moreover, five reviews (two of them systematic), three HTA reports and three position statements were taken into account to analyse ethical and/or organisational issues related to NIPT implementation.

Table 16: Main characteristics of studies included in the ethical analysis, organisational aspects and patients and social aspects domains

Authors, year, countries	Study characteristics	Objective	Study participant characteristics	Outcomes assessed	Assessment element
Oxenford et al. [137], 2017, UK	Research study Mixed method approach (quantitative questionnaires at three points and posttraining qualitative interviews)	To develop and evaluate a training package for health professionals to support the introduction of NIPT into clinical practice	Midwives and other health professionals n=381 (follow-up in 151 and interviews in 35 attendees)	Self-perceived confidence and knowledge	Organisational
Bayón Yusta et al. [138], 2016, Spain	Full HTA report (clinical effectiveness and economic evaluation)	See Appendix 1, Table A7	See Appendix 1, Table A7	See Appendix 1, Table A7	Organisational
Chitty et al. [139], 2016, UK	Cost–benefit analysis	To investigate the benefits and costs of implementation of NIPT	All pregnant women with a T21 risk of at least 1:1000 n=3175	NIPT performance results (uptake rate, number of T21 cases detected, invasive test performed and miscarriages avoided) Cost of NIPT	Ethical
Gregg et al. [140], 2016, USA	Position statement of the American College of Medical Genetics and Genomics	See Appendix 1, Table A1	See Appendix 1, Table A1	See Appendix 1, Table A1	Ethical
Lewis et al. [141], 2016, UK	Cross-sectional survey and semi-structured interviews (face-to-face counselling and written information)	To assess women's experience of being offered NIPT using validated measures of decisional conflict, decisional regret and anxiety	Pregnant women with a T21 risk>1:1000 based on FCT n=582	Preferences and anxiety of pregnant women	Ethical Patient and social
Lewis et al. [142], 2016, UK	Telephone interview-based survey December 2013 to September 2014	To explore women's attitudes towards NIPT and determine factors influencing their decisions around uptake of NIPT	Pregnant women with a standard T21 screening risk>1:1000 were offered NIPT as a contingent test method n=45 (87% accepted NIPT)	Attitudes and preferences of pregnant women	Ethical
Lewis et al. [130], 2016, UK	Validation study (MMIC) Questionnaires and semi-structured interviews at two time points	To validate a modified MMIC instrument for NIPT and measure informed choice among women offered NIPT following T21 screening	All singleton pregnant women ages>16 years who accepted T21 screening as part of routine care n=585 questionnaires and n=45 interviews	Attitudes and preferences of pregnant women	Ethical
Maxwell et al. [143], 2016, Australia	Cost–benefit analysis	To establish the benefits and cost of different FCT cut-off points for NIPT as a contingent screening method	All pregnant women attending for FCT n=115,648	Screen positive rate Detection rate NIPT fee at different FCT cut-off points	Ethical

Authors, year, countries	Study characteristics	Objective	Study participant characteristics	Outcomes assessed	Assessment element
Michie et al. [144], 2016, USA	Qualitative review	To assess the information provided to patients in written patient education and consent documents	n=32 Informed consent documents designed by laboratories and clinics	Information about test performance, screened conditions, test counselling and psychosocial issues and other test considerations	Organisational
Sahlin et al. [145], 2016, Sweden	Questionnaire-based survey January to June 2015	To evaluate pregnant women's awareness, attitudes, preferences for risk information and decision making regarding NIPT	Pregnant women in any week of gestation recruited in waiting rooms of nine different maternity clinics n=1003	Attitudes and preferences of pregnant women	Ethical Patient and social
Van Schendel et al. [146], 2016, Netherlands	Questionnaire-based survey (administered by writing or online after genetic counselling)	To evaluate preferences and decision making among high-risk pregnant women	Pregnant women with an increased risk for fetal trisomies 21, 18 and 13 (cut-off risk≥1:200) n=1091	Preferences of pregnant women	Ethical Patient and social
Allyse et al. [88], 2015, USA	Narrative review	Review international implementation and challenges of NIPT in high- and low-income countries	NA	Clinical, ethical, legal and regulatory issues	Ethical Organisational
Benn et al. [33], 2015, USA, Spain, Hong Kong, Netherlands, Canada, Israel, UK	Position statement of the International Society for Prenatal Diagnosis	See Appendix 1, Table A1	See Appendix 1, Table A1	See Appendix 1, Table A1	Ethical Organisational
Beulen et al. [147], 2015, Netherlands	Discrete choice experiment Web questionnaire-based survey (including minimal gestational age, time to wait for test results, level of information, detection rate, FP rate, miscarriage risk and cost of prenatal test)	To evaluate pregnant women's and healthcare professionals' preferences regarding specific prenatal screening and diagnostic test characteristics	Pregnant women (n=596) Healthcare professionals (n=297)	Attitudes and preferences of pregnant women and healthcare professionals	Ethical Patient and social
Evans et al. [148], 2015, USA	Cost-effectiveness analysis Decision tree analysis	To determine the cost-effectiveness of NIPT as a primary strategy, as a contingent strategy and as a hybrid strategy (all women aged≥35 years and women <35 years who were high-risk on FCT)	NA	Cost per patient Marginal cost per additional case	Ethical
Farrell et al. [149], 2015, USA and Australia	Questionnaire-based survey (administered by investigator) September to December 2012	To assess Latina pregnant women's understanding of NIPT and identify what factors influence uptake/refusal of NIPT to adapt counselling to the needs and interests of this population	Latina pregnant women aged≥18 years were referred to genetic counselling and offered NIPT n=63 (22 women elected to have NIPT and 41 women declined NIPT)	Preferences of pregnant women	Ethical

Authors, year, countries	Study characteristics	Objective	Study participant characteristics	Outcomes assessed	Assessment element
Skirton et al. [150], 2015, UK	Systematic review Websites advertising NIPT for aneuploidies by two Internet search engines (Google UK and Bing)	To investigate the way commercial companies and private health providers are currently marketing NIPT to patients	NA	Attitudes and preferences of pregnant women	Ethical
Tamminga et al. [151], 2015, Netherlands	Discrete choice experiment questionnaire-based survey November 2013 to July 2014	To investigate health professionals' opinions towards offering NIPT as a primary screening test method	Obstetric health professionals received in-service NIPT training n=240	Preferences of health professionals	Ethical Patient and social
Walker et al. [152], 2015, USA	Cost-effectiveness analysis Monte Carlo simulation with one-way and probabilistic sensitivity analysis	To determine the cost-effectiveness of cfDNA as a replacement for integrated screening using a societal cost perspective	Hypothetical cohort of 1,000,000 pregnant women of at least 10 weeks' gestation	ICER	Organisational
Walker et al. [153], 2015, USA	Cost-effectiveness analysis from societal, governmental and payer perspectives Decision-analytic model using microsimulation and probabilistic sensitivity analysis	To determine the cost-effectiveness of contingent and universal NIPT compared with conventional screening	Hypothetical cohort of 1,000,000 pregnant women of at least 12 weeks' gestation	ICER	Organisational
Benn et al. [154], 2014, USA	Questionnaire-based survey March to August 2012	To assess the opinions of fellows of the ACOG on expanded carrier testing and NIPT	Obstetrician-gynaecologists n=222	Preferences of prenatal healthcare professionals	Ethical Patient and social
Beulen et al. [155], 2014, Netherlands	Cost-effectiveness analysis Decision-analytic model	To determine the cost-effectiveness of NIPT as add-on to FCT and as primary screening compared with FCT alone	Theoretical cohort of 180,000 pregnant women	Total and relative costs ICER	Organisational
Chandrasekharan et al. [156], 2014, USA	Narrative review	To analyse factors affecting NIPT implementation in low- and middle-income countries	NA	Regulatory, ethical issues Costs and access Informed decision-making	Organisational
Hulstaert et al. [157], 2014, Belgium	HTA report	See Appendix 1, Table A7	See Appendix 1, Table A7	See Appendix 1, Table A7	Organisational
Institute of Health Economic [158], 2014, Canada	Full HTA report (clinical effectiveness and economic evaluation)	See Appendix 1, Table A7	See Appendix 1, Table A7	See Appendix 1, Table A7	Organisational

Authors, year, countries	Study characteristics	Objective	Study participant characteristics	Outcomes assessed	Assessment element
Kellog et al. [159], 2014, USA	Online questionnaire-based survey (17 questions that included demographic and Likert-scale format attitudinal) October to December 2012	To assess attitudes towards NIPT and what impact would have increased use of NIPT in the future	n=73 Mothers of children with T21	Attitudes of mothers of children with T21	Ethical
Lewis et al. [160], 2014, UK	Cross-sectional questionnaire-based survey (designed by health professionals using a modified Delphi technique)	To assess the views and likely uptake of NIPT for trisomy 21 among potential service users in the UK	n=1131 Women and partners aged>18 years recruited from antenatal clinics and websites	Preferences of pregnant women/parents	Patient and social
Morris et al. [161], 2014, UK	Cost analysis Decision-analytic model	To determine the costs and outcomes of NIPT for T21 as a contingent strategy and as a primary strategy compared with current DS screening	Hypothetical cohort of pregnant women n=10,000	Number of cases of T21 detected, procedure-related miscarriages Total cost	Ethical
Neyt et al. [162], 2014, Belgium	Cost-consequences analysis (time-dependent multistage transition probability model) Short-term time horizon was applied and no discount rate was applied	To estimate the consequences of introducing NIPT for the detection of trisomy 21	All singleton pregnancies n=129,199	Short-term screening costs per case of trisomy 21 diagnosed Incremental cost per extra case of T21 diagnosed	Organisational
Okun et al. [163], 2014, Canada	Cost analysis Scenario modelling	To examine the cost and performance of NIPT in eight distinct scenarios or screening strategies	Population based on a cohort of pregnant women observed for a single year n=144,570	Total programme cost Cost per woman screened, per prenatally diagnosed pregnancy with T21, per additional prenatally diagnosed pregnancy with T21	Ethical
O'Leary et al. [164], 2013, Australia	Cost-effectiveness analysis Decision tree analysis	To analyse the cost-effectiveness and performance of NIPT for high-risk pregnancies following FCT compared with current practice	Singleton pregnant women n=32,478	Diagnostic test uptake Cost of testing pathway, screening, invasive diagnostic testing and NIPT Cost per trisomy 21 case confirmed ICER	Ethical
Sayres et al. [165], 2014, USA	Online questionnaire-based survey (two versions of 25 questions)	To assess expected interest in cfDNA screening for trisomies 13, 18 and 21 among the general public	n=3164 Adults aged≥18 years	Attitudes of pregnant women	Ethical

Authors, year, countries	Study characteristics	Objective	Study participant characteristics	Outcomes assessed	Assessment element
Skirton et al. [166], 2014,UK	Consensus document (guideline) Expert group of health professionals on prenatal diagnosis (n=13) from 11 European countries (Belgium, Czech Republic, Denmark, Finland, Greece, Italy, Netherlands, Spain, Sweden and UK)	To provide prenatal diagnostic testing services that enable families to make informed choices, consistent with their individual needs and values	Women and/or their partners known to genetic services before pregnancy because of significant family history, fetus at risk of genetic condition or abnormal US findings, particularly where the fetal karyotype is normal	NA	Patient and social
Skirton et al. [167], 2013, UK	Systematic review (November 2012) Six relevant electronic databases (CINAHL, MEDLINE, SocIndex, PsycARTICLES, PsycLIT and Web of Science) and hand searching	To investigate factors influencing the clinical use of NIPT	NA	Attitudes and experience of pregnant women Economic analysis Regulation, practice and ethical issues	Ethical

Abbreviations: ACOG=American College of Obstetricians and Gynecologists; cfDNA=cell-free DNA, DS=Down syndrome; FCT=first-trimester combined testing; FP=false positive; HTA=health technology assessment; ICER=incremental cost-effectiveness ratio; MMIC=multidimensional measure of informed choice; NA, not available; NIPT=noninvasive prenatal testing; US=ultrasound.

7.3 Ethical assessment elements

[F0010] – What are the known and estimated benefits and harms for pregnant women when NIPT is implemented or not implemented?

The four basic principles of healthcare ethics include autonomy, nonmaleficence, beneficence and justice. In terms of autonomy, the early availability of results and the increased accuracy of NIPT as a screening test for T21, in comparison with the combined test, is presumed to facilitate a pregnant woman's and her partner's informed choice, which is generally regarded as the main objective of prenatal screening. A study from the UK looking at the introduction of NIPT in the NHS (RAPID study), which used a formal measure of informed choice, found very high rates of informed consent (89%) in 587 women offered NIPT [130]. Around half of the 240 obstetricians surveyed in the Netherlands (47%) were of the opinion that the replacement of FCT by NIPT could simplify counselling, and 64% considered the procedure of the test easier to explain [151]. The vast majority felt that more women would use NIPT, but 49% considered that there could be a risk that pregnant women agreed to screening without fully thinking about their decisions. Further assessments are needed to understand in depth the implications for informed choice, as this can vary depending on many factors, including how the consent and counselling occur.

Establishing the nonmaleficence and beneficence of NIPT is a complex issue because it relies on the value judgement of whether preventing these trisomies could be harmful or good. In essence, it requires weighing of the benefits and harms not only for the pregnant woman and her partner, but also for the family and other involved parties. For NIPT, the benefits and harms can vary substantially depending on the perspective and implementation approach (add-on, total or partial replacement). In general terms, NIPT is liable to lead to more ethical problems when used as a primary test rather than a second-tier test. A systematic review analysing the factors affecting NIPT uptake highlights that the positive aspects perceived by service users are the greater safety for the fetus, earlier information about fetal status and general ease of sample taking [167]. When NIPT is offered as a second-tier test, these benefits have to be weighed against the risk of missing mosaicisms and the nonidentification of other clinically relevant chromosomal anomalies, which could have been detected during the verification of the FCT high-risk results with invasive technologies. Whilst invasive testing can put women at a higher risk than NIPT, recent comparative studies highlight that the additional risk posed by amniocentesis or CVS could be lower than previously estimated, and all this information must be taken into account when ones is informing decision making.

Available evidence supports that the implementation of NIPT as a primary test could increase the detection of T21, T18 and T13 and reduce the number of FP cases in comparison with combined screening, contributing to reduce unnecessary anxiety and procedure-related miscarriages in pregnant women who have tested positive. In a survey performed as part of the implementation study in the UK NHS, women were very much in favour of a test which was safe, accurate and reduced the need for invasive testing, identifying T21 cases which might otherwise be missed [141]. Against this claimed benefit, great uncertainties remain regarding the possibility of missing some of the trisomies because of mosaicisms or technical reasons. Assay failure could also be a potential matter of concern or worry because of the possible association of null results with a higher risk of chromosomal anomalies, as could the loss of information regarding other major defects and other major complications, especially if NIPT replaces NT assessment. In the Swedish survey the positive attitude of women to NIPT or FCT did not reach that of ultrasound examination [145]. In this study, the women who stated that they would not use NIPT were more interested in knowing about other, severer chromosomal anomalies than T21. The questionnaires in the Netherlands also reflected that pregnant women were willing to accept a less accurate test to obtain more information on fetal

chromosomal status or to exclude the risk of procedure risk miscarriage, whilst health professionals put more emphasis on the accuracy of the tests [147]. Around half of the health professionals who were trained to offer NIPT the Netherlands preferred to continue using NT measurements [151].

To comply with the principle of distribution of justice, it should be ensured that NIPT is cost-effective in relation to conventional approaches. The RAPID nonevaluation study performed in the UK, which was based on actual clinical data, established that NIPT as a contingent test, with a risk cut-off point greater than 1 in 150, could improve quality of care, choices for women and overall performance without increasing costs [139]. This and other economic analyses, which establish the cost-effectiveness of NIPT for high-risk women, are based on decision analysis economic modelling, which are known not to be representative of real practice. To date, there are no appropriate real-world comparative studies to establish how the different NIPT algorithms differ with regard to standard approaches in relation to uptake, informed consent, performance or health outcomes (invasive testing performed, miscarriage reduction, detection of other relevant fetal anomalies, etc.), raising important questions regarding the implementation of these technologies in real practice. Taking into account the principle of distribution of justice it should be assessed if it would be ethical to spend resources on technologies which have important uncertainties regarding their implementation, adoption and outcomes. Screening being a morally sensitive issue, because of its association with abortion, the ethical implications of NIPT may differ in different countries, depending on the goals and values acceptable to the society.

When discussing the principle of justice one should also keep in mind that the access to NIPT depends directly on the access to FCT if NIPT is to be used as a second-tier test. In this sense, NIPT will be available only to women who can afford FCT, which widens the gap between high- and low-income settings. Given the higher accuracy of NIPT it could happen that T21 would become largely a problem of poor families, which would increase social stigmatisation. When reimbursement of NIPT as an add-on test is introduced, it is therefore essential to establish general reimbursement for FCT.

[F0011] – What are the benefits and harms of NIPT for relatives, other patients, organisations, commercial entities, societies, etc.?

[F003] – Are there any other hidden or unintended consequences of NIPT and its applications for pregnant women, relatives, other patients, organisations, commercial entities, society, etc.?

[F004] – Does the implementation or use of NIPT affect the pregnant woman's capability and possibility to exercise autonomy?

If NIPT is implemented in the general pregnant population, an increase in the rate of detection of T21, T18 and T13 is expected, and this could lead to an increase in the number of affected fetuses aborted. In this sense, several studies have highlighted concerns regarding the possibility that NIPT could be seen to send a message of stigmatisation to families who live with these conditions and also reduce the availability of services such as medical care, physical therapy, occupational therapy or school programmes [167]. According to an anonymous online survey conducted in the USA, most mothers of children with T21 perceive that NIPT could lead to increased terminations (88%), increased social stigma (57%) and reduced availability of services for T21 individuals (64%) [159].

Because it is a safe and easy test, which is privately available, there is a risk that the test could also be used for minor conditions or even undesired nonmedical traits. This could lead to women deciding to terminate pregnancies for trivial reasons, such as sex selection. In a survey conducted

in the USA, 73% of the obstetricians interviewed believed that NIPT would increase pregnancy terminations for mild diseases [154]. If it is offered directly, it could also happen that women are not appropriately informed and reproductive choices following prenatal screening are made without their really understanding the results. The results of a systematic review showed that the information provided by commercial companies and private health providers is not equally balanced and the need for an invasive test to diagnose aneuploidy is not always underlined [150].

Concerns have also been raised regarding how the informed consent and counselling process will occur in clinical practice, especially if NIPT is to be used as a one-step screening test. Respondents in several studies have cited fears that given the noninvasiveness of the test, it might end up being offered as a routine procedure, depriving women of a well-informed option, not giving them the real chance to decide if they truly desire this information [167].

[F006] – Is there a need for any specific interventions or supportive actions concerning information so as to respect a pregnant woman's autonomy when the technology is used?

Comprehensive NIPT pretest counselling could be complicated by emerging information about the benefits and uncertainties related to this procedure, as well as the potential to detect incidental findings. Professional societies recommend that a trained provider, such as a genetic counsellor, an obstetrician or a maternal-fetal medicine specialist [140], give the posttest counselling. Since NIPT screening has implications different from those of combined screening, clear and accurate consent forms should be developed, providing clinicians with educational materials for explaining, in a neutral manner, the purpose of testing and the potential risks and benefits. In 2017, the Institute for Quality and Efficiency in Health Care was commissioned to prepare such educational materials for Germany.

Different position statements recommend that counselling should be given both before screening, so as to allow women to make a personal decision to accept or decline screening, and after counselling, to discuss positive findings with affected women. This is supported by the findings of a UK study, which established that this multistep process would facilitate informed decision making [142]. In this study, as well as the Dutch implementation study (Trident study), women were given written information in addition to oral counselling [146]. The questionnaire completed by 1091 women who participated in this study revealed that women who made an informed choice (78%) had significantly higher educational levels and adequate health literacy. Women with inadequate health literacy experienced higher posttest result anxiety, highlighting that they might benefit from extra information and/or special counselling aids. A study conducted in the USA among Latina women also found that women who declined NIPT had a lower educational level, suggesting that culturally tailored information could be useful for women to make informed choices [149].

[F0101] – Does NIPT invade the sphere of the pregnant woman/user?

Because of the cytotrophophast origin, NIPT may result in an incidental identification of clinically significant maternal or fetal constitutional chromosomal anomalies or acquired cytogenetic anomalies, including associated malignancies, which might require genetic counselling [33].

[F0012] – How does implementation or withdrawal of the technology affect the distribution of resources?

Some assessments aimed at evaluating the costs of implementing NIPT for T21 performed in different countries (UK, Canada, USA and Australia) show inconsistent results. Whilst some estimate that adding NIPT to current screening programmes would lead to increased costs, others estimate that costs would remain unchanged a result of the reduction in the number of invasive diagnostic tests performed [139, 143, 148, 161, 163, 164]. To date, there are no appropriate studies to assess the real impact of NIPT, leaving important uncertainties regarding the human and financial resources needed to implementation NIPT in a prenatal care programme.

[F0017] – What are the ethical consequences of the choice of endpoints, cut-off values and comparators/controls in the assessment?

[H0012] – Are there factors that could prevent a group or person from gaining access to NIPT?

The level of NIPT implementation in different countries seems to be associated with factors such as the level of education, incomes or insurance coverage. Therefore NIPT uptake is more likely in high-income settings than in countries with low resources. These inequalities may be exacerbated by the NIPT cost, the test being provided mainly in private settings. The availability of genetic laboratories or the viability for transportation of samples to external laboratories might be other factors which could interfere with the access of NIPT, especially in populations from low- and middle-income countries [88, 165].

7.4 Organisational assessment elements

[G0001] – How does the technology affect the current work processes?

[G0100] – What kind of pregnant woman/participation flow is associated with the new technology?

[G0002] – What kind of involvement has to be mobilised for pregnant women/participants and important others and/or carers?

[G0003] – What kind of process ensures proper education and training of staff?

[G0004] – What kind of cooperation and communication of activities has to be mobilised?

[G0012] – In what way is the quality assurance and monitoring system of NIPT organised?

The implementation of NIPT would not require significant changes in the patient workflow or the health professionals involved. However, the current work process of the professionals responsible for screening could change substantially as these might be required to provide additional pretest counselling to inform decision making. To date, little information exists regarding the education and training given to these professionals. The only information comes from the UK RAPID study [137]. This study supports that providing professionals with face-to-face training (lesson plan and PowerPoint presentation) and written factsheets improves confidence and perceived knowledge. Nonetheless 65% of attendees interviewed still had little understanding regarding certain knowledge in specific areas (test turnaround time, FP rates, cfDNA originating from placenta and cell concentration

increases with gestation) [137]. In line with the results of a recent US study, special attention should also be paid to consent documents because it is acknowledged that many of the existing ones do not appropriately reflect psychosocial considerations [144].

In the same way as combined screening, NIPT requires close collaboration and cooperation between all actors involved in the screening process (screening unit, laboratory, manufacturers, hospital, pregnant woman and partner). Samples should be handled and shipped according to manufacturers' instructions and according to good laboratory practices. Training might also be required if the system is to be implemented in standard laboratories. Specific independently developed minimum standards, quality control, proficiency testing and inspection requirements have not yet been developed for NIPT. However, laboratories must adhere to specific standards for laboratory procedures and the protection of patient information confidentiality [33].

[G0005] – How do decentralisation or centralisation requirements influence the implementation of NIPT?

[G0006] – What are the costs of processes related to acquisition and setting up of NIPT?

[G0023] – How does NIPT modify the need for other technologies and use of other resources?

[G0007] – What is the likely budget impact of implementing the technologies being compared?

[G0008] – What management problems and opportunities are attached to NIPT?

Although currently most samples are analysed externally, NIPT could be done in most of the laboratories which are already perform molecular-diagnostic assays. Standard laboratory equipment, in addition to specific equipment and software, would be required in this case.

No information is available regarding costs related to acquisition and setting up of NIPT, and great uncertainty surrounds the actual costs of external testing, as these can differ greatly between laboratories, depending on the specific test and country. According to two narrative reviews in North American countries (USA and Canada) prices range from $795 to $3000 (approximately €665–€2511). In European countries (only provided information regarding Austria, Germany, the UK, Romania, Spain and Switzerland) the cost of NIPT is around €447–€992, with the most expensive test being sold in the UK and the cheapest one being sold in Romania. A noninvasive prenatal test is sold for US$457–US$587 (approximately €382–€491) in China and for US$1492–US$1600 (approximately €1248–1339) in South American countries such as Brazil and Argentina. No information is available about NIPT costs in many low- or middle-income countries [88, 156].

Six full economic evaluations reported on the cost-effectiveness of NIPT for diagnosis of T21 performed in European countries (i.e., the Netherlands, Belgium and Spain). These reports showed a higher incremental cost-effectiveness ratio of NIPT as primary testing than NIPT as a contingent strategy or second-tier testing (cut-off point for NIPT indication between 1 in 200 and 1 in 1000) compared with standard serum screening [138, 155, 157]. In line with these reports, other economic assessment performed by the Institute of Health Economics (reported a lower incremental cost-effectiveness ratio of serum integrated prenatal screening (first- and second-trimester serum markers) plus NIPT or first-trimester quadruple serum screening with NT measurement plus NIPT than NIPT alone for diagnosis of T21 [158]. The remaining two assessments found from a societal perspective that universal NIPT (serum screening is offered to patients in whom NIPT failed and invasive testing is offered to patients with positive NIPT or serum screening results) was a cost-

effective alternative to conventional serum screening (FCT or serum integrated screening); however, from a government or payer perspective, contingent NIPT was a cost-effective alternative to conventional serum screening and less costly than universal NIPT [152, 153].

The opportunities attached to NIPT mainly relate to the alleviation of workload for some professionals. If NIPT totally replaces FCT, it would be expected that biochemistry serum clinical laboratories and cytogenetic and molecular genetics laboratories would receive significantly fewer samples to analyse, although some of the conventional tests might still be required to detect other conditions, such as fetal growth restriction or preeclampsia. The burden of specialists in NT and obstetricians responsible for invasive testing could also be alleviated. Experienced sonographers are limited in number or are unavailable in many rural areas.

[G0009] – Who decides which pregnant women are eligible for NIPT and on what basis?

In principle, all pregnant women would be eligible for NIPT except in those cases where the use of a noninvasive test is not recommended; that is, women of less than 8 or 10 weeks' pregnancy (depending on the test), women who have a chromosomal anomaly, low fetal fraction (<4%) and other clinical situations (see all NIPT contraindications in page 51) in which NIPT accuracy may be reduced. Moreover, some consensus documents and guidelines reported cfDNA screening is not recommended for women with multiple gestations and/or donor oocytes or for diagnosis of microdeletions.

NIPT should be offered by a medical healthcare professional (i.e., obstetrician-gynaecologists, maternal-fetal medicine specialist or other obstetric care providers) following the recommendations mentioned earlier and using a standard approach for genetic counselling that includes information about available options for screening and diagnosing aneuploidies, all this integrated and coordinated in a prenatal screening programme. It should not be offered independently as a direct-to-consumer test by laboratories [33, 38].

7.5 Patient and social assessment elements

[G0010] – How is NIPT accepted?

[H0006] – How do pregnant women perceive NIPT?

[H0100] – What expectations and wishes do pregnant women have with regard to NIPT and what do they expect to gain from the technology?

In general terms, psychosocial research exploring women's and health professionals' attitudes show a general positive view towards NIPT [146, 151, 160], although the available literature points to possible differences in acceptability, which might depend on cultural, social or other factors. Most Swedish (n=1003) and British (n=1131) pregnant women recruited in maternity clinics indicated that they would like to use NIPT (73% and 88%, respectively) [145, 160], in comparison with only 51% in Denmark. These studies support that women's preference regarding NIPT is mainly associated with the early results, the elimination of the procedure-related miscarriage risk and the accuracy of the results, although in the Netherlands a discrete choice experiment reflected a preference for safety over accuracy [147]. The existing literature supports that women and professionals would be very much in favour of replacing FCT and broadening the scope of NIPT to test for all severe disabilities, irrespective of their cause and depending on the woman's and couple's choices [145, 147, 154, 168].

All of the women interviewed in the UK RAPID study expressed their wish that NIPT be publically adopted and offered as a first-line test because they expected NIPT results would be easier to interpret in comparison with FCT results. Having the two sets of results seemed to create some confusion [142]. Ninety-six per cent of women in the nationwide Dutch TRIDENT trial were also glad to be offered NIPT, and 68% were satisfied [146].

[H0002] – What is the burden on carers?

No information was found regarding the burden that NIPT use may cause to carers. However, no additional burden is anticipated.

[H0202] – How are screening options explained to pregnant women?

[H0203] – What specific issues may need to be communicated to pregnant women to increase acceptance of NIPT?

Throughout any prenatal screening programme, screening and diagnostic testing for fetal anomalies available should be offered by pretest and posttest counselling and an informed consent discussion in which the benefits and risks of each option are explained in detail. Skirton et al. [166] suggested that prenatal counselling should take into account beliefs and values related to cultural norms as people from countries with different cultural backgrounds may respond in an uneven way to similar information. To ensure that each woman or all parents receive prenatal counselling adapted to their needs, Skirton et al. [166] produced a set of best practice guidelines for offering genetic testing by a group of experts in prenatal diagnosis from nine European countries (Belgium, Czech Republic, Denmark, Finland, Italy, the Netherlands, Spain, Sweden and the UK). This study concluded that the information should be provided by an appropriately trained health professional in both verbal and written forms, always bearing in mind the local ethical and legal guidelines and including information about the condition (genetic cause, phenotypic features or risk of aneuploidies, etc.), the test (accuracy, limitations, etc.), practical aspects and psychosocial issues. Finally, they recommended that women or parents be given the opportunity to use the information provided on the basis of their personal beliefs.

In the literature retrieved it was not reported if specific issues should be communicated to patients to increase acceptance of NIPT.

8 CONCLUSION

- Existing moderate quality evidence supports that the detection of T21 cases is higher when NIPT replaces FCT as a primary screening test and that this replacement would lead to a reduction in unnecessary invasive testing. However, important uncertainties remain regarding the under-reporting of missed cases given the inappropriate verification of negative results. Data regarding key safety outcomes are also lacking (increase in the number of children born with major anomalies, elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance, etc.). The generalisability of the PPV and NPV is limited by the fact that the prevalence of T21 found in the studies included is not representative of that found in the general pregnant population.

- No data exist to assess the accuracy of NIPT offered as part of the first-trimester fetal combined test.

- The available data suggest that the use of NIPT as an add-on to combined testing for high-risk T21 population screening could also lead to substantial reductions in unnecessary invasive testing, although this needs to be confirmed with real-world data. The performance of the test (test failures, uncertain results) and the uptake of NIPT screening are among the factors that could contribute to change this ratio in real practice.

- There is lack of data to assess the use of NIPT as an add-on to combined testing for high- and intermediate-risk T21 populations.

- The low QoE for T18 and T13 does not allow conclusions to be drawn on these trisomies for any of the screening pathways.

- There is insufficient evidence to establish the accuracy of NIPT for twin pregnancies.

- Appropriately designed studies are required so as to be able to assess the performance of the different test strategies, taking into account detection of all anomalies, abortions, miscarriages and other patient-related outcomes. Important uncertainties remain regarding the best screening pathway.

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APPENDIX 1: METHODS AND DESCRIPTION OF THE EVIDENCE USED

DOCUMENTATION OF THE SEARCH STRATEGIES

Search strategy for identification of clinical guidelines or consensus documents

Search strategy for GIN, Trip database, National Guideline Clearinghouse, Australian Clinical practice guidelines, ACP on line and CPGinfobase on 21th February 2017

#1. Aneuploid*

#2. “Trisomy screening”

#3. "Down syndrome" screening

#4. "Edward syndrome"

#5. "Patau syndrome"

Search strategy for Medline on 21th February 2017

#1. (((((("screen"[TW] OR "Screening"[TW] OR "Screened"[TW] OR "Test"[TW] OR "Tested"[TW] OR "Di-agnostic"[TW] OR "Diagnosis"[TW] OR "Sequencing"[TW] OR "Sequence"[TW])) AND ("Prenatal"[TW] OR "Antenatal")) AND ("aneuploidy"[TW] OR "aneuploidies"[TW] OR "Trisomy"[TW] OR "Trisomies"[TW] OR "Triso-mic"[TW] OR "Down syndrome"[TW] OR "Edward syndrome"[TW] OR "Patau syndrome"[TW])) AND (("Chromosome"[TW] OR "Chromosomal"[TW] OR "DNA"[TW] OR "Cell-free fetal DNA"[TW] OR "cfDNA"[TW] OR "Massively parallel sequencing"[TW] OR "MPS"[TW] OR "Single nucleotide polymorphisms"[TW] OR "SNPs"[TW]) AND ("NIPT"[TW] OR "Non-invasive prenatal testing"[TW] OR "Non-invasive"[TW] OR "Noninvasive"[TW] OR "Blood"[TW] OR "Plasma"[TW]))) AND ("Position statement"[Text Word] OR "Position statements"[Text Word])) AND (Guideline[ptyp] OR Meta-Analysis[ptyp] OR Practice Guideline[ptyp] OR systematic[sb])) AND "2010/01/01"[PDat]: "3000/12/31"[PDat]

Search strategy for Embase on 21th February 2017

#1 (screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence).ti,sh,hw,ab,kw,tw.

#2 (Prenatal or Antenatal).ti,sh,hw,ab,kw,tw.

#3 1 and 2

#4 (aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome").ti,sh,hw,ab,kw,tw.

#5 (Chromosome or Chromosomal or DNA or "Cell-free fetal DNA" or ""cfDNA or "Massively parallel sequencing" or "MPS" or "Single nucleotide polymorphisms" or "SNPs").ti,sh,hw,ab,kw,tw.

#6 3 and 4

#7 ("NIPT" or "Non-invasive prenatal testing" or "Non-invasive" or Noninvasive or Blood or Plasma).ti,sh,hw,ab,kw,tw.

#8 5 and 7

#9 6 and 8

#10 limit 9 to yr="2010 -Current"

#11 limit 10 to consensus development

#12 limit 10 to "conference review"

#13 11 or 12

Search strategy for Web of Science on 21th February 2017

# 1 TI=(aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome") OR TI=(aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome")
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
# 2 TS=(screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence) OR TI=(screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence)
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
# 3 TS=(Prenatal or Antenatal) OR TI=(Prenatal or Antenatal)
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
# 4 #3 AND #2
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
# 5 #4 AND #1
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
# 6 TITLE: ("clinical Practice guideline" OR "clinical guideline" OR consens* OR "position statement" OR "position statments") OR TOPIC: ("clinical Practice guideline" OR "clinical guideline" OR consens* OR "position statement" OR "position statments")

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
# 7 #6 AND #5
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017

Search strategy for Google on 21th February 2017

#1. Aneuploid* "clinical guideline" filetype:pdf

#2. Aneuploid* "clinical practice guideline" filetype:pdf

Search strategy for identification of NIPT systematic reviews or HTA reports

Search strategy for CRD databases on 20th July 2017

#1. (aneuploid* OR trisom):TI AND (screen*):TI FROM 2010 TO 2017

#2. ("Down syndrome"):TI AND (screen*):TI FROM 2010 TO 2017

#3. ("Edward syndrome"):TI AND (screen*):TI FROM 2010 TO 2017

Search strategy for INAHTA

#1. aneuploid* OR trisomy OR "Down syndrome" OR "Edward syndrome" OR “Patau syndrome"

Search strategy for identification of NIPT test accuracy studies

Search strategy for Medline on 10th March 2017

#1. ((((((((((((("screen"[TW] OR "Screening"[TW] OR "Screened"[TW] OR "Test"[TW] OR "Tested"[TW] OR "Diagnostic"[TW] OR "Diagnosis"[TW] OR "Sequencing"[TW] OR "Sequence"[TW])))) AND ((("Prenatal"[TW] OR "Antenatal"[TW])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ((("aneuploidy"[TW] OR "aneuploidies"[TW] OR "Trisomy"[TW] OR "Trisomies"[TW] OR "Trisomic"[TW] OR "Down syndrome"[TW] OR "Edward syndrome"[TW] OR "Patau syndrome"[TW])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ((("Chromosome"[TW] OR "Chromosomal"[TW] OR "DNA"[TW] OR "Cell-free fetal DNA"[TW] OR "cfDNA"[TW] OR "Massively parallel sequencing"[TW] OR "MPS"[TW] OR "Single nucleotide polymorphisms"[TW] OR "SNPs"[TW]) AND ("NIPT"[TW] OR "Non-invasive prenatal testing"[TW] OR "Non-invasive"[TW] OR "Noninvasive"[TW] OR "Blood"[TW] OR "Plasma"[TW])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ((("Sensitivity and Specificity"[MeSH Terms] OR accuracy[TIAB] OR Sensitivity[TIAB] OR specificity[TIAB] OR “false positive”[TIAB] OR “false negative”[TIAB])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) NOT ((((((((((((((("screen"[TW] OR "Screening"[TW] OR "Screened"[TW] OR "Test"[TW] OR "Tested"[TW] OR "Diagnostic"[TW] OR "Diagnosis"[TW] OR "Sequencing"[TW] OR "Sequence"[TW])))) AND ((("Prenatal"[TW] OR "Antenatal"[TW])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ((("aneuploidy"[TW] OR "aneuploidies"[TW] OR "Trisomy"[TW] OR "Trisomies"[TW] OR "Trisomic"[TW] OR "Down syndrome"[TW] OR "Edward syndrome"[TW] OR "Patau

syndrome"[TW])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ((("Chromosome"[TW] OR "Chromosomal"[TW] OR "DNA"[TW] OR "Cell-free fetal DNA"[TW] OR "cfDNA"[TW] OR "Massively parallel sequencing"[TW] OR "MPS"[TW] OR "Single nucleotide polymorphisms"[TW] OR "SNPs"[TW]) AND ("NIPT"[TW] OR "Non-invasive prenatal testing"[TW] OR "Non-invasive"[TW] OR "Noninvasive"[TW] OR "Blood"[TW] OR "Plasma"[TW])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ((("Sensitivity and Specificity"[MeSH Terms] OR accuracy[TIAB] OR Sensitivity[TIAB] OR specificity[TIAB] OR “false positive”[TIAB] OR “false negative”[TIAB])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND (((Addresses[ptyp] OR Autobiography[ptyp] OR Bibliography[ptyp] OR Biography[ptyp] OR Comment[sb] OR Congresses[ptyp] OR Editorial[ptyp] OR Interview[ptyp] OR Lectures[ptyp] OR Legal Cases[ptyp] OR Legislation[ptyp] OR Letter[ptyp])) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))) AND ("2012/01/01"[PDat] : "3000/12/31"[PDat]))
Filters: Publication date from 2012/01/01

Search strategy for Embase on 10th March 2017

#1 (screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence).ti,sh,hw,ab,kw,tw.

#2 (Prenatal or Antenatal).ti,sh,hw,ab,kw,tw.

#3 1 and 2

#4 (aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome").ti,sh,hw,ab,kw,tw.

#5 (Chromosome or Chromosomal or DNA or "Cell-free fetal DNA" or "cfDNA" or "Massively parallel sequencing" or "MPS" or "Single nucleotide polymorphisms" or "SNPs").ti,sh,hw,ab,kw,tw.

#6 3 and 4

#7 ("NIPT" or "Non-invasive prenatal testing" or "Non-invasive" or Noninvasive or Blood or Plasma).ti,sh,hw,ab,kw,tw.

#8 5 and 7

#9 6 and 8

#10 *sensitivity analysis/or *"sensitivity and specificity"/

#11 (accuracy or Sensitivity or specificity or false positive or false negative).ab,kw,sh,ti.

#12 10 or 11

#13 9 and 12

#14 limit 13 to (embase and yr="2012 -Current")

Search strategy for Web of Science on 10th March 2017

 # 1 TI=(aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome") OR TI=(aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome")

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2010-2017

 # 2 TS=(screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence) OR TI=(screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence)

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2010-2017

 # 3 TS=(Prenatal or Antenatal) OR TI=(Prenatal or Antenatal)

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2010-2017

 # 4 #3 AND #2

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2010-2017

 # 5 #4 AND #1

Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2010-2017

 # 6 TS=(accuracy OR Sensitivity OR specificity OR false positive OR false negative) OR TI=(accuracy OR Sensitivity OR specificity OR false positive OR false negative)

 # 7 #5 AND #6

Refined by: [excluding] DOCUMENT TYPES: (PROCEEDINGS PAPER OR MEETING ABSTRACT OR LETTER OR EDITORIAL MATERIAL) Timespan=2012-2017

Search strategy for Cochrane Library on 10th March 2017

#1 screen or Screening or Screened or "Massively parallel sequencing" or MPS or "Single nucleotide polymorphisms" or SNPs:ti,ab,kw (Word variations have been searched)

#2 Prenatal or Antenatal:ti,ab,kw (Word variations have been searched)

#3 #1 and #2

#4 NIPT or "Non-invasive prenatal testing":ti,ab,kw (Word variations have been searched)

#5 #3 or #4

#6 DNA or "Cell-free fetal DNA" or cfDNA:ti,ab,kw (Word variations have been searched)

#7 #5 and #6

#8 Blood or Plasma:ti,ab,kw (Word variations have been searched)

#9 #7 and #8

#10 aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome":ti,ab,kw (Word variations have been searched)

#11 #9 and #10

#12 accuracy or Sensitivity or specificity OR false positive OR false negative:ti,ab,kw (Word variations have been searched)

#13 #11 and #12

#14 MeSH descriptor: [Sensitivity and Specificity] explode all trees

#15 #11 and #14

#16 #13 or #15

Search strategy for identification of qualitative studies

Search strategy for Medline on 10th February 2017

#1. (((((("screen"[TW] OR "Screening"[TW] OR "Screened"[TW] OR "Test"[TW] OR "Tested"[TW] OR "Di-agnostic"[TW] OR "Diagnosis"[TW] OR "Sequencing"[TW] OR "Sequence"[TW])) AND ("Prenatal"[TW] OR "Antenatal"[TW])) AND ("aneuploidy"[TW] OR "aneuploidies"[TW] OR "Trisomy"[TW] OR "Trisomies"[TW] OR "Triso-mic"[TW] OR "Down syndrome"[TW] OR "Edward syndrome"[TW] OR "Patau syndrome"[TW])) AND (("Chromosome"[TW] OR "Chromosomal"[TW] OR "DNA"[TW] OR "Cell-free fetal DNA"[TW] OR "cfDNA"[TW] OR "Massively parallel sequencing"[TW] OR "MPS"[TW] OR "Single nucleotide polymorphisms"[TW] OR "SNPs"[TW]) AND ("NIPT"[TW] OR "Non-invasive prenatal testing"[TW] OR "Non-invasive"[TW] OR "Noninvasive"[TW] OR "Blood"[TW] OR "Plasma"[TW]))) AND (anxiety[Title/Abstract] OR attitudes[Title/Abstract] OR choice[Title/Abstract] OR "clinical imple-mentation"[Title/Abstract] OR Decision-making[Title/Abstract] OR "Decision mak-ing"[Title/Abstract] OR experience*[Title/Abstract] OR "Focus Groups"[Title/Abstract] OR "Fo-cus Group"[Title/Abstract] OR Motivation[Title/Abstract] OR interview*[Title/Abstract] OR "Pa-tient Acceptance of Health Care"[Title/Abstract] OR testimon*[Title/Abstract] OR story-tell*[Title/Abstract] OR (story[TIAB] tell*[TIAB]) OR Patient Education as Topic OR Qualitative Research OR Health Knowledge, Attitudes, Practice OR Health Care Surveys OR "Focus Groups" OR "Interviews as Topic" OR narration[MeSH Terms] OR qualitative[Title/Abstract] OR Views[Title/Abstract] OR "Health Personnel"[Mesh] OR "Health Care Providers"[TIAB] OR "Health Care Provider"[TIAB] OR"Healthcare Providers"[TIAB] OR "Healthcare Provider"[TIAB]) Sort by: PublicationDate Filters: Publication date from 2012/01/01

Search strategy for Embase on 10th February 2017

#1 (screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence).ti,sh,hw,ab,kw,tw.

#2 (Prenatal or Antenatal).ti,sh,hw,ab,kw,tw.

#3 1 and 2

#4 (aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome").ti,sh,hw,ab,kw,tw.

#5 (Chromosome or Chromosomal or DNA or "Cell-free fetal DNA" or "cfDNA" or "Massively parallel sequencing" or "MPS" or "Single nucleotide polymorphisms" or "SNPs").ti,sh,hw,ab,kw,tw.

#6 3 and 4

#7 ("NIPT" or "Non-invasive prenatal testing" or "Non-invasive" or Noninvasive or Blood or Plasma).ti,sh,hw,ab,kw,tw.

#8 5 and 7

#9 6 and 8

#10 (anxiety or attitudes or choice or "clinical implementation" or Decision-making or "Decision making" or experience* or "Focus Groups" or "Focus Group" or Motivation or interview* or "Patient Acceptance of Health Care" or testimon* or storytell* or story tell* or Patient Education as Topic or Qualitative Research or Health Knowledge, Attitudes, Practice or Health Care Surveys or "Focus Groups" or Interview* or narration or qualitative or Views or "Health Personnel" or "Health Care Providers" or "Health Care Provider" or "Healthcare Providers" or "Healthcare Provider").ab,sh,ti.

#11 exp health education/or exp patient education/

#12 exp qualitative research/

#13 exp interview/

#14 exp health care personnel/

#15 OR/10-14

#16 9 AND 15 limit to (embase and yr="2012 -Current")

Search strategy for Web of Science on 10th February 2017

 # 1 TI=(aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome") OR TI=(aneuploidy or aneuploidies or Trisomy or Trisomies or Trisomic or "Down syndrome" or "Edward syndrome" or "Patau syndrome")
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017

 # 2 TS=(screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence) OR TI=(screen or Screening or Screened or Test or Tested or Diagnostic or Diagnosis or Sequencing or Sequence)
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
 # 3 TS=(Prenatal or Antenatal) OR TI=(Prenatal or Antenatal)
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
 # 4 #3 AND #2
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
 # 5 #4 AND #1
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2010-2017
 # 6 TOPIC: (anxiety or attitudes or choice or "clinical implementation" or Decision-making or "Decision making" or experience* or "Focus Groups" or "Focus Group" or Motivation or interview* or "Patient Acceptance of Health Care" or testimon* or storytell* or story tell* or Patient Education as Topic or Qualitative Research or Health Knowledge, Attitudes, Practice or Health Care Surveys or "Focus Groups" or Interview* or narration or qualitative or Views or "Health Personnel" or "Health Care Providers" or "Health Care Provider" or "Healthcare Providers" or "Healthcare Provider") OR TITLE: (anxiety or attitudes or choice or "clinical implementation" or Decision-making or "Decision making" or experience* or "Focus Groups" or "Focus Group" or Motivation or interview* or "Patient Acceptance of Health Care" or testimon* or storytell* or story tell* or Patient Education as Topic or Qualitative Research or Health Knowledge, Attitudes, Practice or Health Care Surveys or "Focus Groups" or Interview* or narration or qualitative or Views or "Health Personnel" or "Health Care Providers" or "Health Care Provider" or "Healthcare Providers" or "Healthcare Provider")
Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC
Timespan=2012-2017
 # 7 #5 AND #6

Search strategy for identification of ongoing studies

Search strategy for ongoing studies databases on 14th July 2017

ClinicalTrials.gov; ICTRP (OMS); EU Clinical Trials Register; UK clinical Trials gateway

#1 aneuploidy OR aneuploidies OR Trisomy OR Trisomies OR Trisomic OR Down syndrome OR Edward syndrome OR Patau syndrome |

#2 Screen*

#3 #1 AND #2

Full-text articles excluded according to selection criteria

References	Reason of exclusion
Cirigliano V, Ordoñez E, Rueda L, Syngelaki A, Nicolaides KH. Performance of the neoBona test: a new paired-end massively parallel shotgun sequencing approach for cell-free DNA-based aneuploidy screening. Ultrasound Obstet Gynecol. 2017; 49(4): 460-464.	Case-control study
Gerundino F, Giachini C, Contini E, Benelli M, Marseglia G, Giuliani C, Marin F, Nannetti G, Lisi E, Sbernini F, Periti E, Cordisco A, Colosi E, D'ambrosio V, Mazzi M, Rossi M, Staderini L, Minuti B, Pelo E, Cicatiello R, Maruotti GM, Sglavo G, Conti A, Frusconi S, Pescucci C, Torricelli F. Validation of a method for noninvasive prenatal testing for fetal aneuploidies risk and considerations for its introduction in the Public Health System. J Matern Fetal Neonatal Med. 2017; 30(6): 710-716.	Proof of principle study Unclear selection criteria and/or indication
Xu C, Wang T, Liu C, Li H, Chen X, Zhu H, Chen S, Xin Q, Tao J, Huang L, Jiang Z. Noninvasive Prenatal Screening of Fetal Aneuploidy without Massively Parallel Sequencing. Clin Chem. 2017; 63(4): 861-869.	Proof of principle study Unclear selection criteria and/or indication
Pescia G, Guex N, Iseli C, Brennan L, Osteras M, Xenarios I, Farinelli L, Conrad B. Cell-free DNA testing of an extended range of chromosomal anomalies: clinical experience with 6,388 consecutive cases. Genet Med. 2017; 19(2): 169-175.	Validation study Unclear selection criteria and/or indication
Crea F, Forman M, Hulme R, Old RW, Ryan D, Mazey R, Risley MD. The IONA® Test: Development of an Automated Cell-Free DNA-Based Screening Test for Fetal Trisomies 13, 18, and 21 That Employs the Ion Proton Semiconductor Sequencing Platform. Fetal Diagn Ther. 2017 Feb 8.	Validation study Unclear selection criteria and/or indication
Taneja PA, Prosen TL, de Feo E, Kruglyak KM, Halks-Miller M, Curnow KJ, Bhatt S. Fetal aneuploidy screening with cell-free DNA in late gestation. J Matern Fetal Neonatal Med. 2017 Feb; 30(3): 338-342. Epub 2016 Apr 28.	Retrospective study Unclear selection criteria and/or indication
Qi G, Yi J, Han B, Liu H, Guo W, Shi C, et al. Noninvasive prenatal testing in routine clinical practice for a high-risk population: Experience from a center. Medicine. 2016;95(41):e5126.	Retrospective study Lack of information regarding index test and reference standard
Manotaya S, Xu H, Uerpairojkit B, Chen F, Charoenvidhya D, Liu H, Petcharaburanin N, Liu Y, Tang S, Wang X, Dansakul S, Thomsopa T, Gao Y, Zhang H, Xu H, Jiang H. Clinical experience from Thailand: noninvasive prenatal testing as screening tests for trisomies 21, 18 and 13 in 4736 pregnancies. Prenat Diagn. 2016 Mar; 36(3):224-31.	Unclear selection criteria and/or indication No appropriate reference standard in negative cases
Togneri F, Court S, Parks M, Clokie S, Hamilton S, Bibb N, et al. Noninvasive prenatal testing (NIPT) for fetal aneuploidy: The experience of an NHS Regional Genetics Laboratory. BJOG: An International Journal of Obstetrics and Gynaecology. 2016; 123(5).	Congress abstract
Neveling K, Tjwan Thung D, Beulen L, van Rens-Buijsman W, Gomes I, van den Heuvel S, Mieloo H, Derks-Prinsen I, Kater-Baats E, Faas BH. Validation of two-channel sequencing-by-synthesis for noninvasive prenatal testing of fetal whole and partial chromosome aberrations. Prenat Diagn. 2016; 36(3): 216-23.	Proof of concept study Unclear selection criteria and/or indication
Ryan A, Hunkapiller N, Banjevic M, Vankayalapati N, Fong N, Jinnett KN, Demko Z, Zimmermann B, Sigurjonsson S, Gross SJ, Hill M. Validation of an Enhanced Version of a Single-Nucleotide Polymorphism-Based Noninvasive Prenatal Test for Detection of Fetal Aneuploidies. Fetal Diagn Ther. 2016; 40(3):219-223.	Technical validation study Unclear selection criteria and/or indication

References	Reason of exclusion
Kim S, Jung H, Han SH, Lee S, Kwon J, Kim MG, Chu H, Han K, Kwak H, Park S, Joo HJ, An M, Ha J, Lee K, Kim BC, Zheng H, Zhu X, Chen H, Bhak J. An adaptive detection method for fetal chromosomal aneuploidy using cell-free DNA from 447 Korean women. BMC Med Genomics. 2016; 9(1): 61.	Technical validation study Unclear selection criteria and/or indication
Papageorghiou AT, Khalil A, Forman M, Hulme R, Mazey R, Mousa HA, Johnstone ED, McKelvey A, Cohen KE, Risley M, Denman W, Kelly B. Clinical evaluation of the IONA test: a non-invasive prenatal screening test for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol. 2016; 47(2): 188-93.	Case control study
Johansen P, Richter SR, Balslev-Harder M, Miltoft CB, Tabor A, Duno M, Kjaergaard S. Open source non-invasive prenatal testing platform and its performance in a public health laboratory. Prenat Diagn. 2016; 36(6): 530-6.	Case-control study
Li B, Sahota DS, Lao TT, Xu J, Hu SQ, Zhang L, Liu QY, Sun Q, Tang D, Ma RM. Applicability of first-trimester combined screening for fetal trisomy 21 in a resource-limited setting in mainland China. BJOG. 2016; 123 Suppl 3: 23-9.	Lack of data to on relevant outcomes
Poon LC, Dumidrascu-Diris D, Francisco C, Fantasia I, Nicolaides KH. IONA test for first-trimester detection of trisomies 21, 18 and 13. Ultrasound Obstet Gynecol. 2016; 47(2): 184-7.	Case-control study
Shen J, Wen Z, Qin X, Shi Y. Noninvasive fetal trisomy detection by multiplexed semiconductor sequencing: a barcoding analysis strategy. J Hum Genet. 2016; 61(3): 247-52.	Validation study Lack of information regarding elegibility criteria/indication
Norton ME, Baer RJ, Wapner RJ, Kuppermann M, Jelliffe-Pawlowski LL, Currier RJ. Cell-free DNA vs sequential screening for the detection of fetal chromosomal anomalies. Am J Obstet Gynecol. 2016; 214(6): 727.e1-6.	Theoretical model for NIPT test accuracy assessment Unclear selection criteria and/or indication
Bestwick JP, Wald NJ. Antenatal reflex DNA screening for trisomy 18 and trisomy 13 in addition to Down's syndrome. J Med Screen. 2016; 23(4): 171-174.	Validation study Unclear selection criteria and/or indication Lack of data on relevant outcomes
Johansen P, Richter SR, Balslev-Harder M, Miltoft CB, Tabor A, Duno M, Kjaergaard S. Open source non-invasive prenatal testing platform and its performance in a public health laboratory. Prenat Diagn. 2016; 36(6): 530-6.	Validation study Lack of information to calculate accuracy measures
Taneja PA, Snyder HL, de Feo E, Kruglyak KM, Halks-Miller M, Curnow KJ, Bhatt S. Noninvasive prenatal testing in the general obstetric population: clinical performance and counseling considerations in over 85 000 cases. Prenat Diagn. 2016; 36(3): 237-43.	Retrospective study Unclear selection criteria and/or indication Lack of information to calculate accuracy measures
Tynan JA, Kim SK, Mazloom AR, Zhao C, McLennan G, Tim R, Liu L, Hannum G, Hull A, Bombard AT, Oeth P, Burcham T, van den Boom D, Ehrich M. Application of risk score analysis to low-coverage whole genome sequencing data for the noninvasive detection of trisomy 21, trisomy 18, and trisomy 13. Prenat Diagn. 2016; 36(1): 56-62.	Retrospective study Unclear selection criteria and/or indication
Chudova DI, Sehnert AJ, Bianchi DW. Copy-Number Variation and False Positive Prenatal Screening Results. N Engl J Med. 2016; 375(1): 97-8.	Lack of data on relevant outcomes

References	Reason of exclusion
Oneda B, Steindl K, Masood R, Reshetnikova I, Krejci P, Baldinger R, et al. Noninvasive prenatal testing: more caution in counseling is needed in high risk pregnancies with ultrasound anomalies. European journal of obstetrics, gynecology, and reproductive biology. 2016;200:72-5.	Lack of data on relevant outcomes
Minarik G, Repiska G, Hyblova M, Nagyova E, Soltys K, Budis J, Duris F, Sysak R, Gerykova Bujalkova M, Vlkova-Izrael B, Biro O, Nagy B, Szemes T. Utilization of Benchtop Next Generation Sequencing Platforms Ion Torrent PGM and MiSeq in Noninvasive Prenatal Testing for Chromosome 21 Trisomy and Testing of Impact of In Silico and Physical Size Selection on Its Analytical Performance. PLoS One. 2015; 10(12): e0144811.	Case-control study
Alberti A, Salomon LJ, Le Lorc'h M, Couloux A, Bussières L, Goupil S, Malan V, Pelletier E, Hyon C, Vialard F, Rozenberg P, Bouhanna P, Oury JF, Schmitz T, Romana S, Weissenbach J, Vekemans M, Ville Y. Non-invasive prenatal testing for trisomy 21 based on analysis of cell-free fetal DNA circulating in the maternal plasma. Prenat Diagn. 2015; 35(5):471-6.	Case-control study
Norem C, Obolensky E, Bijesse E, Turocy J, Blumberg B, Fehlen-Quizon P, et al. Non-invasive prenatal screening for trisomies-2 years experience in a large Health Maintenance Organization (HMO). Prenatal diagnosis. 2015; 35: 62.	Congress abstract
Curnow KJ, Wilkins-Haug L, Ryan A, Kırkızlar E, Stosic M, Hall MP, Sigurjonsson S, Demko Z, Rabinowitz M, Gross SJ. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide poly morphism-based noninvasive prenatal test. Am J Obstet Gynecol. 2015; 212(1): 79.e1-9.	Lack of data on relevant outcomes
Cheng SH, Jiang P, Sun K, Cheng YK, Chan KC, Leung TY, Chiu RW, Lo YM. Noninvasive prenatal testing by nanopore sequencing of maternal plasma DNA: feasibility assessment. Clin Chem. 2015; 61(10): 1305-6.	Validation study Unclear selection criteria and/or indication
Eiben B, Krapp M, Borth H, Kutur N, Kreiselmaier P, Glaubitz R, Deutinger J, Merz E. Single Nucleotide Polymorphism-Based Analysis of Cell-Free Fetal DNA in 3000 Cases from Germany and Austria. Ultrasound Int Open**.** 2015; 1(1): E8-E11.	Retrospective study Unclear selection criteria and/or indication
Hacivelioglu S, Uysal A, Gungor AN, Gencer M, Cakir DU, Cosar E. The effect of maternal polycystic ovary morphology on first-trimester maternal serum biochemical markers of aneuploidy and fetal nuchal translucency thickness. Clin Exp Obstet Gynecol. 2015; 42(1): 32-5.	Lack of data on relevant outcomes
Zhang H, Zhao YY, Song J, Zhu QY, Yang H, Zheng ML, Xuan ZL, Wei Y, Chen Y, Yuan PB, Yu Y, Li DW, Liang JB, Fan L, Chen CJ, Qiao J. Statistical Approach to Decreasing the Error Rate of Noninvasive Prenatal Aneuploid Detection caused by Maternal Copy Number Variation. Sci Rep. 2015; 5: 16106.	Validation study Unclear selection criteria and/or indication
Stokowski R, Wang E, White K, Batey A, Jacobsson B, Brar H, Balanarasimha M, Hollemon D, Sparks A, Nicolaides K, Musci TJ. Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. Prenat Diagn. 2015; 35(12): 1243-6.	Retrospective study Lack of information regarding elegibility criteria
Meck JM, Kramer Dugan E, Matyakhina L, Aviram A, Trunca C, Pineda-Alvarez D, Aradhya S, Klein RT, Cherry AM. Noninvasive prenatal screening for aneuploidy: positive predictive values based on cytogenetic findings. Am J Obstet Gynecol. 2015; 213(2): 214.e1-5.	Retrospective study Lack of information regarding elegibility criteria

References	Reason of exclusion
Wang JC, Sahoo T, Schonberg S, Kopita KA, Ross L, Patek K, Strom CM. Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases. Genet Med. 2015; 17(3): 234-6.	Retrospective study Uncertainty regarding the independent assessement of index test/reference standard
Wald NJ, Huttly WJ, Bestwick JP, Aquilina J, Peregrine E. Reflex antenatal DNA screening for Down syndrome. Prenat Diagn. 2015; 35(11): 1154.	Lack of data on relevant outcomes
Rava RP, Srinivasan A, Sehnert AJ, Bianchi DW. Circulating fetal cell-free DNA fractions differ in autosomal aneuploidies and monosomy X. Clin Chem. 2014; 60(1): 243-50.	Validation study Unclear selection criteria and/or indication Lack of data to calculate accuracy measures
Beamon CJ, Hardisty EE, Harris SC, Vora NL. A single center's experience with noninvasive prenatal testing. Genetics in medicine : official journal of the American College of Medical Genetics. 2014;16(9):681-7.	Retrospective study Uncertainty regarding independent assessment index test/reference standard
Shulman L, Dungan J, Ginsberg N. The use of noninvasive prenatal screening (NIPS) in the assessment of an abnormal fetal ultrasound. Prenatal diagnosis. 2014;34:62.	Congress abstract
Lin G, Gao Y, Yin X, Tan Y, Zhang H, Lu G, et al. Clinical implementation of noninvasive prenatal testing in twin pregnancies with assisted reproductive technique treatment. Prenatal diagnosis. 2014;34:14.	Congress abstracts
del Mar Gil M, Quezada MS, Bregant B, Syngelaki A, Nicolaides KH. Cell-free DNA analysis for trisomy risk assessment in first-trimester twin pregnancies. Fetal Diagn Ther**.** 2014; 35(3): 204-11.	Duplicated data (Pregnant women population included in Bevilacqua et al 2015)
Friel LA, Czerwinski JL, Singletary CN. The impact of noninvasive prenatal testing on the practice of maternal-fetal medicine. Am J Perinatol. 2014; 31(9): 759-64.	Lack of data on relevant outcomes
Li PQ, Zhang J, Fan JH, Zhang YZ, Hou HY. Development of noninvasive prenatal diagnosis of trisomy 21 by RT-MLPA with a new set of SNP markers. Arch Gynecol Obstet. 2014; 289(1): 67-73.	Validation study Lack of information regarding elegibility criteria/indication Lack of data to calculate accuracy measures
Hooks J, Wolfberg AJ, Wang ET, Struble CA, Zahn J, Juneau K, Mohseni M, Huang S, Bogard P, Song K, Oliphant A, Musci TJ. Non-invasive risk assessment of fetal sex chromosome aneuploidy through directed analysis and incorporation of fetal fraction. Prenat Diagn. 2014; 34(5): 496-9.	Case-control study
Hall MP, Hill M, Zimmermann B, Sigurjonsson S, Westemeyer M, Saucier J, Demko Z, Rabinowitz M. Non-invasive prenatal detection of trisomy 13 using a single nucleotide polymorphism- and informatics-based approach. PLoS One. 2014; 9(5): e96677.	Case-control study
Juneau K, Bogard PE, Huang S, Mohseni M, Wang ET, Ryvkin P, Kingsley C,Struble CA, Oliphant A, Zahn JM. Microarray-based cell-free DNA analysis improves noninvasive prenatal testing. Fetal Diagn Ther. 2014; 36(4): 282-6.	Retrospective study Lack of information regarding elegibility criteria/indication (mixed risk population)

References	Reason of exclusion
Dar P, Curnow KJ, Gross SJ, Hall MP, Stosic M, Demko Z, Zimmermann B, Hill M, Sigurjonsson S, Ryan A, Banjevic M, Kolacki PL, Koch SW, Strom CM, Rabinowitz M, Benn P. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. Am J Obstet Gynecol. 2014; 211(5): 527.e1-527.e17.	Retrospective study Lack of information regarding elegibility criteria/indication Uncertainty regarding the independent assessment index test/reference standard
Shaw SW, Hsiao CH, Chen CY, Ren Y, Tian F, Tsai C, Chen M, Cheng PJ. Noninvasive prenatal testing for whole fetal chromosomal aneuploidies: a multicenter prospective cohort trial in Taiwan. Fetal Diagn Ther. 2014; 35(1): 13-7.	Unclear selection criteria and/or indication
Yu SC, Chan KC, Zheng YW, Jiang P, Liao GJ, Sun H, Akolekar R, Leung TY, Go AT, van Vugt JM, Minekawa R, Oudejans CB, Nicolaides KH, Chiu RW, Lo YM. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. Proc Natl Acad Sci U S A. 2014; 111(23): 8583-8.	Retrospective study Lack of information regarding elegibility criteria/indication Uncertainty regarding the independent assessment index test/reference standard
Bijok J, Gorzelnik K, Massalska D, Ilnicka A, Pawłowska B, Zimowski JG,Kucińska-Chahwan A, Jakiel G, Roszkowski T. [Non-invasive prenatal diagnosis of the most common aneuploidies with cell-free fetal DNA in maternal serum--preliminary results]. Ginekol Pol. 2014; 85(3): 208-13.	Article in Polish
Syngelaki A, Pergament E, Homfray T, Akolekar R, Nicolaides KH. Replacing the combined test by cell-free DNA testing in screening for trisomies 21, 18 and 13: impact on the diagnosis of other chromosomal anomalies. Fetal Diagn Ther. 2014; 35(3): 174-84.	Retrospective study Lack of information regarding elegibility criteria/indication Uncertainty regarding the independent assessment index test/reference standard
Hall MP, Hill M, Zimmermann B, Sigurjonsson S, Westemeyer M, Saucier J, Demko Z, Rabinowitz M. Non-invasive prenatal detection of trisomy 13 using a single nucleotide polymorphism- and informatics-based approach. PLoS One**.** 2014; 9(5): e96677.	Case-control study
Liao C, Yin AH, Peng CF, Fu F, Yang JX, Li R, Chen YY, Luo DH, Zhang YL, Ou YM, Li J, Wu J, Mai MQ, Hou R, Wu F, Luo H, Li DZ, Liu HL, Zhang XZ, Zhang K. Noninvasive prenatal diagnosis of common aneuploidies by semiconductor sequencing. Proc Natl Acad Sci U S A. 2014; 111(20): 7415-20.	Validation study Lack of information regarding elegibility criteria/indication
Nicolaides KH, Musci TJ, Struble CA, Syngelaki A, Gil MM. Assessment of fetal sex chromosome aneuploidy using directed cell-free DNA analysis. Fetal Diagn Ther. 2014; 35(1): 1-6.	Case-control study
Liao C, Fu YG, Huang SY, Fu F, Xie GE. Rapid noninvasive prenatal diagnosis of Down syndrome with Ion Proton. Prenatal diagnosis**.** 2013; 33: 76-7.	Congress abstract
Hofmann W, Entezami M, Haug K, Blank C, Wustemann M, Schulze B, et al. Diagnostic accuracy for the noninvasive prenatal detection of common autosomal aneuploidies. Prenatal diagnosis. 2013; 33: 75.	Congress abstract
Nicolaides K, Syngelaki A, Ashoor G, Musci T, Wang E, Song K. Clinical performance comparison of Harmony™ Prenatal Test and first-trimester combined screening in general pregnancy population. Prenatal diagnosis. 2013; 33: 3.	Congress abstract
Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM. First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. Ultrasound Obstet Gynecol. 2013; 42(1): 41-50.	Case-control study

References	Reason of exclusion
Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. Prenat Diagn. 2013; 33(7): 662-6.	Lack of data on relevant outcomes
Futch T, Spinosa J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. Prenat Diagn. 2013; 33(6): 569-74.	Retrospective study Lack of information regarding elegibility criteria/indication
Brar H, Wang E, Struble C, Musci TJ, Norton ME. The fetal fraction of cell-free DNA in maternal plasma is not affected by a priori risk of fetal trisomy. J Matern Fetal Neonatal Med. 2013; 26(2): 143-5.	Duplicated (a *post hoc* analysis of NICE study population)
Guex N, Iseli C, Syngelaki A, Deluen C, Pescia G, Nicolaides KH, Xenarios I, Conrad B. A robust second-generation genome-wide test for fetal aneuploidy based on shotgun sequencing cell-free DNA in maternal blood. Prenat Diagn. 2013; 33(7): 707-10.	Case control study Lack of independent assessment of index test/reference standard
Ashoor G, Syngelaki A, Wang E, Struble C, Oliphant A, Song K, Nicolaides KH. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. Ultrasound Obstet Gynecol. 2013; 41(1): 21-5.	Case-control study
Gorduza EV, Popescu R, Caba L, Ivanov I, Martiniuc V, Nedelea F, et al. Prenatal diagnosis of 21 trisomy by quantification of methylated fetal DNA in maternal blood: study on 10 pregnancies. Revista Romana De Medicina De Laborator. 2013; 21(3-4): 275-84.	Lack of data on relevant outcomess
Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. Ultrasound Obstet Gynecol. 2013; 42(1): 34-40.	Duplicated data (Pregnant women population included on Quezada et al)
van den Oever JM, Balkassmi S, Johansson LF, Adama van Scheltema PN, Suijkerbuijk RF, Hoffer MJ, Sinke RJ, Bakker E, Sikkema-Raddatz B, Boon EM. Successful noninvasive trisomy 18 detection using single molecule sequencing. Clin Chem. 2013; 59(4): 705-9.	Retrospective study (both selected euploid and aneuploid samples) Lack of information regarding elegibility criteria/indication (mixed risk population)
Fairbrother G, Johnson S, Musci TJ, Song K. Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. Prenat Diagn**.** 2013; 33(6): 580-3.	Lack of reference standard testing
Ashoor G, Syngelaki A, Poon LC, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound Obstet Gynecol. 2013; 41(1): 26-32.	Lack of data on relevant outcomes
Fairbrother G, Johnson S, Musci T, Song K. Clinical experience of Harmony™ Prenatal Test, a noninvasive prenatal test, in a general screening population. Prenatal diagnosis. 2013; 33: 78.	Congress abstract
Bianchi D, Platt L, Goldberg J, Abuhamad A, Sehnert A, Rava R. Whole genome maternal plasma DNA sequencing accurately detects autosomal and sex chromosome aneuploidies. Prenatal diagnosis. 2012;32:3-4.	Congress abstract
Jiang FM, Ren JH, Chen F, Zhou YQ, Xie JS, Dan S, et al. Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. BMC medical genomics. 2012;5.	Unclear selection criteria and/or indication
Lau TK, Chan MK, Lo PSS, Chan HYC, Chan WSK, Koo TY, et al. Clinical utility of noninvasive fetal trisomy (NIFTY) test – early experience. Journal of Maternal-Fetal & Neonatal Medicine. 2012;25(10):1856-9.	Lack of information regarding elegibiity criteria/indication (mixed-risk population)

References	Reason of exclusion
Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP; Maternal Blood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. 2012; 119(5): 890-901.	Nested case-control study
Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol. 2012; 207(5): 374.e1-6.	Retrospective study Lack of information regarding elegibility criteria/indication
Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, Nelson SF, Canick JA. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med. 2012; 14(3): 296-305.	Nested case-control study
Dan S, Wang W, Ren J, Li Y, Hu H, Xu Z, Lau TK, Xie J, Zhao W, Huang H, Xie J, Sun L, Zhang X, Wang W, Liao S, Qiang R, Cao J, Zhang Q, Zhou Y, Zhu H, Zhong M, Guo Y, Lin L, Gao Z, Yao H, Zhang H, Zhao L, Jiang F, Chen F, Jiang H, Li S, Li Y, Wang J, Wang J, Duan T, Su Y, Zhang X. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. Prenat Diagn**.** 2012;32(13): 1225-32.	Unclear selection criteria and/or indication
Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012; 206(4): 319.e1-9.	Validation study Lack of data on relevant outcomes
Canick JA, Kloza EM, Lambert-Messerlian GM, Haddow JE, Ehrich M, van den Boom D, Bombard AT, Deciu C, Palomaki GE. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenat Diagn. 2012; 32(8): 730-4.	Validation study Lack of data on relevant outcomes
Stumm M, Entezami M, Trunk N, Beck M, Löcherbach J, Wegner RD, Hagen A, Becker R, Hofmann W. Noninvasive prenatal detection of chromosomal aneuploidies using different next generation sequencing strategies and algorithms. Prenat Diagn. 2012; 32(6): 569-77.	Validation study Unclear selection criteria and/or indication Lack of data on relevant outcomes
Zimmermann B, Hill M, Gemelos G, Demko Z, Banjevic M, Baner J, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. Prenatal diagnosis. 2012; 32(13): 1233-41.	Proof of principle study Lack of data on relevant outcomes
Song K, Ashoor G, Syngelaki A, Wagner M, Birdir C, Struble C, et al. Clinical evaluation of a directed cfDNA analysis method for non-invasive prenatal fetal trisomy detection. Prenatal Diagnosis. 2012; 32: 16-7.	Congress abstract
van den Oever JM, Balkassmi S, Verweij EJ, van Iterson M, Adama van Scheltema PN, Oepkes D, van Lith JM, Hoffer MJ, den Dunnen JT, Bakker E, Boon EM. Single molecule sequencing of free DNA from maternal plasma for noninvasive trisomy 21 detection. Clin Chem**.** 2012; 58(4): 699-706.	Validation study (both selected euploid and aneuploid samples) Lack of data on relevant outcomes
Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol. 2012; 206(4): 322.e1-5.	Nested case-control study

References	Reason of exclusion
Sparks AB, Wang ET, Struble CA, Barrett W, Stokowski R, McBride C, Zahn J, Lee K, Shen N, Doshi J, Sun M, Garrison J, Sandler J, Hollemon D, Pattee P, Tomita-Mitchell A, Mitchell M, Stuelpnagel J, Song K, Oliphant A. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn. 2012; 32(1): 3-9.	Validation study (both selected euploid and aneuploid samples) Lack of data on relevant outcomes
Sehnert AJ, Rhees B, Comstock D, de Feo E, Heilek G, Burke J, Rava RP. Optimal detection of fetal chromosomal anomalies by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem. 2011; 57(7): 1042-9.	Unclear selection criteria and/or indication
Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, Lun FM, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ. 2011; 342: c7401.	Validation study (archived maternal samples from euploid and aneuploid cases) sampling Lack of data on relevant outcomes
Chen EZ, Chiu RW, Sun H, Akolekar R, Chan KC, Leung TY, Jiang P, Zheng YW, Lun FM, Chan LY, Jin Y, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. PLoS One. 2011; 6(7): e21791.	Case-control study
Tong YK, Jin S, Chiu RW, Ding C, Chan KC, Leung TY, Yu L, Lau TK, Lo YM.Noninvasive prenatal detection of trisomy 21 by an epigenetic-genetic chromosome-dosage approach. Clin Chem. 2010; 56(1): 90-8.	Technical validation study (both selected euploid and aneuploid samples) Lack of data on relevant outcomes
Ghanta S, Mitchell ME, Ames M, Hidestrand M, Simpson P, Goetsch M, Thilly WG, Struble CA, Tomita-Mitchell A. Non-invasive prenatal detection of trisomy 21 using tandem single nucleotide polymorphisms. PLoS One. 2010; 5(10): e13184.	Validation study (both selected euploid and aneuploid samples) Lack of data on relevant outcomes
Alberry MS, Maddocks DG, Hadi MA, Metawi H, Hunt LP, Abdel-Fattah SA, Avent ND, Soothill PW. Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction. Am J Obstet Gynecol. 2009; 200(1): 98.e1-6.	Lack of data on relevant outcomes
Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, Foo CH, Xie B, Tsui NB, Lun FM, Zee BC, Lau TK, Cantor CR, Lo YM. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A. 2008; 105(51): 20458-63.	Proof of principle study (both selected aneuploid and euploid samples) Lack of data on relevant outcomes
Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A. 2008; 105(42): 16266-71.	Proof of principle study (both selected aneuploid and euploid samples) Lack of data on relevant outcomes
Dhallan R, Guo X, Emche S, Damewood M, Bayliss P, Cronin M, Barry J, Betz J, Franz K, Gold K, Vallecillo B, Varney J. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. Lancet. 2007; 369(9560): 474-81.	Proof or principle study Lack of information on elegibility criteria/indication Uncertainty regarding the independent assessment of index test/reference standard

DESCRIPTION OF THE EVIDENCE USED

Guidelines for diagnosis and management

Table A1: Overview of guidelines

Name of society/ organisation issuing guidance	Date of issue	Country/ies to which applicable	Summary of recommendations related to NIPT	Level of evidence
The American College of Medical Genetics and Genomics (ACGM) Noninvasive prenatal screening for fetal aneuploidy 2016 update: a position Statement of the ACGM	July 2016	USA	ACGM recommends: Allowing patients to select diagnostic or screening approaches for the detection of fetal aneuploidy and/or genomic changes that are consistent with their personal goals and preferences. Informing all women that diagnostic testing (CVS or amniocentesis) is an option for the detection of chromosome anomalies and clinically significant selected copy variants. Informing all pregnant women that NIPS is the most sensitive option for traditionally screened aneuploides (T21, T18 and T13). Referring patients to a trained genetics professional when an increased risk of aneuploidy is reported after NIPT. Offering diagnostic testing when a positive screening test result is reported after NIPS Laboratories should not offer screening for autosomal aneuploidies other than those involving chromosomes 13, 18 and 21. Offering diagnostic testing for a no-call NIPS result due to low fetal fraction if maternal blood for NIPS was drawn at an appropriate gestational age. A repeat blood draw is not considered appropriate. Offering aneuploidy screening other than NIPT in cases of significant obesity. Informing patients that a no-call result may be due to long stretches of homozygosity, which could be due to either uniparental disomy or parental consanguinity is received. Offering diagnostic testing with chromosomic microarrays when a no-call result is obtained. In pregnancies with multiple gestations and/or donor oocyts, testing laboratories should be contacted regarding the validity of NIPT before it is offered.	
The American College of Obstetricians and	May 2016	USA	Because cfDNA is a screening test with the potential for false positive and false negative results, such testing should not be used as a substitute for diagnostic testing.	A

Name of society/ organisation issuing guidance	Date of issue	Country/ies to which applicable	Summary of recommendations related to NIPT	Level of evidence
Gynecologists (ACOG) and Society for Maternal-Fetal Medicine (SMFM) "Practice Bullletin Number 163: Clinical Management Guidelines for obstetrician-gynecologists. Screening for fetal aneuploidy"			All women with a positive NIPT result should have a diagnostic procedure before any irreversible action, such as pregnancy termination. Women whose cfDNA screening are not reported, are undeterminate, or uninterpretable should receive councelling and be offered comprehensive ultrasound evaluation and diagnostic testing because of increase risk of aneuploidy. NIPT for microdeletions have not been validated clinically and are not recommended at this time. Some women who receive a positive test result from traditional screening may prefer to have NIPT screening rather than undergoe definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some foetuses with aneuploidy.	A A B C
The ACOG and SMFM "Committee Opinion Number 640:cell free DNA screening for fetal Aneuploidy"	September 2015	USA	A discussion of risks, benefits and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing should occur with all patients Although patients may choose cfDNA analysis regardless of her risk status, they should understand limitations and benefits in the context of alternative options. Given the risk for inaccurate results, a diagnostic test should be recommended for a patient who has a positive cfDNA test result. Parallel or simultaneous screening is not cost-effective and should not be performed. However, use of cfDNA screening as a follow up test for patients with a positive traditional screening result is reasonable for patients who want to avoid a diagnostic test. Management decisions, including termination of pregnancy should not be based on cfDNA screening alone. Patients whose results are not reported, indeterminate or uninterpretable should receive further counselling and should be offered comprehensive ultrasound evaluation and diagnostic testing. If a fetal structural anomaly is identified on ultrasound examination, diagnostic testing should be offered. Patients should be counselled that a negative cfDNA test result does not ensure unaffected pregnancy. CfDNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects; patients who are undergoing cfDNA should be offered maternal serum-fetoprotein screening or ultrasound evaluation. Before offering NIPT family history should be reviewed in order to determine if the patient should be offered other forms of screening Conclusion statement	

Name of society/ organisation issuing guidance	Date of issue	Country/ies to which applicable	Summary of recommendations related to NIPT	Level of evidence
			A discussion of risks, benefits and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing, should occur with all patients. Such a discussion should include the advisability and applicability of cell-free DNA and other screening tests and the interpretation of test results, based on patient risk stratification.	
European Society of Human Genetics (ESHG) and American Society of Human Genetics (ASHG) Position document on prenatal screening	October 2015	USA and Europe	The main options for using NIPT in practice are: 1) NIPT as a second test after combined first trimester screening using the current high risk cut off point. 2) NIPT as a replacement for combined first trimester screening. 3) NIPT as a second test after adapted risk cut off points (intermediate risk).	
Austrian Society of Obstetrics and Gynecology, Austrian Society of Ultrasound in Medicine, Austrian Society of Pre-and Perinatal Medicine, ASHG, German Society of Ultrasound in Medicine, Fetal Medicine Foundation Germany, Swiss Society of Ultrasound in Medicine "Cell-Free DNA testing for fetal chromosomal anomalies in clinical practice: Austrian German-Swiss Recommendations for NIPT	October 2015	Austria Germany Switzerland	CfDNA testing should be offered only after, or in conjunction with, a qualified ultrasound and following appropriate counselling about the nature, scope and significance of the test. CfDNA tests are sceening tests. A high-risk cfDNA testing result should always be confirmed by an invasive diagnostic test before a clinical consequence is drawn from the findings CfDNA tests can be used as secondary screening test for trisomy 21 (DS) for the reduction of invasive procedures after a high or intermediate risk result from first trimester combined tes (1 in 1,000 or >1:500). It should be noted that, even when cfDNA testing is used as a secondary screening, invasive testing is still the method of choice when the adjusted risk for trisomy 21 after combined test is >1:10 or the fetal nuchal translucency thickness is >3.5 mm or a fetal malformation is present. CfDNA can also be used as a primary screening method for fetal trisomy 21 in pregnant women of every age and risk group In general, it should be noted that the performance of cfDNA screening for trisomy 18 and trisomy 13 is lower than that for trisomy 21	
The ISPD "Position Statement from the chromosome anomaly screening Committee on behalf of the Board of the ISPD	April 2015	International	The following protocol options are currently considered appropriate: 1. CfDNA screening as a primary test offered to all pregnant women. 2. CfDNA secondary to a high risk assessment based on serum and ultrasound and screening protocols. 3. CfDNA contingently offered to a broader group of women ascertained as having high or intermediate risks by conventional screening. Contingent provision could also include a protocol in which women with very high risks are offered invasive diagnosis, while those with intermediate risk cfDNA. 4. Ultrasound at 11 to 13 completed weeks combined with serum markers at 9 to 13 weeks´ gestation.	

Name of society/ organisation issuing guidance	Date of issue	Country/ies to which applicable	Summary of recommendations related to NIPT	Level of evidence
			5. Extending option 4 to include other trimester serum or sonographic markers 6. A contingent test whereby women with borderline risks from option 4 have option 5 at a specialist centre. 7. Four maternal markers (quadruple test) at 15 to 19 weeks, for women who first attend after 13 weeks 6 days gestation. 8. Combining options 4 and 7 in either stepwise or contingent protocol, provided all markers are included in final risk assessment. Integrated screening can be offered when CVS is not available. 9. Contingent second trimester ultrasound to modify risks for aneuploidy for women having options 4, 7 or 8.	
Italian Ministry of Health-Higher Health Council of Italy (Consiglio Superiori de Sanita)	May 2015	Italy	NIPT is not a diagnostic test. NIPT investigates the probability of a foetus being affected by the most common aneuploidies, with specificity and sensitivity, which are significantly higher than combined testing. NIPT defines the presence of the specific fetal disease on a probability basis. Therefore, any positive results must be confirmed by invasive technique (chorionic villus sampling/amniocentesis NIPT should be preceded by ultrasound and pre-test counceling. Results are reliable if obtained from a percentage of free fetal DNA that is not less than 4% of total free DNA present in maternal plasma. The investigation is currently targeted and validated for major autosomal aneuoploides (T21, T18, T13). The chromosomal anomalies investigated concern only a portion, although relevant (50-70%) of the chromosome aberrations which might be present in the foetus. NIPT can be performed in twin pregnancies, even after gamete donation. Generally, results indicative of a “low risk of trisomy” should be considered reassuring for the mother. However, the results of the screening could refer to genetic characteristics of the cytotrophoblast (placenta), that in rare cases may be inconsistent with those of the foetus (feto-placental discrepancy) Since NIPT represents the most sensitive non-invasive test for prenatal diagnosis, it is necessary that its introduction-as first or second-choice test for the detection of major autosomal aneuploidies must be set up at central and regional level It is necessary to provide information campaigns to the public and training to prefessionals, to ensure equity in access. Currently, NIPT-based screening has no reason to be extended to diseases other than T21, T18 and T13	

Name of society/ organisation issuing guidance	Date of issue	Country/ies to which applicable	Summary of recommendations related to NIPT	Level of evidence
The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) ISUOG consensus statement on the impact of NIPT on prenatal ultrasound practice	2014	International	All women should be offered a first-trimester ultrasound scan, regardless of their intention to undergo NIPT. Pre-test counselling is essential. Various options should be explained clearly to women, discussing the pros and cos of each, including the expected performance and adverse effects. Following a normal early pregnancy scan, three options should be considered for women who wish to have further risk assessment for T21 and, to a lesser extent, T13 and T18. 1) Screening strategies based on individual risk calculated from maternal age and nuchal translucency and/or maternal markers and/or other ultrasound markers in the first trimester. At this moment, ISUOG endorsed this strategy. 2) Invasive testing based on background risk (including for example, maternal age and history of aneuploidy), with no other individual calculation or risk 3) NIPT as first-line screening test. NIPT is not a diagnostic test and confirmatory invasive testing is required NIPT has not been evaluated extensively in low risk populations First-trimester risk estimates for T21, T18 and T13 based on nuchal translucency measurements and maternal biochemistry should not be computed in a woman who has already received a normal NIPT results. NIPT may be discussed as an alternative to invasive testing following an abnormal result on combined screening or offered to patients who are not sufficiently reassured by an intermediate result. The role of NIPT as an alternative to standard invasive testing in women considered to be at very high risk (>1:10) after combined screening but with no ultrasound anomaly should be evaluated in prospective studies. Expert opinion suggests that NIPT should not replace invasive testing. In the presence of a fetal structural anomaly, the indications for fetal karyotyping and/or microarray testing should not be modified by an normal NIPT result. Accuracy of NIPT in twin pregnancies should be investigated further. Variations in NIPT performance by different providers should be investigated further. The so called “genetic sonogram” which includes looking for soft markers of trisomy 21 should not be performed in women with normal NIPT results due to its high false positive rate and poor positive predictive value. Prospective, publicly funded studies assessing the cost-effectiveness of various screening strategies should be performed as a matter of urgency.	

Name of society/ organisation issuing guidance	Date of issue	Country/ies to which applicable	Summary of recommendations related to NIPT	Level of evidence
BeSHG (Belgium Society for Human Genetics), approved by College of Medical Genetics	December 2014	Belgium	The use of NIPT for prenatal screening in a general Belgian obstetric population results in the smallest number of missed diagnoses of fetal trisomy 21. Morever the number of invasive tests that will have to be performed as a result of a positive screening test will be much lower than in the current situation using the combined first trimester screening as the primary screening instrument. Therefore, NIPT is currently the best choice as a first tier prenatal screening tool for trisomy 13, 18 and 21. Good clinical practice with NIPT: NIPT is the first tier screening tool for prenatal screening for fetal trisomy 13, 18 and 21 Pre-test counselling with information about the different screening options and their possibilities and limitations is required. Informed consent has to be obtained. NIPT does not replace the first trimester fetal ultrasound for measurement of the nuchal translucency and identification of fetal malformations; fetal ultrasound should be performed before NIPT screening to ascertain whether there is an indication for another prenatal test or additional genetic counselling. In case of ultrasound anomalies, including NT> 95 percentile, invasive techniques are indicated. Acquiring pre-NIPT family history by means of pedigree information is standard of practice to make sure no other prenatal test is indicated. Referral of a patient with positive NIPT for invasive testing, preferably amniocentesis is necessary. Accreditation of genetic labs offering NIPT and regular peer revie on an national level is required. NIPT should be performed with caution in case of an increased in maternal BMI (>30) and in case of multiple pregnancy. NIPT is not indicated in the patient has undergone any of the following treatments in the past 3 months: blood transfusions, immunotherapy, stem cell transplant or organ transplantation.	

Abbreviations: CVS-chorionic villus sampling, T21-trisomy 21, T18-trisomy 18, T13-trisomy 13, NIPS-non-invasive prenatal screening, NIPT-non-invasive prenatal testing, cfDNA-cell free DNA, NT-nuchal translucency, BMI-body mass index

Evidence tables of individual studies included for clinical effectiveness and safety domains

Table A2: Characteristics of included studies on general pregnant population

Author(s): Sarno et al [39]	
Study characteristics	Study design: prospective DTA trial Year of publication: 2016 Study's registration number in clinical trial database: not reported Country/ies of recruitment: UK Setting: routine prenatal screening Data collection period: October 2012 to August 2015 Target population: high-risk pregnant population (singleton or twin pregnancies). Target condition prevalence in the enrolled population: Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): Harmony™ Prenatal Test/CSS Country where samples were analysed: USA Cut off for NIPT: risk score for T21, 18 and T13 was ranged between >99% to <0.01%
Population characteristics	Maternal age in years (median [169]): 36.3 [33.2-39.3] Gestational age in weeks (median [169]): 11.9 [10.6-12.9] BMI in Kg/m^2 (median [169]): 23.3 [21.2-26.5] Pregnancy by ART (no [% pts]): 1015 (9.5%) Inclusion criteria: singleton or twin pregnant women at 11+0 to 13+6 weeks' gestation who received NIPT as an option following FCT or as part of routine screening. All pregnant women had undergone FCT. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 10698 Population excluded: 0 Population included: 10698 -with NIPT result: 10530 -with comparator result: no intervention -with reference standard result: not reported

<table>
<tr><td></td><td>Reference standard (% pts): fetal karyotype (not specified method used)
Sample processing protocol
Maternal blood samples were collected at enrolment centres and sent to laboratory for its analysis.</td></tr>
<tr><td rowspan="2">Outcomes</td><td>Performance of NIPT for T21, T18 and T13
-Test failure (% samples): 316 samples (2.9%) in the first analysis. In 235 samples cfDNA testing was repeated and this provided results in 148 cases (test failure after 2nd NIPT testing: 1.5% or 168/10698).
-Uncertain results rate (% samples): NA</td></tr>
<tr><td>Diagnostic accuracy measures for all trisomies in pregnancies with outcome data, excluding women with low fetal fractions, test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA</th></tr><tr><td>TP</td><td>205</td></tr><tr><td>TN</td><td>10288</td></tr><tr><td>FP</td><td>23 (FP rate= 0.22%)</td></tr><tr><td>FN*</td><td>14 (FN rate= 6.4%)</td></tr><tr><td>S*</td><td>93.6 (89.6-96.2)</td></tr><tr><td>Sp</td><td>99.8 (99.7-99.9)</td></tr><tr><td>PPV</td><td>89.9 (85.3-93.2)</td></tr><tr><td>NPV</td><td>99.9 (99.8-99.9)</td></tr></table>*FN rate T21=1.26%, FN rate T18= 10.8% and FN rate T13= 46.7%; S T21= 98.7%, S T18= 89.1% and S T13= 53.3%.
Additional population relevant outcomes
Safety
-Increase in the number of children born with other major unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies of uncertain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing cfDNA vs. standard screening):NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Norton et al [31]	
Study characteristics	**Non-invasive Examination of Trisomy-NEXT study** Study design: prospective, blinded, multicentre comparative DTA trial Year of publication: 2015 Study's registration number in clinical trial database: NCT01511458 Country/ies of recruitment: USA, Canada, Sweden, Belgium, Netherland, Italy Setting: routine prenatal-screening Data collection period: March 2012- April 2013 Target population: unselected general pregnancy population Target condition prevalence in the enrolled population (n=15841 pts): 1/417 for T21, 1/1584 for T18, 1/2640 for T13 and 1/5280 or lower for other aneuploidies i.e. 45,X, marker chromosomes, unbalanced translocations, balanced translocations, deletion 7p, deletion/duplication 5p, 1q41 deletion and isochromosome Yp. Comparator: standard screening (measurement of nuchal translucency and biochemical analytes i.e. serum pregnancy-associated plasma protein A and total or free beta submit of human chorionic gonadotropin) Cut off point comparator: mid-trimester risk of at least 1/270 for T21 and at least 1/150 for T18 and T13 Index test (trademark/technique type): Harmony Prenatal Test/CSS Country where samples were analysed: USA (Ariosa Clinical Laboratory) Cut off for NIPT: 1/100 or higher was clasificated as high risk
Population characteristics	Maternal age in years (mean [169]): 31 [18-28] Gestational age in weeks (median [169]): 12.5 [10.0-14.3] Maternal weight (median [169]: 65.8 [31.8-172.4] Pregnancy by assisted reproductive techniques (% pts): 3.0 Inclusion criteria: populations were at least 18 years of age and had a singleton pregnancy between 10.0 and 14.3 weeks of gestation (mean gestational age: 12.5) Exclusion criteria: populationsoutside the gestational-age window, no standard screening result, known maternal aneuploidy or cancer, donor oocytes conception, twin pregnancies or an empty gestational sac identified on ultrasonography
Study protocol	*Population enrollment flow* Population enrolled: 18955 Population excluded: 3114 Reasons for exclusion: -did not meet the eligibility criteria or meet exclusion criteria (n: 445) -blood-collection or labeling error (n: 384) -absence of a result on standard screening (n: 308)

-absence of a result on cfDNA (n: 488)
-were lost to follow-up (n: 1489)
Population included: 15841
-with NIPT result: 15841
-with comparator result: 15841
-with reference standard results: 625/15481 (3.9 %)
Reference standard (% pts): CVS (21.6), amniocentesis (67.5), products of conception (2.6) and newborn (8.3)
Sample processing protocol
Blood samples were collected into cell-free DNA BCT tubes (Streck), sent to sponsor (Ariosa Diagnostic) and analysed at 7 days after collection. The analyses and interpretation of cfDNA data were performed in a blinded fashion with respect to result of ultrasonographic and standard screening.

Outcomes

Performance of NIPT test for T21, T18 and T13

-Test failure (% samples): 3% (488/16329)
-Uncertain results rate (% samples): NA

Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding women with low fetal fractions, test failures and miscarriages (calculated based on study results)

Variable	Standard screening	cfDNA test[1,2]
TP	30	38
TN	14,949	15,794
FP	854 (FPrate: 5.4%)	9 (FPrate: 0.06%)
FN	8 (FN rate: 21%)	0
S	78.9 (62.7-90.4)	100 (90.7-100)*
Sp	94.6 (94.2-94.9)	99.9 (99.9-100)**
PPV	3.4 (2.3-4.8)	80.9 (66.7-90.9)**
NPV	99.9 (99.9-100)	100 (99.9-100)***
AUC	0.958	0.999**

[1]In low risk population (n=14,957), there were 8 TP cases, 8 FP cases, no FN cases, S=100% [63.1-100], Sp=99.9% [99.9-99.94], PPV=50.0% [24.7-75.3] and NPV= 100% [99.9-100]

[2]In women with maternal age < 35 yrs (n=11,994), there were 19 TP cases, 6 FP cases, no FN cases, S=100% [82.4-100], Sp=99.9% [99.9-99.94], PPV=76.0% [54.9-90.6] and NPV= 100% [99.9-100];

*p=0.008, **p<0.001, ***p=0.005

Diagnostic accuracy measures for T21 in pregnancies with outcome data, including women with low fetal fractions and test failures (calculated based on study results)

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA test
TP	41
TN	15794
FP	494 (FP rate: 3%)
FN	0
S	100 (91.4-100)
Sp	97.0 (96.7-97.2)
PPV	7.7 (5.7-10.2)
NPV	100 (100-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA test
TP	38
TN	16279
FP	9 (FPrate: 0.05%)
FN	3 (FNrate: 7.3%)
S	92.7 (80.6-97.5)
Sp	99.9 (99-100)
PPV	80.8 (67.5-89.6)
NPV	100 (99.9-100)

Diagnostic accuracy measures for T18, for pregnancies with outcome data, excluding women with low fetal fraction, test failures and miscarriages (calculated based on study results)

Variable	Standard screening	cfDNA Testing
TP	8	9
TN	15,782	15,830
FP	49 (FP rate: 0.3%)	1 (FP rate: 0.006%)
FN	2 (FN rate: 20%)	1 (FN rate: 10%)
S	80.0 (44.4-97.5)	90.0 (55.5-99.7)
Sp	99.7 (99.6-99.8)	100 (99.9-100)*
PPV	14.0 (6.2-25.8)	90.0 (55.5-99.7)*
NPV	100 (99.9-100)	100 (99.9-100)

*p<0.001

Diagnostic accuracy measures for T18 in pregnancies with outcome data, including women with low fetal fraction and test failures (calculated based on study results)

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA
TP	10
TN	15830
FP	489 (FP rate: 2.9%)
FN	1 (FN rate: 9%)
S	90.9 (62.3-98.4)
Sp	97.0 (96.7-97.3)
PPV	2.0 (1.1-3.6)
NPV	100 (100-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA
TP	9
TN	16319
FP	1 (FP rate: 0.006%)
FN	2 (FN rate: 18.2%)
S	81.8 (52.3-94.9)
Sp	99.9 (99.9-100)
PPV	90.0 (59.6-98.2)
NPV	100 (100-100)

Diagnostic accuracy measures for T13, for pregnancies with outcome data, excluding women with low fetal fraction, test failures and miscarriages (calculated based on study results)

Variable	Standard screening	cfDNA Testing
TP	1	2
TN	11,155	11,181
FP	28 (FP rate:0.25%)	2 (FP rate: 0.018%)
FN	1 (FN rate: 50%)	0
S	50.0 (1.2-98.7)	100 (15.8-100)
Sp	99.7 (99.6-99.8)	100 (99.9-100)*
PPV	3.4 (0.1-17.8)	50.0 (6.8-93.2)
NPV	100 (99.9-100)	100 (99.9-100)

*p<0.001

Diagnostic accuracy measures for T13 in pregnancies with outcome data, including women with low fetal fraction and test failures (calculated based on study results

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA
TP	4
TN	11181
FP	490 (FP rate: 4.2%)
FN	0
S	100 (51.0-100)
Sp	95.8 (95.4-96.2)
PPV	0.8 (0.3-2.1)
NPV	100 (100-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA
TP	2
TN	11667
FP	2 (FP rate: 0.02%)
FN	2 (FN rate: 50%)
S	50.0 (15.0-85.09
Sp	100 (99.9-100)
PPV	50.0 (15.0-85.0)
NPV	100 (99.9-100)

Additional population relevant outcomes

Safety

-Increase in the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies of uncertain significance: NA

Effectiveness

	-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NIPT diagnosed 8 more cases of T18, 1 more case of T18 and 1 more case of T13 than first trimester standard screening. It is unknown if these cases would have been diagnosed during the progression of the pregnancy or if they were livebirths. -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing cfDNA vs. standard screening):NA Estimations assuming all high risk patients would undergoe Invasive testing: 5.3 for T21, 0.3 for T18 and 0.16 for T13. -Change in uptake of prenatal screening: NA

Author(s): Pérez-Pedregosa et al [41]	
Study characteristics	Study design: Comparative prospective observational DTA trial, one centre (cross-sectional design) Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: Spain Setting: routine prenatal screening Data collection period: not reported Target population: general pregnancy population Target condition prevalence in the enrolled population: 1/41 for T21 and 1/193 for T18 Comparator: standard screening that includes serum biochemical assays (PAPP-A and free β-hCG) with NT Cut off point comparator: high-risk>1/100 and low-risk=1/101 to 1/1000 (PRISCA 4.0 Typolog software) Index test (trademark/technique type): Harmony™ Prenatal Test/CSS Country where samples were analysed: USA (Ariosa Diagnostic) Cut off for NIPT: high-risk score>99% and low-risk score<0.01% (Fetal –Fraction) Optimized Risk of Trisomy Evaluation-FORTE algorithm)
Population characterisitics	Maternal age in years (mean [169]): 36.5 [22-47] Gestational age in weeks (median [169]): not reported Maternal weight (median [169]: not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women with singleton pregnancy and a gestational age at least 10 weeks Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 582 Population excluded: 3 (test failure) Population included: 579 -with NIPT result: 579 -with comparator result: 581 -with reference standard result: not reported With reference standard results: 100% (CVS or amniocentesis in population with high risk for any chromosopathy and neonatal examination or telephone contact in the rest of women). *Sample processing protocol* Maternal blood was collected in enrollment center and sent to Ariosa Diagnostics.

Outcomes

Performance of NIPT for T21 and T18

-Test failure (% samples): 0.5 (3 samples)

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in all included cases (exclusion of miscarriages)

Variable	Standard screening[1]	cfDNA
TP	12	14
TN	529	565
FP	38 (FP rate: 6.7%)	0
FN	2 (FN rate: 14.3%)	0
S	85.7 (56.1-97.4)	100 (73.2-100)
Sp	93.2 (90.7-95.1)	100 (99.1-100)
PPV	24 (13.5-38.4)	100 (73.2-100)
NPV	99.6	100 (99.1-100)

[1]581 population included; [2] 579 included population

Diagnostic accuracy measures for Trisomy 18 in all included cases (exclusion of miscarriages)

Variable	Standard screening	cfDNA
TP	3	3
TN	574	576
FP	5 (FP rate: 0.86%)	0
FN	0	0
S	100 (43.8-100)	100 (43.8-100)
Sp	99.1 (98.0-99.6)	100 (99.3-100)
PPV	37.5 (13.7-69.4)	100 (43.8-100)
NPV	100 (99.3-100)	100 (99.1-100)

**Calculated based on data provided (Sp noted in study=98.9 [97.6-99.5]*

Diagnostic accuracy measures for Trisomy 13

No data provided for T13

Additional population relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing: NA (calculated from highr risk cfDNA vs. standard screening, %): 1.4% for T21 and 0.85% for T18

-Change in uptake of prenatal screening: NA

Author(s): Quezada et al [42]	
Study characteristics	Study design: prospective, comparative DTA trial (cross-sectional design) Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: United Kingdom (UK) Setting: routine prenatal-screening Data collection period: October 2012-January 2014 Target population: general pregnancy population Target condition prevalence in the enrolled population: 1/85 for T21, 1/290 for T18 and 1/581 for T13 Comparator: standard screening that includes serum biochemical assays (PAPP-A and free β-hCG), NT and fetal CRL. It was performed at 11-13 week's gestation Cut off point comparator: population was classified as high risk at estimated risk≥1/100 (cut-off recommendation by the UK National Screening Committee for invasive testing) Index test (trademark/technique type): Harmony™ Prenatal Test/CSS Country where samples were analysed: USA (Ariosa Diagnostics) Cut off for NIPT: risk score for trisomy>99% or <1/10000
Population characteristics	Maternal age in years (mean [169]): 36.9 [20.4-51.9] Gestational age in weeks (median [169]): 10+4 [10+0-11+6] Maternal weight (median [169]): 62.8 [40.5-137.7] Pregnancy by assisted reproductive techniques (% pts): 16.1 Inclusion criteria: women with singleton pregnancies and a live foetus Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Populations enrolled: 2905 Populations excluded: 0 Populations included: 2905 -with NIPT result: 2851 -with comparator result: 2863 -with reference standard result: 2857 Reference standard (% pts): invasive fetal karyotyping (CVS, amniocentesis or neonate blood) or neonate phenotype examination No fetal tissue karyotyping available for pregnancies resulting in miscarriage or stillbirth *Sample processing protocol*

Maternal blood was collected in Streck cell-free DNA BCT™ tubes and sent to the Ariosa Diagnostics. Invasive testing results were obtained from laboratories and pregnancy outcomes were obtained from obstetricians, general practitioners or the population.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (%, n samples): 1.9 (54 samples) (38 cases with fetal fraction<4% and 15 cases of assays failure)

-Uncertain results rate (%, n samples): not reported

Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	Standard combined test[1]	cfDNA[2]
TP	34	32
TN	2663	2734
FP	139 (FP rate: 4.96%)	1 (FP rate: 0.04%)
FN	0	0
S	100 (89.8-100)	100 (89.3-100)
Sp	95.0 (94.2-95.8)	99.9 (99.8-100)
PPV	19.7 (14.4-26.2)	96.9 (84.7-99.5)
NPV	100 (99.9-100)	100 (99.9-100)

[1]Trisomic status unknown in 69 samples (52 with low-risk, 5 with high-risk and 12 with no results)

[2]Trisomic status unknown in 69 samples (65 with low-risk, 1 with high-risk and 3 with no results)

Diagnostic accuracy measures for T21 in pregnancies with outcome data, including test failures (calculated based on study results)

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA
TP	34
TN	2734
FP	50 (FP rate: 1.79%)
FN	0
S	100 (89.8-100)
Sp	98.2 (97.6-98.6)
PPV	40.5 (80.6-51.2)
NPV	100 (99.9-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA
TP	32
TN	2783
FP	1 (FP rate: 0.035%)
FN	2 (FN rate: 5.88%)
S	94.1 (80.9-98.4)
Sp	99.9 (99.8-100)
PPV	96.9 (84.7-99.5)
NPV	99.9 (99.7-100)

Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	*Standard screening*	*cfDNA*
TP	10	9
TN	2663	2730
FP	163 (FP rate: 5.8%)	5 (FP rate: 0.18%)
FN	0	1 (FN rate: 10%)
S	100 (72.2-100)	90.0 (59.6-98.2)
Sp	94.2 (93.3-95)	99.8 (99.6-99.91)
PPV	5.8 (3.2-10.3)	64.3 (38.3-83.7)
NPV	100 (99.9-100)	99.9 (99.8-100)

Diagnostic accuracy measures for T13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	*Standard screening*	*cfDNA*
TP	5	2
TN	2663	2730
FP	168 (FP rate: 5.9%)	2 (FP rate: 0.07%)
FN	0	3 (FN rate: 60%)
S	100 (56.6-100)	40.0 (11.8-76.9)
Sp	94.1 (93.1-94.9)	99.9 (99.7-100)
PPV	2.9 (1.2-6.6)	50.0 (15.0-85)
NPV	100 (99.9-100)	99.9 (99.7-100)

	Additional population relevant outcomes *Safety* -Increase in the number of children born with other major unconfirmed chromosomal anomalies: NA -Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies of uncertain significance: NA *Effectiveness* - Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NIPT detected four less cases of aneuploidies (one case of T18 and 3 cases of T13), although it is unknown if these were live births -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages related to invasive testing: NA -Reduction in uptake of invasive testing: NA Estimations based on high risk cfDNA vs. standard screening, %): 4.13 for T21, 4.16 for T18 and 4.10 for T13 -Change in uptake of prenatal screening: NA

Author(s): Zhang et al [43]	
Study characteristics	Study design: propective, multicentre DTA study (cross-sectional design) Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China Setting: routine prenatal care Data collection period: January 2012- August 2013 Target population: high-risk pregnant population Target condition prevalence in the enrolled population: 1/205 for T21, 1/882 for T18 and 1/6696 for T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): BGI laboratories; Illumina HiSeq2000 platforms with The Fetal Copy-number Analysis through Maternal Plasma Sequencing (FCAPS) algorithm/MPS Country where samples were analysed: laboratories of BGI-Health, China Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 30.9 [18-46] Gestational age in weeks (median [169]): 18.7 [9-36] 94.13% samples were collected during the second trimester Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women at least 18 years old with singleton or twin pregnancy at ≥9 weeks' gestation Population with NIPT testing result were classified into high or low-risk group attending to the following criteria: High-risk group: advanced maternal age >35 years of age, positive conventional Down syndrome screening test result (cut-off 1/270 or 1/300, depending on hospital's criteria), abnormal sonographic findings, family history of aneuploidy or previous pregnancy with a trisomic foetus (n= 72382). Low-risk group: none of high-risk factors mentioned above (n=40287) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Populations enrolled: 147314 (802 twin pregnancy and 146156 singleton pregnancy) Populations excluded: 356 Reasons for exclusion: -absence of a result on cfDNA (testing failure): 145 -inappropriate sample (inadequate volume, contamination, obtained before 9 week' gestation or improper labeling): 211

<table>
<tr><td></td><td colspan="2">Populations included: 146958
-with NIPT result: 146958
-with reference standard result: 112669
Reference standard (% pts): karyotyping (1055, 66.8% positive NIPT results) or clinical follow-up (111605)
Sample processing protocol
Maternal blood samples were collected into EDTA tubes at each enrolment center and sent to laboratories of BGI-Health for its analysis</td></tr>
<tr><td rowspan="3">Outcomes</td><td colspan="2">Diagnostic performance of NIPT for T21, T18 and T13
-Test failure (% samples): (145 samples (0.098%)
-Inappropriate sample (n,%): (211, 0.14%)
-Uncertain results rate (% samples): 326 (0.2%)</td></tr>
<tr><td>Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures, uncertain results and miscarriages

<table>
<tr><th>Variable</th><th>cfDNA testing[1,2,3]</th></tr>
<tr><td>TP</td><td>720</td></tr>
<tr><td>TN*</td><td>111594</td></tr>
<tr><td>FP</td><td>61 (FP rate: 0.05%)</td></tr>
<tr><td>FN</td><td>6 (FN rate: 0.83%)</td></tr>
<tr><td>S</td><td>99.2 (98.5-99.8)</td></tr>
<tr><td>Sp</td><td>99.9 (99.9-99.9)</td></tr>
<tr><td>PPV</td><td>92.2 (90.3-94.1)</td></tr>
<tr><td>NPV</td><td>100 (99.9-100)</td></tr>
</table>
[1] Karyotyping or follow-up confirmation was not available for 34289 samples due to follow up loss, elective termination or pregnancy loss (512 samples from positive NIPT results cohort and 33777 samples from negative NIPT result cohort).
[2]In twin pregnancies (n=404), there were 5 TP cases, 2 FP cases, no FN cases, S=100 [47.82-100], Sp=99.50 [98.20-99.94], PPV=71.43 [29.04-96.33] and NPV= 100 [99.08-100].
[3] If underteminate results are considered as FP: PPV would be 65,04
*4605 birth defects irrelevant to trisomy (facial anomalies, cardiac anomalies, hearing impairment, heel blood testing anomalies and physical examination anomalies).</td><td>Comparison NIPT accuracy for T21 high-risk vs. low-risk pregnancies

<table>
<tr><th rowspan="2">Variable</th><th colspan="2">cfDNA testing</th></tr>
<tr><th>High-risk</th><th>Low-risk</th></tr>
<tr><td>TP</td><td>624</td><td>96</td></tr>
<tr><td>TN</td><td>NA</td><td>NA</td></tr>
<tr><td>FP</td><td>39</td><td>22</td></tr>
<tr><td>FN</td><td>5</td><td>1</td></tr>
<tr><td>S</td><td>99.2 (98.5-99.9)</td><td>98.9 (94.4-100)</td></tr>
<tr><td>Sp</td><td>99.95 (99.9-99.9)</td><td>99.9 (99.9-100)</td></tr>
<tr><td>PPV*</td><td>94.12 (92.3-95.9)</td><td>81.4 (74.3-88.4)</td></tr>
<tr><td>NPV</td><td>100 (99.9-100)</td><td>100 (99.9-100)</td></tr>
</table>
NA: not available; *p<0.00001</td></tr>
<tr><td>Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding test failures, uncertain results and miscarriages</td><td>Diagnostic accuracy measures for T13 in pregnancies with outcome data, excluding test failures, uncertain results and miscarriages</td></tr>
</table>

Variable	cfDNA testing
TP	167
TN	111594
FP	51 (FP rate: 0.05%)
FN	3 (FN rate: 1.77%)
S	98.24 (94.93-99.63)
Sp	99.95 (99.94-99.97)
PPV	76.61 (70.99-82.23)
NPV	100 (99.99-100)

Variable	cfDNA Testing
TP	22
TN	111594
FP	45 (FP rate: 0.04%)
FN	0
S	100 (84.56-100)
Sp	99.9 (99.95-99.97)
NPV	32.8 (21.59-44.08)
NPV	100 (99.99-100)

Additional population relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA

Effectiveness-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: Na

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Bianchi et al [30]	
Study characteristics	**Comparison of Aneuploidy Risk Evaluations (CARE) study** Study design: prospective, blinded, multicentre comparative DTA study (cross sectional design) Year of publication: 2014 Study's registration number in clinical trial database: NCT01663350 Country/ies of recruitment: United States (21 medical centers) Setting: routine prenatal-screening Data collection period: July 2012- January 2013 Target population: general pregnancy population Target condition prevalence in the enrolled population (n: 1914 pts): 1/383 for T21, 1/957 for T18 and 1/1914 for T13 Comparator: standard screening that included serum biochemical assays at first trimester (PAPP-A and hCG) or second trimester (maternal serum alpha-fetoprotein, hCG, unconjugated estriol and inhibin A) with or without NT Cut off point comparator: the risk classification was determined on the basis on first or second-trimester results. Cut of point was not reported. Index test (trademark/technique type): Verifi™Prenatal Test (Illumina HiSeq 2000 instruments) Country where samples were analysed: USA (Verinata Health) Cut off for NIPT: Samples with a normalized chromosome value ≥4.0 were classified as affected and samples with value≤3.0 were classified as unaffected.
Population characteristics	Maternal age in years (mean [SD]): 29.6±5.54 Gestational age in weeks (median [SD]): 20.3±8.6 Body-mass index (median [SD]: 28.7±6.96 Pregnancy by assisted reproductive techniques (% pts): 3.4 Inclusion criteria: pregnant women had to be at least 18 years of age and had to be carrying a foetus with a gestational age of at least 8 weeks. All populations had planned to undergo or had completed standard prenatal serum screening for fetal aneuploidies. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Populations recruited: 2042 (ineligible due to maternal age < 18 years (n=1), withdraw consent (n=1) and insufficient blood volume or late receipt (n=8). Populations enrolled: 2042 -Populations excluded: 128 Reasons for exclusion: -no clinical outcome: 72 -no live birth, no karyotype: 24 -no result on cfDNA testing: 17 -no result on standard screening: 39

Populations included: 1914

Reference standard (% pts): newborn physical examination (1857) and karyotype (57)(CVS in 10, amniocentesis in 38, testing of products of conception in 3 and postnatal evaluation in 6)

Sample processing protocol

Blood samples were collected in a cfDNA blood collection tube (Streck) in enrolment sites and sent to Verinata Health. A sample was eligible for analysis if it was received within 5 days after the sample was obtained and contained at least 7 ml of blood. All personnel were unaware of clinical data and outcomes.

All cytogenetic reports were generated in accredited laboratories and reviewed by independent, board-certified cytogeneticist who were unaware of the results of cfDNA testing.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 0.9 (18/2042)

-Uncertain results are (% samples): not reported

Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding women with absence or uncertain screening results, cfDNA test failures and miscarriages (n=1952)

Variable	Standard[2] screening	cfDNA Testing[1]
TP	3	5
TN	1840	1941
FP	69 (FP rate: 3.6%)	6 (FP rate: 0.3)*
FN	0	0
S	100 (29.2-100)	100 (47.8-100)
Sp	96.4 (95.4-97.2)	99.7 (99.3-99.9)
PPV	4.2 (0.9-11.7)	45.5 (16.7-76.6)
NPV	100 (99.8-100)	100 (99.8-100)

[1] 1952 analysed for cfDNA test performance; 28% of results obtained in 3rd trimester

[2] 1912 analysed for standard screening test performance
*p<0.001

Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding women with absence or uncertain screening results, cfDNA test failure and miscarriages

Variable	Standard screening[2]	cfDNA Testing[1]
TP	1	2
TN	1894	1947
FP	11 (FP rate: 0.6%)	3 (FP rate: 0.2%)*
FN	0	0
S	100 (2.5-100)	100 (15.8-100)
Sp	99.4 (99.0-99.7)	99.8 (99.6-100)
PPV	8.3 (0.2-38.5)	40.0 (5.3-85.3)
NPV	100 (99.8-100)	100 (99.8-100)

[1] 1952 analysed for cfDNA test performance; 28% of results obtained in 3rd trimester

[2] 1906 analysed for standard screening test
*p=0.03

Diagnostic accuracy measures for T13 in pregnancies with outcome data, excluding women with absence or uncertain screening results, cfDNA test failures and miscarriages

Variable	Standard screening	cfDNA Testing
TP	1	1
TN	892	1910
FP	6 (FP rate: 0.67%)	3 (FP rate: 0.16%)
FN	0	0
S	100	100 (20.7-100)
Sp	99.3	99.8 (99.5-99.9)
PPV	14	25 (4.6-69.9)
NPV	100	100 (99.8-100)

1914 analysed for cfDNA test performance; 28% of results obtained in 3rd trimester

[2] analysed for standard screening test performance
*p=0.059

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies:NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: 0

	-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing: NA Estimations based on high risk cfDNA vs. standard screening, (%): 3.46 for T21 and 0.42 for T18. -Change in uptake of prenatal screening: NA

Author(s): Comas et al [40]	
Study characteristics	Study design: prospective, unicentre, DTA study (cross sectional design) Year of publication: 2015 Country/ies of recruitment: Spain Setting: routine prenatal-screening Data collection period: January to December 2013 Target population: general pregnancy population Prevalence of T21 in the enrolled population: 4/333 (1.2%) Index test (trademark/technique type): Panorama™ Prenatal Test (Natera Inc.) or Harmony™ (Ariosa diagnostics) Country where samples were analysed: Natera and Ariosa laboratories Cut off for NIPT: NA
Population characteristics	Maternal age in years (mean [SD]): 37 (21-46) Gestational age in weeks (mean): 14.6 (9.5-23.5) Body-mass index (mean: 22.9 (17-1-42.4) Pregnancy by assisted reproductive techniques (% pts): NA Inclusion criteria: All singleton pregnant women were offered NIPT in addition to FCT Exclusion criteria: cases with ultrasound anomalies or those at high risk for other conditions
Study protocol	*Population enrollment flow* Patients included: 333 (83.5% considered of low risk (referred for anxiety) and 16.5% high risk pregnancies With NIPT result: 324 With reference standard result: 315 (94.5% pts) Reference standard: karyotyping for all high risk patients (n=5); follow up rest (n=310) *Sample processing protocol* Maternal blood samples were collected into bcttm tubes at each enrolment center and sent to laboratories the same day

Outcomes

Performance of NIPT for T21

-Test failure: 1.2%

Diagnostic accuracy measures for T21 in pregnancies with outcome data, women with absence or uncertain screening results, cfDNA test failures and miscarriages (n=329)

	Variable							
Test cfDNA	TP	TN	FP	FN	S	Sp	PPV	NPV
Harmony™	0	116	1	0	0	99.1	0	100
Panorama™	4	203	0	0	100	100	100	
Total	4	319	1 (FP rate: 0.3%)	0	100 (51-100)	99.6 (98.3-99)	80 (37.6-96.4)	100

Harmony (n=117); Panorama (n=207)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other relevant unconfirmed chromosomal anomalies:NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: 0

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing: NA

Author(s): Song et al [45]	
Study characteristics	Study design: Comparative DTA study (prospective cohort) Year of publication: 2013 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China (Peking Union Medical College) Setting: routine prenatal care Data collection period: April 2011-December 2011 Target population: general pregnant population Target condition prevalence in the enrolled population: 1/239 for T21, 1/958 for T18, 1/1916 for T13, 1/958 for 45,X and no cases for 47,XXY Comparator: triple serum screening in the second trimester (α-fetoprotein, free β-hCG and unconjugated estriol) Cut off point comparator: 1/270 Index test (trademark/technique type): Berry Genomics laboratories, HiSeq2000, Illumina/MSP Country where samples were analysed: Berry Genomics Co, Ltd, China Cut off for NIPT: sample with Z-score≥3 was classified as aneuploidy. GC corrected normalized chromosome representation NCR_{gc} value was also used to classify sample trisomy status.
Population characteristics	Maternal age in years (mean [SD]): 29.03±2.70 Gestational age in weeks (mean [SD]): 16.57±1.56 Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): 0.8 Inclusion criteria: pregnant women < 35 years Exclusion criteria: not reported.
Study protocol	*Population enrollment flow* Population enrolled: 1916 Population excluded: 175 Reason for exclusion: missed follow up and failures with no follow up information -absence of a result on cfDNA and no birth follow up (64) -No birth outcome information (111) Population included: 1741 -with NIPT result: 1741 -with comparator result: 1741 -with reference standard result: 217 by invasive testing and 1805 by neonatal follow-up. Reference standard (n or % pts): amniocentesis (190), CVS (10), cordocentesis (2) or birth follow-up including physical details on neonates.

<table>
<tr><td></td><td colspan="3">Sample processing protocol
Blood samples were collected into EDTA tubes at each enrolment institution and sent to Berry Genomics Co, Ltd. for its analysis. Plasma DNA was extracted using the QIAamp Circulating Nucleic Acid Kit from Qiagen.
Results analysis was performed in a blinded fashion.</td></tr>
<tr><td>Outcomes</td><td colspan="3">Performance of NIPT for T21, T18, T13, 45,X syndrome and 47, XXY syndrome
-Test failure (% samples): 73 samples (3.8%)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td></td><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>8</td></tr><tr><td>TN</td><td>1733</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (59.8-100)</td></tr><tr><td>Sp</td><td>100 (99.7-100)</td></tr><tr><td>PPV</td><td>100 (59.8-100)</td></tr><tr><td>NPV</td><td>100 (99.7-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>2</td></tr><tr><td>TN</td><td>1738</td></tr><tr><td>FP</td><td>1 (FP rate: 0.06%)</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (19.79-100)</td></tr><tr><td>Sp</td><td>99.9 (99.6-99.99)</td></tr><tr><td>PPV</td><td>66.7 (20.8-93.1)</td></tr><tr><td>NPV</td><td>100 (99.8-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA Testing</th></tr><tr><td>TP</td><td>1</td></tr><tr><td>TN</td><td>1740</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (5.5-100)</td></tr><tr><td>Sp</td><td>100 (99.7-100)</td></tr><tr><td>PPV</td><td>100 (5.5-100)</td></tr><tr><td>NPV</td><td>100 (99.7-100)</td></tr></table></td></tr>
<tr><td></td><td colspan="3">Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: serum screening false negative rate for all of these trisomies reached 45.45% in comparison to 0% of NIPT testing (5 FN cases by serum screening vs. 0 FN cases by NIPT)
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: According to number of TP or PPV for 13,18 and 21 trisomies, NIPT identified the most of aneuploid cases and therefore it could avoid unnecessary miscarriages or still birth of affected foetuses
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): FP cfDNA for 13,18 and 21 trisomies was 0.06% vs. 14% for serum screening. Aneuploidies screening by NIPT, it was needed 237 invasive testing less than by serum screening (reduction of 3.07%)
-Reduction in the number of miscarriage related to invasive testing: NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Pergament et al [44]	
Study characteristics	Study design: DTA study (prospective cohort) Year of publication: 2014 Study's registration number in clinical trial database: not reported Country/ies of recruitment: USA (36 prenatal care centers) Setting: routine prenatal-screening Data collection period: not reported Target population: general pregnancy population (high and low risk) Target condition prevalence in the enrolled population: 1/17 for T21, 1/39 for T18, 1/80 for T13 and 1/97 for monosomy X Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): The Next-generation Aneuploidy Test Using SNPs (Natera Inc.) Country where samples were analysed: USA Cut off for NIPT: not reported
Population characteristics	Maternal age in years (median [169]): 30.0 [18-47] Gestational age in weeks (median [169]): 14.3 [7.6-40.6] Maternal weight (median [169]: not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women were ≥18 years of age with a singleton pregnancy of at least 7 weeks of gestation and signed an informed consent NIPT indication (% pts): -High risk (defined after positive serum screen, ultrasound anomaly and/or maternal age≥35 years): 51.0 -Low risk (defined as maternal age<35 years and lacking any reported high-risk indications): 49.0 Exclusion criteria: confirmed sex chromosome anomaly (47,XXX/XXY/XYY), confirmed triplody or confirmed fetal mosaicism
Study protocol	*Patient enrollment flow* Patient enrolled: 1,064 (543 with high-risk and 521 with low-risk) Patient excluded: 13 Reason for exclusion: -not meet the eligibility criteria or met exclusion criteria: confirmed triploidy (6), fetal mosaic (3), 47,XXY (2), 47,XXX (1) and 47,XYY (1) Patient included: 1,051 -with NIPT result: 966 (85 samples were excluded for non-fulfillment of quality control named "no-calls" for the following reasons: low fetal fraction, low amount of input cel-free DNA, contamination, the presence of regions of loss of heterozygosity (LOH) in maternal DNA exceeding 25% of the chromosome or poor fit of the data to the model)

-with reference standard result: 1064

Reference standard (% pts): amniocentesis/CVS (44.1), products of conception (42.8) or genetic testing of cord blood, buccal sample, saliva (13.2).

Sample processing protocol

Cell-free DNA analysis was blinded to sample karyotype.

Outcomes

Performance of NIPT for T21, T18, T13 and monosomy X

Test failure (% samples): 8.1 (85/1051 samples)

Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures (calculated based on study results)

Variable	cfDNA Testing
TP	58
TN	905
FP	0
FN	0
S	100 (93.8-100)
Sp	100 (99.6-100)
PPV	100 (93.8-100)
NPV	100 (99.6-100)

Diagnostic accuracy measures for T21 in pregnancies with outcome data, including test failures (calculated based on study results)

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA Testing
TP	66
TN	905
FP	77 (FP rate: 7.8%)
FN	0
S	100 (94.5-100)
Sp	92.2 (90.3-93.7)
PPV	46.2 (38.2-54.3)
NPV	100 (99.6-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA Testing
TP	58
TN	982
FP	0
FN	8 (FN rate: 12.1%)
S	87.9 (77.9-93.7)
Sp	100 (99.6-100)
PPV	100 (93.8-100)
NPV	99.2 (98.4-99.6)

Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	25
TN	939
FP	1 (FP rate: 0.1%)
FN	1 (FN rate: 3.84%)
S	96 (79.7-99.9)
Sp	99.9 (99.4-100]
PPV	96.1 (79.7-99.9)
NPV	99.9 (99.4-100)

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Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, including test failures and miscarriages (calculated based on study results)

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA Testing
TP	32
TN	939
FP	79 (FP rate: 7.7%)
FN	1 (FN rate: 3.03%)
S	97.0 (84.7-99.59
Sp	92.2 (90.4-93.7)
PPV	28.8 (21.2-37.9)
NPV	99.9 (99.4-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA Testing
TP	25
TN	1017
FP	1 (FP rate: 0.098%)
FN	8 (FN rate: 24.2%)
S	75.8 (59.0-87.2)
Sp	99.9 (99.4-100)
PPV	96.1 (81.1-99.3)
NPV	99.2 (98.5-99.6)

Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	12
TN	953
FP	0
FN	0
S	100 (75.7-100)
Sp	100 (99.6-100)
PPV	100 (75.7-100)
NPV	100 (99.6-100)

Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, including test failures and miscarriages (calculated based on study results)

Considering cases with no NIPT test results as high-risk of aneuploidies

Variable	cfDNA Testing
TP	14
TN	953
FP	83 (FP rate: 8%)
FN	0
S	100 (78.5-100)
Sp	92.0 (90.2-93.5)
PPV	14.4 (8.8-22.8)
NPV	100 (99.6-100)

Considering cases with no NIPT test results as low-risk of aneuploidies

Variable	cfDNA Testing
TP	12
TN	1036
FP	0
FN	2 (FN rate: 14.28%)
S	85.7 (60.1-96.0)
Sp	100 (99.6-100)
PPV	100 (75.7-100)
NPV	99.8 (99.3-99.9)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Change in uptake of prenatal screening: NA

Abbreviations: NA: not available; S: sensitivity; Sp: specificity; TP: true positive; TN: true negative; FP: false positive; FN: false negative; PPV: positive predictive value; NPV: negative predictive value. FPR: false positive rate and FNR: false negative rate.

Table A3: Characteristics of included studies on high-risk pregnant population

Author(s): Persico et al [49]	
Study characteristics	Study design: multicentre prospective DTA study Year of publication: 2016 Study's registration number in clinical trial database: not reported Country/ies of recruitment: Italy Setting: routine prenatal screening Data collection period: March to December 2014 Target population: high-risk pregnant population Target condition prevalence in the enrolled population (including cases with no result for cfDNA test): 1/7 for T21, 1/17 for T18 and 1/43 for T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): not reported/SNPs Country where samples were analysed: USA Cut off for NIPT: >1/100
Population characteristics	Maternal age in years (median [169]): 36 [20-46] Gestational age in weeks (median [169]): not reported Maternal weight in Kg (median [169]): not reported Pregnancy by assisted reproductive techniques (no [% pts]): not reported Inclusion criteria: women with singleton pregnancies and an estimated risk for trisomies 21, 18 or 13 after first-trimester combined screening ≥1/250 (combination of maternal age, fetal NT thickness, fetal heart rate and maternal serum free β-hCG and pregnancy-associated plasma protein-A at 11-13 weeks' gestation). Exclusion criteria: not reported.
Study protocol	*Population enrollment flow* Population enrolled: 259 Population excluded: 0 Population included: 259 -with NIPT result: 249 -with comparator result: no intervention -with reference standard result: 259 Reference standard (% pts): amniocentesis, CVS. Microarray- CGH analysis was performed in selected cases (based on clinician's decision, including NT thickness≥3.5 mm and/or evidence of a major structural defect on ultrasound)(n=32)

Sample processing protocol

Maternal blood samples were collected into Cell-Free DNA BCT tubes at enrolment centres and sent to Natera, Inc. where sequencing analysis was performed.

It was not reported if karyotyping and sequencing analysis were performed in a blinded fashion.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 10 samples (3.9%)(fetal fraction <4% in 8 cases and failed internal quality control in 2).

-Uncertain results rate (% samples): NA

Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	35
TN	213
FP	0
FN	1 (FN rate: 2.8%)
S	97.2 (65.8-99.5)
Sp	100 (98.2-100)
PPV	100 (90.1-100)
NPV	99.5 (97.4-99.9)

Diagnostic accuracy measures for T21 in pregnancies with outcome data, including test failures (calculated based on study results)

Considering no results cfDNA test cases as high risk of aneuploidy

Variable	cfDNA testing
TP	36
TN	213
FP	9 (FP rate: 4%)
FN	1 (FN rate: 2.7%)
S	97.3 (86.2-99.5)
Sp	95.9 (92.5-97.9)
PPV	80.0 (66.2-89.1)
NPV	99.5 (97.4-99.9)

Considering no results cfDNA test cases as low risk of aneuploidy

Variable	cfDNA testing
TP	35
TN	222
FP	0
FN	2 (FN rate: 5.4%)
S	94.6 (82.3-98.5)
Sp	100 (98.3-100)
PPV	100 (90.1-100)
NPV	99.1 (96.8-99.8)

Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNAcfDNA testing
TP	13
TN	236
FP	0
FN	0
S	100 (77.2-100)
Sp	100 (98.4-100)
PPV	100 (77.2-100)
NPV	100 (98.4-100)

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Diagnostic accuracy measures for T18 in pregnancies with outcome data, including test failures (calculated based on study results)

Considering no results cfDNA test cases as high risk of aneuploidy

Variable	cfDNAcfDNA testing
TP	15
TN	236
FP	8 (FP rate: 3.28%)
FN	0
S	100 (79.6-100)
Sp	96.7 (93.7-98.3)
PPV	65.2 (44.9-81.2)
NPV	100 (98.4-100)

Considering no results cfDNAcfDNA test cases as low risk of aneuploidy

Variable	cfDNAcfDNA testing
TP	13
TN	244
FP	0
FN	2 (FN rate: 13.3%)
S	86.7 (62.1-96.3)
Sp	100 (98.4-100)
PPV	100 (77.2-100)
NPV	99.2 (97.1-99.8)

Diagnostic accuracy measures for T13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	5
TN	244
FP	0
FN	0
S	100 (56.6-100)
Sp	100 (98.4-100)
PPV	100 (56.6-100)
NPV	100 (98.4-100)

Diagnostic accuracy measures for T13 in pregnancies with outcome data, including test failures (calculated based on study results)

Considering no results cfDNA test cases as high risk of aneuploidy

Variable	cfDNA testing
TP	6
TN	244
FP	9 (FP rate: 3.6%)
FN	0
S	100 (61.0-100)
Sp	96.4 (93.4-98.1)
PPV	40.0 (19.8-64.3)
NPV	100 (98.4-100)

Considering no results cfDNA test cases as low risk of aneuploidy

Variable	cfDNA testing
TP	5
TN	253
FP	0
FN	1 (FN rate: 16.7%)
S	83.3 (43.6-97.0)
Sp	100 (98.5-100)
PPV	100 (56.6-100)
NPV	99.6 (97.8-99.9)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: Invasive testing detected 8 anomalies not targeted by NIPT (11,3%)

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with unsignificant results: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Oepkes et al [48]	
Study characteristics	Study design: Observational, multicentre,prospective DTA study (TRIDENT study) Year of publication: 2016 Study's registration number in clinical trial database: not reported Country/ies of recruitment: The Netherlands Setting: routine prenatal screening Data collection period: April-September 2014 Target population: high-risk pregnant population Target condition prevalence in the enrolled population: 1/48 for T21, 1/347 for each T18 and T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): Illumina HiSeq 2500 or Life Technologies 5500 W SOLiD Country where samples were analysed: The Netherlands Cut off for NIPT: Z-score>3.0 or WISECONDOR algorithm
Population characteristics	Maternal age in years (no [%]): <36= 603 [43] ≥36= 703 [51] Unknown= 84 [6] Gestational age in weeks (median [169]): not reported Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: all pregnant women with high risk for T21, T18 or T13 based on FCT result (trisomy risk≥1:200)(n=1211) or medical history (previous child with such a trisomy or a balanced translocation in one of the parents)(n=179) Exclusion criteria: multiple pregnancies, vanishing twins, fetal nuchal translucency ≥3.5 mm or other structural anomalies, chromosome anomaly or history of maternal malignancy, gestational age <10+0 weeks, women <18 years old and inability to give informed consent.
Study protocol	*Population enrollment flow* Population enrolled: 1390 Population excluded: 0 Population included: 1390 -with NIPT result: 1386 (one women underwent amniocentesis and four samples were indeterminate results) -with comparator result: no intervention -with reference standard result: 53 sample confirmed by invasive testing and 1376 confirmed by neonatal follow-up as well Reference standard (% pts): invasive testing (amniocentesis or CVS), ultrasound data, genetic testing in products of conception (cord blood, placenta), birth data and data of postnatal examination

<table>
<tr><td></td><td>Sample processing protocol
Maternal blood samples were collected into EDTA or cfDNA BCT tubes at enrolment centres and sent to one of all eight University Hospital Genetics Laboratories where sequencing analysis was performed.
It was not reported if NIPT and karyotyping analysis were performed in a blinded fashion.</td></tr>
<tr><td>Study protocol</td><td>Population enrollment flow
Population enrolled: 1390
Population excluded: 0
Population included: 1390
-with NIPT result: 1386 (one women underwent amniocentesis and four samples were indeterminate results)
-with comparator result: no intervention
-with reference standard result: 53 sample confirmed by invasive testing and 1376 confirmed by neonatal follow-up as well
Reference standard (% pts): invasive testing (amniocentesis or CVS), ultrasound data, genetic testing in products of conception (cord blood, placenta), birth data and data of postnatal examination
Sample processing protocol
Maternal blood samples were collected into EDTA or cfDNA BCT tubes at enrolment centres and sent to one of all eight University Hospital Genetics Laboratories where sequencing analysis was performed.
It was not reported if NIPT and karyotyping analysis were performed in a blinded fashion.</td></tr>
<tr><td>Outcomes</td><td>Performance of NIPT for T21, T18 and T13
-Test failure (% samples): 4/1390 (0.3%)
-Uncertain results rate (% samples): NA</td></tr>
</table>

Diagnostic accuracy measures for T21 in pregnancies with outcome data, including test failures and miscarriages for abnormal results (calculated based on study results)

Variable	cfDNA testing
TP	29
TN	1354
FP	2 (FP rate: 0.15%)
FN	1 (FN rate: 3.33%)
S	96.7 (83.3-99.4)
Sp	99.9 (99.5-100)
PPV	93.5 (79.3-98.2)
NPV	99.9 (99.6-100)

Diagnostic accuracy measures for T18 in pregnancies with outcome data, including test failures and miscarriages for abnormal results (calculated based on study results)

Variable	cfDNA testing
TP	4
TN	1381
FP	1 (FP rate: 0.07%)
FN	0
S	100 (51-100)
Sp	99.9 (99.6-100)
PPV	80.0 (37.6-96.4)
NPV	100 (99.7-100)

Diagnostic accuracy measures for T13 in pregnancies with outcome data, including test failures and miscarriages for abnormal results (calculated based on study results)

Variable	cfDNA testing
TP	4
TN	1380
FP	2 (FP rate: 0.14%)
FN	0
S	100 (51-100)
Sp	99.9 (99.5-100)
PPV	66.7 (30-90.3)
NPV	100 (99.7-100)

	Additional patient relevant outcomes *Safety* -Increase the number of children born with other major unconfirmed chromosomal anomalies: NA -Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with unclear significance: NA *Effectiveness* -Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages related to invasive testing: NA -Reduction in uptake of invasive testing: Estimations based on figures of women with FCT who would elect to undergo invasive testing: 62% -Change in uptake of prenatal screening: NA

Author(s): Zhang et al [50]	
Study characteristics	Study design: Observational prospective DTA study Year of publication: 2016 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China (Obstetrics and Gynecology Hospital of Fundan University) Setting: routine prenatal screening, Data collection period: January 2012- December 2013 Target population: high-risk pregnant population Target condition prevalence in the enrolled population: 1/29 for T21, 1/87 for each T18 and Turner syndrome Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type):): Verifi™™ Prenatal Test (Illumina HiSeq™ 2000) Country where samples were analysed: not reported Cut off for NIPT: cut-off point, Z=3
Population characteristics	Maternal age in years (mean [SD]): 37.48±2.17 Gestational age in weeks (median [169]): 19.0 [12.4-32.5] Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: advanced maternal age pregnant women with aged≥35 years at the time of delivery, single birth, high-risk of DS or single abnormal multiple of the median, elevated fetal nuchal translucency in the early pregnancy, a soft marker in the genetic scan, or cardiac structural anomalies in the second-trimester genetic sonography or not suitable for invasive prenatal diagnosis (human immunodeficiency virus infection, placenta previa, low-set placenta, oligohydramnios, Rh-negative blood type, a history of abortion, threatened abortion or precious pregnancy) Exclusion criteria: pregnant women with chromosomal disease, received allogenic blood transfusion, organ transplantation, stem cell therapy or with a gestational age of <12 weeks
Study protocol	*Population enrollment flow* Population enrolled: 87 Population excluded: 0 Population included: 87 -with NIPT result: 87 -with comparator result: NA -with reference standard result: 87 Reference standard (% pts): amniocentesis (24.1%), neonatal blood karyotyping (42.3%) or follow-up examination of newborn *Sample processing protocol*

<table>
<tr><td></td><td colspan="2">Maternal blood samples were collected into EDTA and anticoagulant tubes.
Sequencing analysis was performed by personnel who were blinded to the study.</td></tr>
<tr><td>Outcomes</td><td colspan="2">Performance of NIPT for T21, T18 and other SCAs
-Test failure (% samples): not reported
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td></td><td>Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>3</td></tr><tr><td>TN</td><td>84</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (43.8-100)</td></tr><tr><td>Sp</td><td>100 (95.6-100)</td></tr><tr><td>PPV</td><td>100 (43.8-100)</td></tr><tr><td>NPV</td><td>100 (95.6-100)</td></tr></table></td><td>Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>1</td></tr><tr><td>TN</td><td>86</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (20.7-100)</td></tr><tr><td>Sp</td><td>100 (95.7-100)</td></tr><tr><td>PPV</td><td>100 (20.7-100)</td></tr><tr><td>NPV</td><td>100 95.7-100)</td></tr></table></td></tr>
<tr><td></td><td colspan="2">Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Ma et al [47]	
Study characteristics	Study design: multicentre DTA study (only prospective samples considered for analysis) Year of publication: 2016 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China (18 centres) Setting: routine prenatal care Data collection period: February to May 2014 Target population: high-risk population Target condition prevalence in the enrolled population: 1/64 for T21, 1/305 for T18 and 1/2,439 for T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): BGISEQ-1000/combinatorial probe-anchor ligation sequencing-cPAL platform-based NIPT testing Country where samples were analysed: clinical laboratory of BGI-China Cut off for NIPT: If Z-score>3 or <3, the sample was classified as high-risk of aneuploidy and if Z-score=-3 to 3, the sample was considered as low-risk.
Population characteristics	Inclusion criteria: pregnant women who meet at least one high-risk factor, i.e. advanced maternal age, high risk of biochemical screening result, abnormal ultrasound markers and previous adverse pregnant history. Exclusion criteria: cases without karyotyping confirmation or follow-up results.
Study protocol	-NIPT test failure: 4 samples Population included: 2,425 samples -with NIPT result: 2,425 samples -with comparator result: no intervention -with reference standard result: 2,425 samples Referenece standard (% pts): karyotype testing (G-banding)(amniocentesis, CVS or percutaneous umbilical cord blood sampling) or postnatal follow-up *Sample processing protocol* Blood samples were collected into EDTA tubes at each recruitment centre and sent to reference laboratory for its analysis.

<table>
<tr><td></td><td colspan="3">Sequencing analysis was performed in a blinded fashion to respect medical information or previous testing results.</td></tr>
<tr><td rowspan="3">Outcomes</td><td colspan="3">Performance of NIPT for T21, T18 and T13
-Test failure (% samples): 0.60% (0.00%-2.70%) (14 samples)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>38</td></tr><tr><td>TN</td><td>2,387</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (90.8-100)</td></tr><tr><td>Sp</td><td>99.8 (99.4-100)</td></tr><tr><td>PPV</td><td>100 (90.8-100)</td></tr><tr><td>NPV</td><td>100 (99.4-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>8</td></tr><tr><td>TN</td><td>2,378</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (60.3-100)</td></tr><tr><td>Sp</td><td>100 (99.9-100)</td></tr><tr><td>PPV</td><td>100 (60.3-100)</td></tr><tr><td>NPV</td><td>100 (99.9-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA Testing</th></tr><tr><td>TP</td><td>1</td></tr><tr><td>TN</td><td>2,378</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (2.5-100)</td></tr><tr><td>Sp</td><td>100 (99.9-100)</td></tr><tr><td>PPV</td><td>100 (2.5-100)</td></tr><tr><td>NPV</td><td>100 (99.9-100)</td></tr></table></td></tr>
<tr><td colspan="3">Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Kim et al [46]	
Study characteristics	Study design: Prospective observationalDTA study Year of publication: 2016 Study's registration number in clinical trial database: not reported Country/ies of recruitment: Republic of Korea Setting: routine prenatal care Data collection period: December 2014- April 2015 Target population: high-risk pregnant population Target condition prevalence in the enrolled population: 1/20 for T21 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): not reported/ion semiconductor-based sequencing i.e. Ion Torrent PGM or Proton platforms Country where samples were analysed: not reported Cut off for NIPT: Z-score>2.07 indicates a high risk of trisomy for the PGM system and >2.10 for Proton
Population characteristics	Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: singleton pregnant women underwent invasive testing based on first trimester serum screening (PAPP-A and hGC) and or ultrasonography (NT). The second-trimester serum screening (quadruple screening) was used to evaluate and define aneuploidy risk. Exclusion criteria: not reported
Study protocol	-with comparator result: no intervention -with reference standard result: 101 Reference standard (% pts): amniocentesis (100%) *Sample processing protocol* Maternal blood samples were collected at each enrolment centers previously karyotyping testing and stored into BCT™ tubes. One mL of plasma was used to extract cfDNA using QIAmp Circulating Nucleic Acid Kit. The karyotyping results were blinded

<table>
<tr><td>Outcomes</td><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>5</td></tr>
<tr><td>TN</td><td>96</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (47.8-100)</td></tr>
<tr><td>Sp</td><td>100 (96.2-100)</td></tr>
<tr><td>PPV*</td><td>100 (47.8-100)</td></tr>
<tr><td>NPV*</td><td>100 (96.2-100)</td></tr>
</table>
*Although authors' study identified sequencing quality difference between both platforms, these did not affect the final Z-score results and therefore accuracy of each one platform</td></tr>
<tr><td></td><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Song et al [57]	
Study characteristics	Study design: DTA study (prospective cohort) Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China (Pekin Union Medical College Hospital) Setting: routine prenatal care Data collection period: May 2012-August 2013 Target population: high-risk population Target condition prevalence in the enrolled population: 1/106 for T21, 1/213 for T18, T13, 47,XXY and 45,x/47,XXX Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): Illumina HiSeq 2000 platform/ Country where samples were analysed: Berry Genomics, China Cut off for NIPT: normal Z-score range=-3<Z<3
Population characteristics	Maternal age in years (median [169]): 37.25 (35-45) 85.8% women were <40 years of age Gestational age in weeks (median [169]): 9+6 (8+0 to 12+6) 52% samples were collected between 8+0 to 9+6 week Maternal weight (mean [SD]): 60.02±8.83 Pregnancy by ART (% pts): 6.1 Previous miscarriage (% pts): 19.8 Inclusion criteria: advanced maternal age (≥35 years) and singleton pregnancy Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 213 Population excluded: 1 Reason for exclusion: -not meet quality control standard due to hemolysis Population included: 212 -with NIPT result: 212 -with comparator result: no intervention -with reference standard result: 178

<table>
<tr><td></td><td>References standard (% pts): CVS or amniocentesis. Negative NIPT test results were confirmed by karyotyping and followed to birth and neonates were assessed clinically by pediatrician.
Sample processing protocol
Blood samples were collected into Streck tubes at enrolment institution and sent to Berry Genomics for its analysis. NIPT testing and karyotyping were performed in a double-blinded manner.</td></tr>
<tr><td>Outcomes</td><td>Performance of NIPT for T21, T18, T13 and SCA
-Test failure (% samples): 1 sample (0.5%)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td></td><td>
Diagnostic accuracy measures for Trisomy 21 (n=178) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>2</td></tr>
<tr><td>TN</td><td>176</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (19.79-100)</td></tr>
<tr><td>Sp</td><td>100 (97.35-100)</td></tr>
<tr><td>PPV</td><td>100 (19.79-100)</td></tr>
<tr><td>NPV</td><td>100 (97.35-100)</td></tr>
</table>
Diagnostic accuracy measures for Trisomy 18 (n=178) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>1</td></tr>
<tr><td>TN</td><td>179</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (5.46-100)</td></tr>
<tr><td>Sp</td><td>100 (97.35-100)</td></tr>
<tr><td>PPV</td><td>100 (5.46-100)</td></tr>
<tr><td>NPV</td><td>100 97.35-100)</td></tr>
</table>
Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA Testing</th></tr>
<tr><td>TP</td><td>1</td></tr>
<tr><td>TN</td><td>179</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (5.46-100)</td></tr>
<tr><td>Sp</td><td>100 (97.35-100)</td></tr>
<tr><td>PPV</td><td>100 (5.46-100)</td></tr>
<tr><td>NPV</td><td>100 (97.35-100)</td></tr>
</table>
</td></tr>
<tr><td></td><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed major chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Wang et al [58]	
Study characteristics	Study design: Prospective observational DTA study Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China (Lyanyungang Maternal and Child Hospital) Setting: routine prenatal care Data collection period: January 2013- December 2013 Target population: high-risk population Target condition prevalence in the enrolled population: 1/38 for T21 and 1/306 for T18 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): Illumina HiSeq2000 Country where samples were analysed: China Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean no[%]): -18 to 25 years, 299 [22.86] -26 to 35 years, 318 [40] -36 to 46 years, 300 [37.14] Gestational age in weeks (median [169]): Maternal weight in Kg (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: abnormal result on FCT (alfa-fetoprotein and beta-human chorionic gonadotropin)(n=521, 56.82%), advanced maternal age (≥35 years)(n=300, 32.72%), abnormal ultrasound findings (n=6, 0.65%) and others (abnormal amniotic fluid volume, adverse pregnancy history obtained from medical records and single umbilical artery)(n= 90, 9.81%) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 917 Population excluded: 0 Population included: 917 -with NIPT result: 917 -with comparator result: no intervention -with reference standard result: 917

<table>
<tr><td></td><td colspan="2">Reference standard (% pts): conventional karyotyping analysis or FISH and neonatal follow-up
Sample processing protocol
Maternal blood samples were collected into EDTA tubes at enrolment institution and sent to Berry Genomics for its analysis.
It was not reported if sequencing or karyotyping analysis were performed in a blinded fashion.</td></tr>
<tr><td rowspan="3">Outcomes</td><td colspan="2">Performance of NIPT for T21 and T18
-Test failure (% samples): NA
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>24</td></tr><tr><td>TN</td><td>882</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (86.2-100)</td></tr><tr><td>Sp</td><td>100 (99.6-100)</td></tr><tr><td>PPV</td><td>100 (86.2-100)</td></tr><tr><td>NPV</td><td>100 (99.6-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>3</td></tr><tr><td>TN</td><td>882</td></tr><tr><td>FP</td><td>1 (FP rate: 0.11%)</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (43.8-100)</td></tr><tr><td>Sp</td><td>99.88 (99.4-100)</td></tr><tr><td>PPV</td><td>75 (30.1-95.4)</td></tr><tr><td>NPV</td><td>100 (99.6-100)</td></tr></table></td></tr>
<tr><td colspan="2">Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Sánchez-Usabiaga et al [56]	
Study characteristics	Study design: Prospective observational DTA study Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: México Setting: routine prenatal care Data collection period: March 2013-February 2015 Target population: high-risk population Target condition prevalence in the enrolled population: 1/67 for T21, 1/270 for T18 and 1/270 for T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): not reported/SNPs Country where samples were analysed: Natera, San Carlos, USA Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 35 (21-45) Gestational age in weeks (median [169]): 11.85 (9-26.3) Maternal weight in Kg (median [169]): 60.9 (45-78) Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: singleton pregnant women at least 9 week of gestation with advanced maternal age (≥35 years old)(n=114), positive FCT (biochemical and ultrasonographic markers)(n=72) and maternal anxiety (n=84). Exclusion criteria: women with multiple pregnancies by donor oocytes or surrogate mother, previous bone marrow transplant or did not give written consent informed
Study protocol	*Population enrollment flow* Population enrolled: 270 Population excluded: 4 Reason for exclusion: -absence of a result on cfDNA: 4 Population included: 266 -with NIPT result: 266 -with comparator result: no intervention -with reference standard result: 214 Reference standard (% pts): CVS, amniocentesis (n=6) or neonatal examination at birth (n=208)

<table>
<tr><td></td><td colspan="3">Sample processing protocol
Maternal blood samples were collected at enrolment institution and sent to Natera for its analysis</td></tr>
<tr><td>Outcomes</td><td colspan="3">Performance of NIPT for T21, T18 and T13
-Test failure (% samples): 4 samples (1%)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td></td><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>4</td></tr><tr><td>TN</td><td>256</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (51.0-100)</td></tr><tr><td>Sp</td><td>100 (98.5-100)</td></tr><tr><td>PPV</td><td>100 (51.0-100)</td></tr><tr><td>NPV</td><td>100 (98.5-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>1</td></tr><tr><td>TN</td><td>256</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (20.7-100)</td></tr><tr><td>Sp</td><td>100 (98.5-100)</td></tr><tr><td>PPV</td><td>100 (20.7-100)</td></tr><tr><td>NPV</td><td>100 (98.5-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA Testing</th></tr><tr><td>TP</td><td>1</td></tr><tr><td>TN</td><td>256</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (20.7-100)</td></tr><tr><td>Sp</td><td>100 (98.5-100)</td></tr><tr><td>PPV</td><td>100 (20.7-100)</td></tr><tr><td>NPV</td><td>100 (98.5-100)</td></tr></table></td></tr>
<tr><td></td><td colspan="3">Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Benachi et al [51]	
Study characteristics	Study design: multicentre DTA study (prospective cohort)(29 centres) Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: France Setting: routine prenatal care Data collection period: December 2012 to October 2013 Target population: high-risk population Target condition prevalence in the enrolled population: 1/17 for T21, 1/40 for T18 and 1/74 for T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): HiSeq1500/MPS Country where samples were analysed: not reported Cut off for NIPT: Z-score for T21=3 and Z-score for T18 and T13=3.95
Population characteristics	Maternal age in years (mean [SD]): 35 [30-39] Gestational age in weeks (median [SD]): 15.1 [10.2-34.6] BMI in Kg/m^2 (median [169]): 23 [21-27] Pregnancy by ART(% pts): not reported Inclusion criteria: -Pregnant women were more 10 weeks of gestation and had a singleton or twin -With or without fetal ultrasound findings -Maternal age>38 years -Maternal serum screening or history of pregnancy with trisomy and who were willing to undergo invasive procedures Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 900 (893 singleton and 7 twin) Population excluded: 8 Reason for exclusion: -Definitive karyotype not available: 8 Population included: 892 -with NIPT result: 886 -with comparator result: no intervention -with reference standard result: 892

<table>
<tr><td></td><td>Reference standard (% pts): amniocentesis or CVS
Sample processing protocol
Maternal blood samples were collected into EDTA tubes at each enrolment institution and sent to the clinical laboratory.
CfDNA was extracted from plasma samples by QIAamp DSP Circulating Nucleic Acid Kit.
NIPT was performed in a blinded fashion respect to the fetal karyotype. It was not reported if karyotype analysis was blinded.</td></tr>
<tr><td>Outcomes</td><td>Performance of NIPT for T21
-Test failure (% samples): 6 samples (0.7%) (fetal fraction<4% or atypical result, i.e. positive Z-score for more than one chromosome). By CVS were confirmed two cases of triploidy and one cases of monosomy X.
-Uncertain results rate (% samples): not reported
Forty-five cases with negative NIPT result, were classified as pathogenic (42 cases with 14 sex chromosomal anomalies and 3 cases of 18 trisomy).</td></tr>
<tr><td></td><td>
Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>76</td></tr>
<tr><td>TN</td><td>815</td></tr>
<tr><td>FP</td><td>1</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (95.3-100)</td></tr>
<tr><td>Sp</td><td>99.9 (99.3-100)</td></tr>
<tr><td>PPV</td><td>98.7 (93.0-99.8)</td></tr>
<tr><td>NPV</td><td>100 (99.5-100)</td></tr>
</table>
Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>22</td></tr>
<tr><td>TN</td><td>860</td></tr>
<tr><td>FP</td><td>1</td></tr>
<tr><td>FN</td><td>3</td></tr>
<tr><td>S</td><td>88.0 (68.8-97.5)</td></tr>
<tr><td>Sp</td><td>99.9 (99.4-100)</td></tr>
<tr><td>PPV</td><td>95.6 (79.0-99.2)</td></tr>
<tr><td>NPV</td><td>99.6 (99.0-99.9)</td></tr>
</table>
Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>12</td></tr>
<tr><td>TN</td><td>873</td></tr>
<tr><td>FP</td><td>1</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (73.5-100)</td></tr>
<tr><td>Sp</td><td>99.9 (99.4-100)</td></tr>
<tr><td>PPV</td><td>92.3 (66.7-98.6)</td></tr>
<tr><td>NPV</td><td>100 (99.6-100)</td></tr>
</table>
</td></tr>
<tr><td></td><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with major other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA</td></tr>
</table>

	-Change in uptake of prenatal screening: NA

Author(s): Ke et al [53]	
Study characteristics	Study design: DTA study (prospective cohort) Year of publication: 2015 Study's registration number in clinical trial database: not reported Country/ies of recruitment: China Setting: routine prenatal care Data collection period: March 2012- May 2013 Target population: high-risk pregnant population Target condition prevalence in the enrolled population: 1/138 for T21, 1/390 for T18 and 1/2340 for T13 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): not reported Country where samples were analysed: Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 2061 women were <35 years and 279 were ≥35 years Gestational age in weeks (median [169]): 80 women at 12-14 week of gestation, 2239 at 15-20 week and 21 at ≥24 week Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: singleton pregnant women at 12-14 week of gestation with high-risk of aneuploidies due to the following reasons, i.e. over age 35 (n=147), history of abnormal pregnancy including children with DS and repeated spontaneous abortion, stillbirth in pregnancy periods, abnormal serological screening for DS at early and mid-pregnancy (n=1189) or abnormal screening for fetal NT (n=72). Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 2340 Population excluded: 0 Population included: 2340 -with NIPT result: 2340 -with comparator result: no intervention -with reference standard result: 2340 (24 samples by karyotyping and 2316 by follow-up) Reference standard (% pts): amniocentesis or neonatal follow-up *Sample processing protocol* Maternal blood samples were collected into an EDTA tubes at the enrolment institution. Fetal DNA in maternal plasma was extracted by QIAamp Circulation Nucleic Acid Kit (Qiagen).

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 0 samples

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in all pregnancies (calculated based on study results)

Variable	cfDNA testing
TP	17
TN	2316
FP	0
FN	0
S	100 (81.6-100)
Sp	100 (99.8-100)
PPV	100 (81.6-100)
NPV	100 (99.8-100)

Diagnostic accuracy measures for Trisomy 18 in all included pregnancies (calculated based on study results)

Variable	cfDNA testing
TP	6
TN	2316
FP	0
FN	0
S	100 (61-100)
Sp	100 (99.8-100)
PPV	100 (61.0-100)
NPV	100 (99.8-100)

Diagnostic accuracy measures for Trisomy 13 in all included pregnancies(calculated based on study results)

Variable	cfDNA Testing
TP	1
TN	2316
FP	0
FN	0
S	100 (20.7-100)
Sp	100 (99.8-100)
NPV	100 (20.7-100)
NPV	100 (99.8-100)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Lee et al [54]	
Study characteristics	Study design: DTA study (prospec-tive cohort) Year of publication: 2015 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Korea (Asan Medical Center, Seoul, Ko-rea) Setting: routine prenatal screening Data collection period: August 2014-February 2015 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/19 for T21, 1/46 for T18 and 1/93 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): MiSeq and NextSeq, Illumi-na/random massively parallel shotgun sequencing-Momguard protocol Country where samples were ana-lysed: LabGenomics Clinical La-boratory, Korea Cut off for NIPT: Z-score>4 for a chromosome indicates a higher risk of aneuploidy than standard set. If Z-score is between 2.5 and 4, it was considered as intermediate risk for T21 and T18. For T13, cut-off for high risk was Z-score=2.8 and 1.9 for intermediate risk.
Population characteristics	Maternal age in years (mean [169]): 32 (21-43) Gestational age in weeks (median [169]): 21.2 (8.2-31.1) Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women with sin-gleton or twin pregnancy who were >18 years old, gestational age >8 weeks and who met at least one of the following additional criteria: advanced maternal age (≥35 years), a positive serum biochemical screening test, the presence of fetal anomalies detected by ultrasound, or a personal/family history of fetal aneuploidy. It were also included multiple gestation (n: 2 twin preg-nancies) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 93 Population excluded: 1 Reason for exclusion: -absence of a result on cfDNA Population included: 92 -with NIPT result: 92 -with comparator result: no inter-vention -with reference standard result: 93

<table>
<tr><td></td><td>Reference standard (% pts): amni-ocentesis, CVS, cordocentesis, neonatal peripheral blood or prod-ucts of conception. It was not re-ported sampled percentage ana-lysed with each method.
Sample processing protocol
Maternal blood samples were col-lected into Streck DNA BCT tubes and sent to LabGenomics Clinical Laboratory.
All clinical data and NIPT results were blinded to the laboratory in-vestigators.</td></tr>
<tr><td>Outcomes</td><td>Performance of NIPT for T21, T18 and T13
-Test failure (% samples): 1.07% (1 sample)
-Uncertain results rate (% samples): not reported
Diagnostic accuracy measures for Trisomy 21 in all pregnancies with karyotyping (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>5</td></tr><tr><td>TN</td><td>87</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (56.6-100)</td></tr><tr><td>Sp</td><td>100 (95.8-100)</td></tr><tr><td>PPV</td><td>100 (56.6-100)</td></tr><tr><td>NPV</td><td>100 (95.8-100)</td></tr></table>Diagnostic accuracy measures for Trisomy 18 in pregnancies with karyotyping (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>2</td></tr><tr><td>TN</td><td>90</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (34.2-100)</td></tr><tr><td>Sp</td><td>100 (95.9-100)</td></tr><tr><td>PPV</td><td>100 (34.2-100)</td></tr><tr><td>NPV</td><td>100 (95.9-100)</td></tr></table>Diagnostic accuracy measures for Trisomy 13 in all pregnancies with karyotyping (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA Testing</th></tr><tr><td>TP</td><td>1</td></tr><tr><td>TN</td><td>91</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (20.7-100)</td></tr><tr><td>Sp</td><td>100 (95.9-100)</td></tr><tr><td>PPV</td><td>100 (20.7-100)</td></tr><tr><td>NPV</td><td>100 (95.9-100)</td></tr></table>Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: An additional 10 chromosome anomalies were de-tected by cytogenetic analysis (10.8%)
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Sago et al [55]	
Study characteristics	Study design: multicentre DTA study (prospective cohort) Year of publication: 2015 Study's registration number in clini-cal trial database: University Medi-cal Information Network clinical trials, UMIN000009338 Country/ies of recruitment: Japan Setting: routine prenatal-screening Data collection period: April 2013-March 2014 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/110 for T21, 1/0228 for T18 and 1/860 for T13 Comparator: no intervention Cut off point comparator: NA Index test (trademark/technique type): MaternityT Plus/MPS Country where samples were ana-lysed: USA (Sequenom, Inc.) Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 38.3 [21-48] Gestational age in weeks (median [169]): 13.3 [10.0-19.9] BMI (median [169]): 20.9 [14.1-37.0] Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women with sin-gleton pregnancy at least 10 weeks of gestation with increased risk of aneuploidy, i.e. maternal age≥35 years of age, foetuses with ultraso-nographic or maternal serum mark-er findings indicating an increased risk of aneuploidy, history of children affected by trisomy, or a parent car-rying a balanced Robertsonian translocation with an increased risk of trisomy 13 or trisomy 21. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 7740 Population excluded: 0 Reason for exclusion: -absence of a result on cfDNA (n=4) Population included: 7736 -with NIPT result: 7736 -with comparator result: NA -with reference standard result: 131 Reference standard (% pts): CVS or amniocentesis

	Sample processing protocol Blood samples were collected at each institution and sent to Se-quenom, Inc. for its analysis. It was not reported if sequencing and karyotyping analysis was per-formed in a blinded fashion respect to ultrasonographic and standard screening results.
Outcomes	**Performance of NIPT for T21, T18 and T13** -Test failure (% samples): 0.05 (4 samples) -Uncertain results rate (% samples): NA PPV for Trisomy 21 in women who have undergone invasive testing: 95.9% (70/73) PPV for Trisomy 18 in women who have undergone invasive testing: 81% (34/42) PPV for Trisomy 13 in women who have undergone invasive testing: 81.8% (9/11) In 1638 women followed after birth, it was detected one false-negative case of T18.
	Additional patient relevant outcomes *Safety* -Increase the number of children born with other major unconfirmed chromosomal anomalies: not reported cases with other anomalies -Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with uncertain significance: not reported cases with other anomalies *Effectiveness* -Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA -Change in uptake of prenatal screening: NA

Author(s): Hernandez-Gomez et al [52]	
Study characteristics	Study design: DTA study (prospec-tive observational cohort) Year of publication: 2015 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Mexico (Hospital Ángeles Loma) Setting: routine prenatal care Data collection period: August 2013-January 2015 Target population: high-risk popula-tion Target condition prevalence in the enrolled population: 1/21 for T18 and 1/21 for monosomy Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Harmony® Prenatal Test Country where samples were ana-lysed: Ariosa Diagnostic, USA Cut off for NIPT: risk score for 13, 18 and 21 trisomy>99% or <1/10000
Population characteristics	Maternal age in years (mean [169]): 37.1 [23-46] Gestational age in weeks (median [169]): 13.3 [10.0-18.6] Maternal age (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: singleton pregnant women who meet any of the follow-ing indications, i.e. advanced mater-nal age (n=30), positive FCT (n=3), recurrent previous miscarriage (n=3), clinical decision (n=6) or ma-ternal anxiety due to X chromo-some-linked recessive disorder (n=1) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 42 Population excluded: 0 Population included: 42 -with NIPT result: 41 (1 case of failure test because of low fetal fraction) -with comparator result: no inter-vention -with reference standard result: 41 Reference standard (% pts): amni-ocentesis (n=4) or neonatal follow-up (n=37) *Sample processing protocol* Blood samples were collected at enrolment center and sent to Ariosa Diagnostic for its analysis. The cfDNA of each sample was isolated and quantified using the DANSR assay and the FORTE algo-rithm was used to estimate the risk or OR of 13, 18 and 21 trisomy in each sample.

<table>
<tr><td>Outcomes</td><td>
Performance of NIPT for T18 and Monosomy X
-Test failure (% samples): 1 sample (2.8%)
-Uncertain results rate (% samples): not reported
</td></tr>
<tr><td></td><td>
Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP*</td><td>2</td></tr>
<tr><td>TN</td><td>37</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (34.2-100)</td></tr>
<tr><td>Sp</td><td>100 (90.6-100)</td></tr>
<tr><td>PPV</td><td>100 (34.2-100)</td></tr>
<tr><td>NPV</td><td>100 (90.6-100)</td></tr>
</table>
* One T18 case was not confirmed by karyotyping because of miscarriage but ultrasonography findings FCT were concordant with presence of NIPT result. Another T18 case was confirmed by amniocentesis (47, XX,+18).
</td></tr>
<tr><td></td><td>
Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA
</td></tr>
</table>

Author(s): Korostelev et al [60]	
Study characteristics	Study design: DTA study (prospec-tive cohort) Year of publication: 2014 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Russia Setting: routine prenatal care Data collection period: 2012-2014 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/37 for T21, 1/864 for T18 and 1/576 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): not reported/SNPs using NATUS algorithm Country where samples were ana-lysed: Natera laboratories, USA Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 34.4 (26-45) Gestational age in weeks (median [169]): 14 (3-33) Maternal weight (median [169]): not reported Pregnancy by ART (% pts): not re-ported Inclusion criteria: singleton pregnant women who had high-risk of aneu-ploidies according to the FCT results (risk score<1/250, 53.8%), age of the women (≥35 years, 27.9%) or the woman's wish with results of the screening being normal (11%) and other reasons (bad reproductive history in 3.6% of populations or undergone the IVF procedure in 2.4% of populations) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 1968 Population excluded: 240 Reasons for exclusion: NA Population included: 1728 -with NIPT result: 1728 -with comparator result: no intervention -with reference standard result: 681 57 (invasive testing); 624 (follow up) Reference standard (% pts): karyo-typing, CMA (n=241) or neonatal examination at birth. *Sample processing protocol* Maternal blood samples were col-lected at enrolment institution and sent to Natera for its analysis.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 0 samples

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	47
TN	682
FP	0
FN	0
S	100 (92.4-100)
Sp	100 (99.4-100)
PPV	100 (92.4-100)
NPV	100 (99.4-100)

Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	2
TN	680
FP	0
FN	0
S	100 (34.2-100)
Sp	100 (99.4-100)
PPV	100 (34.2-100)
NPV	100 99.4-100)

Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	3
TN	679
FP	0
FN	1 (FN rate: 25%)
S	75 (30.1-95.4)
Sp	100 (99.4-100)
PPV	100 (43.8-100)
NPV	99.9 (99.2-100)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: Using invasive testing one case of microdeletion 22q13.31-q13.33 associated with PhelanMcDermid syndrome and two cases of small microdeletions associated dominant diseases were detected. None of them were identified by NIPT test.

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Stumm et al [62]	
Study characteristics	Study design: Blinded DTA study (prospective observational cohort) Year of publication: 2014 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Germa-ny and Switzerland (5 centres) Setting: routine prenatal care Data collection period: not reported Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/13 for T21, 1/65 for T18 and 1/104 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): LifeCodexx (Illumina HiS-eq2000/random MPS) Country where samples were ana-lysed: LifeCodexx AG and GATC Biotech AG Cut off for NIPT: sample with Z-score≥3 was classified as high risk of T21. If Z-score<3, sample was considered as negative result for T21. The cut-off Z-score for T18 and T13 was 3.9 and 3.2 respec-tively
Population characteristics	Maternal age in years (mean [169]): 36.0 [19-47] Gestational age in weeks (mean [169]): 15.6 [11+0 – 32+1) Maternal weight (median [range]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: singleton pregnant women at the age of at least 18 years who signed informed consent with indication for invasive testing due to any of following reasons: advanced maternal age (>35 years old)(n=363), positive maternal se-rum markers (n=58), ultrasound anomaly (n=205), positive family history for prenatally diagnosable disease (n=11), parental chromo-somal aberration (n=2) or other risk factors (n=78), performance of a conventional karyotyping procedure and blood drawn after the invasive procedure. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 522 consecu-tive women Population excluded: 18 Reason for exclusion: -no informed consent: 9 -no karyotyping analysis: 8 -previously analyzed: 1 Population included: 504 -with NIPT result: 472 (failed se-quencing quality criteria in 14 sam-ples and failed libraries in 18 sam-ples) -with comparator result: no inter-vention

-with reference standard result: 472

Reference standard (% pts): amni-ocentesis (69.1), CVS (30.3) or cordocentesis (0.6)

Sample processing protocol

Blood samples were collected into either EDTA (n=166) or cell-free DNA BCT™ (n=306) tubes at each study center prior to invasive pro-cedure and sent to LifeCodexx AG or GATC Biotech AG for its analy-sis, which was blinded to popula-tion clinical information including karyotyping. CfDNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (Qi-agen). Sequencing data analysis was performed using two bioinformatics algorithm DAP.21 focused on de-tection T21 only and DAP.plus which detects T18 and T13 in addi-tion to T21 and is based on guano-sine-cytosine (GC) normalization.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 32 samples (6.3%)

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	40
TN	430
FP	0
FN	2 (FN rate: 4.8%)
S	95.2 (84.2-95.7)
Sp	100 (99.1-100)
PPV	100 (91.2-100)
NPV	99.5 (98.3-99.9)

Diagnostic accuracy measures for Trisomy 18 (only algorithm DAP.plus) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	8
TN	463
FP	1 (FP rate: 0.21%)
FN	0
S	100 (67.7-100)
Sp	99.8 (98.8-100)
PPV	88.9 (56.5-98)
NPV	100 (99.2-100)

Diagnostic accuracy measures for Trisomy 13 (only algorithm DAP.plus) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	5
TN	467
FP	0
FN	0
S	100 (56.6-100)
Sp	100 (99.2-100)
PPV	100 (56.6-100)
NPV	100 (99.2-100)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-clear significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

	-Change in uptake of prenatal screening: NA

Author(s): Jeon et al [59]	
Study characteristics	Study design: DTA study (prospec-tive cohort) Year of publication: 2014 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: China (Xiamen Maternal & Child Health Care Hospital) Setting: routine prenatal care Data collection period: March 2012-October 2013 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/14 for T21 and 1/39 for T18 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Ion Proton™ System Country where samples were ana-lysed: not reported Cut off for NIPT: Sample Z-score>2.459 was considered indic-ative of T18 and sample Z-score>2.566 was classified as high-risk of T21
Population characteristics	Maternal age in years (mean [SD]): 30.73±4.99 42% of women were ≥35 years old Gestational age in weeks (% popula-tions): 12-21 weeks: 73.6% ≥22 weeks: 26.5 % Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women who were ≥19 years old, had a singleton preg-nancy with at least 12 weeks' gesta-tion and classified as high-risk of aneuploidies by FCT screening in combination with nuchal translucen-cy measurement or second trimester serum screening alone or in combi-nation with FTS results. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 155 Population excluded: 0 Population included:155 -with NIPT result: 155 -with comparator result: no inter-vention -with reference standard result: 155

<table>
<tr><td></td><td colspan="2">Reference standard (% pts): amni-ocentesis
Sample processing protocol
Maternal blood samples were col-lected into cfDNA BCT™ tubes at each enrolment institution.
Plasma cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit (Qiagen).</td></tr>
<tr><td rowspan="3">Outcomes</td><td colspan="2">Performance of NIPT for T21 and T18
Test failure (% samples): 0 samples
Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>11</td></tr><tr><td>TN</td><td>139</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (71.5-100)</td></tr><tr><td>Sp</td><td>100 (97.5-100)</td></tr><tr><td>PPV</td><td>100 (71.5-100)</td></tr><tr><td>NPV</td><td>100 (97.5-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>5</td></tr><tr><td>TN</td><td>139</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (47.8-100)</td></tr><tr><td>Sp</td><td>100 (97.6-100)</td></tr><tr><td>PPV</td><td>100 (47.8-100)</td></tr><tr><td>NPV</td><td>100 (97.6-100)</td></tr></table></td></tr>
<tr><td colspan="2">Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Porreco et al [61]	
Study characteristics	Study design: Multicentre, blinded DTA study (prospective cohort) Year of publication: 2014 Study's registration number in clini-cal trial database: NCT00847990 Country/ies of recruitment: USA Setting: routine prenatal care Data collection period: September 2009-April 2011 Target population: high-risk popula-tion Target condition prevalence in the enrolled population: 1/30 for T21, 1/107 for T18 and 1/260 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): HiSeq 2000, Illumina Inc. Country where samples were ana-lysed: USA Cut off for NIPT: Z-scores at or above 3 were considered indicative of trisomy 21 and Z-scores of at or above 3.95 were considered in-dicative of trisomies 18 and 13
Population characteristics	Maternal age in years (mean [SD]): 35.1 [5.6] Gestational age in weeks (mean [SD]): 16.3 [3.5] Maternal weight (median [169]): 156.9 [36.7] Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: singleton pregnant women were at least 18 years of age and had made the decision to pursue invasive prenatal diagnosis by CVS or amniocentesis due to high risk of aneuploidies because of met at least one of the following conditions: advanced maternal age (≥35 years of age), positive result on first or second trimester serum screening test, presence of fetal anomaly on ultrasound or per-sonal or familiar history of a chro-mosome anomaly Exclusion criteria: inability to give written informed consent, multiple gestation or fetal demise of an addi-tional embryo during the current pregnancy at ≥8 weeks of gestation
Study protocol	*Population enrollment flow* Population enrolled: 4170 Population excluded: 740 Reason for exclusion: -insufficient sample volume: 320 -outside laboratory processing window: 120 -laboratory quality control set: 270 -incomplete report forms: 24

<table>
<tr><td></td><td>
-no invasive procedure performed: 6
Population included: 3430
-with NIPT result: 3376 (54 samples with insufficient quality criteria due to low fetal DNA fraction, insuffi-cient library concentration/total accounts or amplification bias).
-with comparator result: no inter-vention
-with reference standard result: 3322 (56 samples with complex karyotypes)
Reference standard (% pts): CVS (25%) or amniocentesis (75%)
Sample processing protocol
Maternal blood samples were col-lected into EDTA tubes at each participant institution and sent to external laboratory of study spon-sor (Sequenom Inc.) for its analy-sis.
Karyotyping analysis was per-formed by an independent com-mercial laboratory.
All database including NIPT test results, karyotyping analysis results and populations demographic information were stored separately until its statistical analysis
</td></tr>
<tr><td>Outcomes</td><td>
Performance of NIPT for T21, T18, T13
-Test failure (% samples): 54 samples (1.29%)
-Uncertain results rate (% samples): not reported
<table>
<tr>
<td>Diagnostic accuracy measures for Trisomy 21 (n=3322) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>137</td></tr>
<tr><td>TN</td><td>3182</td></tr>
<tr><td>FP</td><td>3 (FP rate: 0.09%)</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (97.34-100)</td></tr>
<tr><td>Sp</td><td>99.9 (99.72-99.98)</td></tr>
<tr><td>PPV</td><td>97.9 (93.8-99.56)</td></tr>
<tr><td>NPV</td><td>100 (99.98-100)</td></tr>
</table>
</td>
<td>Diagnostic accuracy measures for Trisomy 18 (n=3322) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>36</td></tr>
<tr><td>TN</td><td>3283</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>3 (FN rate: 7.7%)</td></tr>
<tr><td>S</td><td>92.3 (79.13-98.38)</td></tr>
<tr><td>Sp</td><td>100 (99.89-100)</td></tr>
<tr><td>PPV</td><td>100 (90.26-100)</td></tr>
<tr><td>NPV</td><td>99.9 (99.73-99.9)</td></tr>
</table>
</td>
<td>Diagnostic accuracy measures for Trisomy 13 (n=3322) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA Testing</th></tr>
<tr><td>TP</td><td>14</td></tr>
<tr><td>TN</td><td>3306</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>2 (FN rate: 12.3%)</td></tr>
<tr><td>S</td><td>87.5 (61.65-98.45)</td></tr>
<tr><td>Sp</td><td>100 (99.89-100)</td></tr>
<tr><td>PPV</td><td>100 (76.84-100)</td></tr>
<tr><td>NPV</td><td>99.9 (99.78-99.9)</td></tr>
</table>
</td>
</tr>
</table>
Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA
</td></tr>
</table>

	-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA *Effectiveness* -Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA -Change in uptake of prenatal screening: NA

Author(s): Zhou et al [64]	
Study characteristics	Study design: Blinded DTA study (prospective cohort) Year of publication: 2014 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: China Setting: routine prenatal care Data collection period: September- July 2013 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/214 for T21, 1/770 for T18 and 1/3852 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): not reported Country where samples were ana-lysed: clinical laboratory at BGI-Shenzhen Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 40.4% or 3108/7701 wom-en with advanced age, ≥35 years and 59.6% or 4596/7701 women with <35 years Gestational age in weeks (median [169]): not reported Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: women with sin-gleton pregnancies at 12 to 14 week of gestation who met the following indications: advanced age, high risk for Down syndrome based on screening results (32.1%, 2472/7701) and presence de ab-normal ultrasonographic soft mark-ers or ultrasound anomalies, as well as women with no prior DS screen-ings (56.6%, 4361/7701). Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 7705 Population excluded: 4 Reason for exclusion: - absence of a result on cfDNA Population included: 7701 -with NIPT result: 7701 -with comparator result: no inter-vention -with reference standard result: 54 amniocentesis and 3894 follow-ups Reference standard (% pts): amni-ocentesis or neonatal follow-up

Sample processing protocol

Sample collection, molecular test and trisomy analysis were per-formed in a clinical laboratory at BGI-Shenzhen.

Fetal karyotyping was performed blindly following NIPT.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 0,05% (4 samples))

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	36
TN	3918
FP	2 (FP rate: 0.05% [0.02-0.10])
FN	0
S	100 (72.2-100)
Sp	99.9 [99.8-100]
PPV	94.7 [82.7-98.5]
NPV	100 (99.9-100)

Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	10
TN	3926
FP	2 (FP rate: 0.05% [0.02-0.10])
FN	0
S	100 (72.1-100)
Sp	99.9 [99.8-100]
PPV	83.3 [55.2-95.3]
NPV	100 (99.9-100)

Diagnostic accuracy for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	2
TN	3944
FP	2 (FP rate: 0.05% [0.02-0.10])
FN	0
S	100 (34.2-100)
Sp	99.9 [99.8-100]
PPV	50.0 [15.0-100]
NPV	100 (99.9-100)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Willems et al [63]	
Study characteristics	Study design: Blinded DTA study (prospective cohort) Year of publication: 2014 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Belgium and The Netherlands Setting: routine prenatal screening Data collection period: March to December 2013 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/59 for T21, 1/750 for T18 and 1/1500 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Harmony™ Prenatal Test/CSS Country where samples were ana-lysed: USA (Ariosa Diagnostic) Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [SD]): 36±3 Gestational age in weeks (mean [SD]): 13±2 Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: pregnant women who had indication for NIPT testing i.e. an elevated FCT risk (>1/200 in the Netherlands and >1/300 in Bel-gium) (22%), advanced maternal age (>37 years at expected day of delivery) without increased risk on FTS or no FTS done (40.06%), other indications (previous pregnancies with chromosome anomalies in one or both parents or family)(3.27%) or none of these indications (34.73%) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 3000 consecu-tive women Population excluded: 32 Reason for exclusion: -absence of a result on cfDNA: 32 samples Population included: 2968 -with NIPT result: 2968 -with comparator result: NA -with reference standard result: 47 Reference standard (% pts): amni-ocentesis or CVS

Sample processing protocol

Maternal blood samples were col-lected into Streck DNA BCT tubes and sent to Ariosa Diagnostics for its analysis.

It was not reported if sequencing and karyotyping analysis was per-formed in a blinded fashion respect to ultrasonographic and standard screening results.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 1.06% or 32 samples (27 2nd/3rd analysis samples and 5 no 2nd sample)

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	51
TN	2911
FP	0
FN	1 (FN rate: 1.92%)
S	98 (89.9-99.7)
Sp	100 (99.9-100)
PPV	100 (93.0-100)
NPV	99.9 (99.8-100)

Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	4
TN	2911
FP	0
FN	1 (FN rate: 20%)
S	80 (37.6-96.4)
Sp	100 (99.9-100)
PPV	100 (51.0-100)
NPV	99.9 (99.8-100)

Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	3
TN	2911
FP	0
FN	0
S	100 (43.8-100)
Sp	100 (99.9-100)
PPV	100 (51.0-100)
NPV	100 (99.8-100)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Liang et al [65]	
Study characteristics	Study design: DTA study (prospec-tive cohort) Year of publication: 2013 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: China Setting: routine prenatal care Data collection period: March 2009-June 2011 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/11 for T21, 1/31 for T18, 1/109 for T13, 1/435 for T13 and 1/54 for SCA Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): HiSeq2000, Illumina/MPS. For DNA library preparation was used the modified ChIP seq proto-col and data analysis was per-formed by guanosine-citosine (GC) correction algorithm. Country where samples were ana-lysed: Berry Genomics, China Cut off point for NIPT: T21, Z score=3 T18, Z score= 5,91 T13, Z score= 5,72 T9, Z score= 7,45 Sex chromosomal aneuploidy, Z score= -2,91 to 2,91/<3
Population characteristics	Maternal age in years (mean [SD]): 31±5.9 31% women were ≥35 years of age Gestational age in weeks (median [169]): 21+3 (11+3 to 39+3) 11+0 to 14+6=0.23% 15+0 to 20+6=40% 21+0 to 39+6=59.77% Maternal weight (median [169]): not reported Pregnancy by ART (% pts): not re-ported Inclusion criteria: pregnant women who had indication for invasive di-agnostic procedure, i.e. advanced maternal age (≥35 years old) (19.31%), positive serum screening (49.89%), ultrasound anomaly (14.48%), prior aneuploid pregnancy (0.92%) and more than one indica-tion (15.40%) Exclusion criteria: not reported

<table>
<tr><td>Study protocol</td><td colspan="3">
Population enrollment flow
Population enrolled: 435
Population excluded: 23
Reason for exclusion:
-failed sequencing quality control: 12
-missing karyotyping: 11
Population included: 412
-with NIPT result: 412
-with comparator result: no inter-vention
-with reference standard result: 412
Reference standard (% pts): amni-ocentesis and other method not specified
Sample processing protocol
Maternal blood samples were col-lected into EDTA tubes at each enrolment institution and sent to Berry Genomics for its analysis. Plasma was extracted using the QIAamp Circulating Nucleic Acid kit (Qiagen). The karyotyping was conducted in the participant cen-tres.
Karyotyping information were con-ducted in a blinded fashion and Berry Genomics obtained sequenc-ing results independently.
</td></tr>
<tr><td>Outcomes</td><td colspan="3">
Performance of NIPT for T21, T18, T13
-Test failure (% samples): 12 samples (2.76%)
-Uncertain results rate (% samples): not reported
</td></tr>
<tr><td></td><td>
Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>40</td></tr>
<tr><td>TN</td><td>372</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (91.2-100)</td></tr>
<tr><td>Sp</td><td>100 (99.0-100)</td></tr>
<tr><td>PPV</td><td>100 (91.2-100)</td></tr>
<tr><td>NPV</td><td>100 (99.9-100)</td></tr>
</table>
</td><td>
Diagnostic accuracy for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA testing</th></tr>
<tr><td>TP</td><td>14</td></tr>
<tr><td>TN</td><td>398</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (78.5-100)</td></tr>
<tr><td>Sp</td><td>100 (99.0-100)</td></tr>
<tr><td>PPV</td><td>100 (78.5-100)</td></tr>
<tr><td>NPV</td><td>100 (99.0-100)</td></tr>
</table>
</td><td>
Diagnostic accuracy measures for Trisomy 13* in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table>
<tr><th>Variable</th><th>cfDNA Testing</th></tr>
<tr><td>TP</td><td>4</td></tr>
<tr><td>TN</td><td>407</td></tr>
<tr><td>FP</td><td>1 (FP rate: 0.24%)</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (51.0-100)</td></tr>
<tr><td>Sp</td><td>99.75 (98.6-100)</td></tr>
<tr><td>PPV</td><td>80 (37.6-96.4)</td></tr>
<tr><td>NPV</td><td>100 (99.1-100)</td></tr>
</table>
*Including a twin sample
</td></tr>
</table>

	Additional patient relevant outcomes *Safety* -Increase the number of children born with other major unconfirmed chromosomal anomalies: NA -Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA *Effectiveness* -Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA -Change in uptake of prenatal screening: NA

Author(s): Verweij et al [67]	
Study characteristics	Study design: DTA study (prospec-tive cohort) European Non-Invasive Trisomy Evaluation, EU-NITE study Year of publication: 2013 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Norway, Sweden and Netherlands Setting: routine prenatal care Data collection period: May 2011 to March 2012 Target population: high-risk popula-tion Target condition prevalence in the enrolled population: 1/33 for T21 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): not reported Country where samples were ana-lysed: Ariosa Diagnostic, USA Cut off for NIPT: 1/100 (1%) was fixed as the threshold for classify-ing a sample as high-risk versus low risk
Population characteristics	Maternal age in years (median [169]): 36.4 [20-47] Gestational age in weeks (median [169]): 14.0 [10-28] Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: pregnant women ≥18 years of age with an increased risk for T21 based on first trimester screening (serum screening, nuchal translucency measurement and/or maternal age), choosing to undergo invasive testing due to presence of ultrasound fetal anomalies or requiring invasive testing for psy-chosocial or anxiety reasons. Exclusion criteria: women with >1 foetus, an invasive testing per-formed prior of the blood sampling, history or active significant malig-nancy requiring major surgery or systemic chemotherapy or language restriction with failure to understand the study information.
Study protocol	*Population enrollment flow* Population enrolled: 595 consecutive women Population excluded: 75 Reason for exclusion: -no meet inclusion criteria: 21 -insufficient plasma volume: 19 -logistic problems: 11 -other chromosome anomalies besides T21: 24

<table>
<tr><td></td><td>Population included: 520
-with NIPT result: 504 (absence of a result on cfDNA due to low fetal fraction in 7 samples, 9 with assays failure and one additional samples with no accurate result)
-with comparator result: no intervention
-with reference standard result: 503
Reference standard (% pts): CVS (n=280) or amniocentesis (n=240)
Sample processing protocol
Blood samples were collected into two cell-free DNA BCT™ tubes at each enrolment centers just prior to the invasive testing and sent to Ariosa Diagnostic, Inc. for its analysis.
The cfDNA of each sample was isolated and quantified using the DANSR assay and the FORTE algorithm was used to estimate the risk of T21 in each sample (OR of T21).
The laboratory personnel were blinded to the clinical information.
Invasive analysis was performed by respective certified genetic laboratories of the participating centers.</td></tr>
<tr><td rowspan="3">Outcomes</td><td>Performance of NIPT for T21
-Test failure (% samples): 16 samples (3.07%)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>17</td></tr><tr><td>TN</td><td>486</td></tr><tr><td>FP</td><td>0</td></tr><tr><td>FN</td><td>1 (FN rate: 5.6%)</td></tr><tr><td>S</td><td>94.4 (72.7-99.9)</td></tr><tr><td>Sp</td><td>100 (99.4-100)</td></tr><tr><td>PPV</td><td>100 (81.6-100)</td></tr><tr><td>NPV</td><td>99.8 (99.8-100)</td></tr></table></td></tr>
<tr><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with other major unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies with un-certain significance: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA</td></tr>
</table>

	-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA -Change in uptake of prenatal screening: NA

Author(s): Nicolaides et al [66]	
Study characteristics	Study design: DTA study (prospec-tive cohort) Year of publication: 2013 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: United Kingdom Setting: routine prenatal care Data collection period: not reported Target population: high-risk popula-tion Target condition prevalence in the enrolled population: 1/10 for T21, 1/81 for T18, 1/121 for Turner syn-drome and 1/242 for each T13 and triploid (69,XXX) Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): not reported/targeted se-quencing of SNPs with NATUS (Next-generation Aneuploidy Test Using SNPs) algorithm Country where samples were ana-lysed: Natera Inc. USA Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean [169]): 35.7 [18.5-46.5] Gestational age in weeks (median [169]): 13.1 [11.3-13.9] Maternal weight (median [169]): not reported Pregnancy by ART (% pts): not re-ported Inclusion criteria: singleton pregnant women at 11-13 week of gestation were undergoing fetal karyotyping because positive FCT result (risk for T13, T18 or T21>1/300)(n=227) or previous aneuploidy pregnancy (n=6) and advanced maternal age (n=5), or women were undergoing invasive testing for sickle cell dis-ease. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 242 Population excluded: 13 Reason for exclusion: -absence of a result on cfDNA (insufficient total DNA, insufficient cfDNA fraction or high noise level) Population included: 229 -with NIPT result: 229 -with comparator result: no intervention -with reference standard result: 229

Reference standard (% pts): CVS

Sample processing protocol

Maternal blood samples were collected previously CVS, into Streck cell-free DNA BCT™ tubes from enrolment center and sent to the laboratory of Natera Inc. for its analysis.

Sequencing analysis was blinded to the fetal karyotype information.

Outcomes

Performance of NIPT for T21, T18 and T13

-Test failure (% samples): 13 samples (5.4%)

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	25
TN	204
FP	0
FN	NA
S	100 [86.7-100]
Sp	100 [98.2-100]
PPV	100 (86.7-100)
NPV	100 (98.2-100)

Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	3
TN	226
FP	0
FN	0
S	100 (43.8-100)
Sp	100 (98.3-100)
PPV	100 (43.8-100)
NPV	100 (98.3-100)

Diagnostic accuracy measures for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	1
TN	228
FP	0
FN	0
S	100 (20.7-100)
Sp	100 (98.3-100)
PPV	100 (20.7-100)
NPV	100 (98.3-100)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Norton et al [69]	
Study characteristics	Study design: multicentre blinded DTA study Year of publication: 2012 Study's registration number in clinical trial database: not reported Country/ies of recruitment: United States of America (USA), The Netherlands and Sweden Setting: routine prenatal care Data collection period: August 2010 to November 2011 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/49 for T21 and 1/108 for 1/108 Comparator: no intervention Cut off point comparator: no intervention Index test (trademark/technique type): trademark was not speci-fied/it was used Digital Analysis of Selected Regions-DANRS sequencing method and Fetal-fraction Optimized Risk of Trisomy Evaluation-FORTE analysis algorithm Country where samples were analysed: not reported Cut off for NIPT: 1/100 (1%) [99/100 or 99% to 1/10,000 or 0.01%]
Population characteristics	Maternal age in years (mean [169]): 34.3 [18-50] Gestational age in weeks (median [169]): 16.9 [10.0-38.7] Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: pregnant women aged ≥18 years, at gestational age≥10 weeks, with singleton preg-nancy and who were planned to undergo invasive prenatal diagnosis for any indication (not specified). Exclusion criteria: women with >1 foetus, presence of known aneuploidy, active malignancy or a history of metastatic cancer or who had already received CVS or amniocentesis during current pregnancy.
Study protocol	*Population enrollment flow* Population enrolled: 4002 Population excluded: 774 Reason for exclusion: -samples used for assay development: 433 -failed inclusion/exclusion criteria: 237 -insufficient sample volume: 84 -incorrect sample labeling: 20 Population included: 3228 -with NIPT result: 3080 (148 samples were excluded due to low fetal fraction (n=57) and assay failure (n=91)) -with comparator result: no intervention

<table>
<tr><td></td><td colspan="3">-with reference standard result: 3080
Reference standard (% pts): CVS (25.3%) or amniocentesis (74.7%)
Sample processing protocol
Maternal blood samples were collected into Cell-free BCT tubes at each institution and sent to laboratory for its analysis. The laboratory personnel who performed the analyses were blinded to the clinical information associated with each sample.</td></tr>
<tr><td>Outcomes</td><td colspan="3">Performance of NIPT for T21 and T18
-Test failure (% samples): 148 samples (4.8%)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td></td><td>Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>81</td></tr><tr><td>TN</td><td>2887</td></tr><tr><td>FP</td><td>1 (FP rate: 0.03% [0.002-0.20])</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (95.5-100)</td></tr><tr><td>Sp</td><td>99.97 (99.8-99.99)</td></tr><tr><td>PPV</td><td>98.8 (93.4-99.8)</td></tr><tr><td>NPV</td><td>100 (99.9-100)</td></tr></table></td><td>Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>37</td></tr><tr><td>TN</td><td>2888</td></tr><tr><td>FP</td><td>2 (FP rate: 0.07% [0.02-0.25])</td></tr><tr><td>FN</td><td>1 (FN rate: 2.6%)</td></tr><tr><td>S</td><td>97.4 (86.5-99.9)</td></tr><tr><td>Sp</td><td>99.9 (99.7-99.9)</td></tr><tr><td>PPV</td><td>94.9 (83.1-96.6)</td></tr><tr><td>NPV</td><td>99.9 (99.8-100)</td></tr></table></td><td>NIPT testing performance at other cut-off points (main changes in comparison to 1/100 cut-off point used previously)
-Cut-off point=1/1000 (0.1%)
S for T21 or T18 did not change but Sp decreased (99.90% and 99.79% respectively) and therefore FP rate increased, i.e. added two FP cases more for T21 and four FP cases more for T18.
-Cut-off point=1/300 (0.33%)
S for T21 or T18 did not change but Sp decreased (99.90% and 99.86% respectively) and therefore positive false rate increased two FP cases more for each trisomy.
- Cut-off point=1/10 (10%)
S for T21 did not change. However, S for T18 decreased (94.7%) and therefore there was one FN case more. Sp for T18 did not change, whereas Sp for T21 increased and there were not FP cases.</td></tr>
<tr><td></td><td colspan="3">Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Lau et al [68]	
Study characteristics	Study design: Blinded DTA study (prospective cohort) Year of publication: 2012 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Japan Setting: routine prenatal screening Data collection period: not reported Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/9 for T21, 1/11 for T18, 1/54 for T13, 1/13 for Turner syndrome and 1/108 for Klinefelter syndrome Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Verifi™™ Prenatal Test (Illu-mina HiSeq™ 2000) Country where samples were ana-lysed: Japan (enrolment center) Cut off for NIPT: sample with z-score>3 was classified as having at increased risk for assessed trisomy
Population characteristics	Maternal age in years (mean [SD]): 37±4.3 Gestational age in weeks (median [169]): 12+5 [11+4 to 28+0] Maternal weight (median [169]): not reported Pregnancy by ART (% pts): not re-ported Inclusion criteria: singleton pregnant women with indication for diagnostic procedures i.e. high-risk for FCT (47.2%), positive first trimester so-nographic markers (22.2%), other structural anomalies (18.5%), ma-ternal anxiety (11.1%) or previous trisomy 21 (0.9%). Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 108 Population excluded: 0 Population included: 108 -with NIPT result: 108 -with comparator result: NA -with reference standard result: 108 Reference standard (% pts): CVS (94.4%) or amniocentesis (5.6%) *Sample processing protocol* Maternal blood samples were col-lected into EDTA tubes. DNA se-quencing and data analysis was performed in the enrolment center. Investigators who carried out the DNA extraction, sequencing and data analysis were blinded to the karyotyping information

Outcomes

Performance of NIPT for T21, T18, T13

-Test failure (% samples): 0

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	11
TN	97
FP	0
FN	0
S*	100 (20.7-100)
Sp	100 (96.2-100)
PPV	100 (20.7-100)
NPV	100 (96.2-100)

*Using the z-score method with adjustment by GC correction, PPV=100%; using the Z-score method with adjustment by internal reference in this study, PPV= 100%

Diagnostic accuracy measures for Trisomy 18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	10
TN	98
FP	0
FN	6 (FN rate: 37.5%)
S*	62.5 (38.6-81.5)
Sp	100 (96.2-100)
PPV	100 (72.2-100)
NPV	94.2 (88.0-97.3)

*Using the z-score method with adjustment by GC correction, PPV=100%; using the Z-score method with adjustment by internal reference in this study, PPV= 90%

Diagnostic accuracy for Trisomy 13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	0
TN	106
FP	0
FN	2 (FN rate: 100%)
S*	0
Sp	100
PPV	0
NPV	98.1

*Using the z-score method with adjustment by GC correction, PPV=100%; using the Z-score method with adjustment by internal reference in this study, PPV= 100%

Additional patient relevant outcomes

Safety

-Increase the number of children born with other unconfirmed chromosomal anomalies: NA

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA

Effectiveness

-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA

-Reduction in the number of miscarriage related to invasive testing: NA

-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA

-Change in uptake of prenatal screening: NA

Author(s): Ehrich et al [70]	
Study characteristics	Study design: DTA study Year of publication: 2011 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: United States of America Setting: routine prenatal care Data collection period: August 2009- May 2009 Target population: high-risk popula-tion Target condition prevalence in the enrolled population: 1/12 for T21 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): GAIIx sequencer by Ilumi-na/MPSS in combination with anal-ysis software CASAVA version 1.6 Country where samples were ana-lysed: USA Cut off for NIPT: Z-score=2.5
Population characteristics	Maternal age in years (mean [169])(n=448): 37 [18-47] Gestational age in weeks (median [169])(n=448): 16 [8-36] Maternal weight in lbs (median [169])(n=425): 153 (96-314) Pregnancy by assisted reproductive techniques (% pts): not reported Inclusion criteria: pregnant women who were at high risk for fetal trisomy 21 or DS based on positive serum biochemical screening test (n=133/441), advanced maternal age (≥35 years at the estimated date of delivery)(n=306/448), fetal ultrasound finding suggestive of DS (n=57/441), a personal/family history of DS (n=23/441) or not specified (n=45/441) Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 480 Population excluded: 31 Reason for exclusion: -plasma volume<3.5 ml=9 -processing error=4 -failed quality control=18 Population included: 449 -with NIPT result: 449 -with comparator result: no inter-vention

<table>
<tr><td></td><td>-with reference standard result: 449
Reference standard (% pts): CVS (19%) or amniocentesis (80.9%)
Sample processing protocol
Maternal blood samples were col-lected into EDTA-K2 spray-dried Vacutainer at enrolment institution and sent to Sequenom Inc. for its analysis. Plasma DNA was extract-ed by QIAamp Circulating Nucleic Acid Kit from Qiagen and amount of extracted DNA was determinated by fetal quantifier assay (FQA).
Sample, demographics and karyo-type results were unknown to the laboratory investigators and data analysts until after completion of all sample testing and submission for review.</td></tr>
<tr><td rowspan="3">Outcomes</td><td>Test performance
-Test failure (% samples): 18 samples (3.75%)(failed quality control)
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Performance for Trisomy 21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>39</td></tr><tr><td>TN</td><td>409</td></tr><tr><td>FP</td><td>1 (FP rate: 0.24%)</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (89-100)</td></tr><tr><td>Sp</td><td>99.7 (98.5-100)</td></tr><tr><td>PPV</td><td>100 (87.1-99.6)</td></tr><tr><td>NPV</td><td>100 (99.1-100)</td></tr></table>The study’s authors reassessed accuracy NIPT test previous performing of quality control (QC) in 467 samples, excluding only the 13 samples with analytic failure with the aim of assessing value of QC. Therefore, S=97% (86-100), Sp=99.8 (98.5-100) and one FN case.</td></tr>
<tr><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with other unconfirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Abbreviations: NA: not available; S: sensitivity; Sp: specificity; TP: true positive; TN: true negative; FP: false positive; FN: false negative; PPV: positive predictive value; NPV: negative predictive value.

Table A4: Characteristics of included studies on high-intermediate pregnant population

Author(s): Gil et al [71]	
Study characteristics	Study design: prospective DTA study Year of publication: 2016 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: United Kingdom (UK) Setting: routine prenatal screening Data collection period: October 2013 to February 2015 Target population: high-risk preg-nant population Target condition prevalence in the enrolled population: 1/282 for T21, 1/577 for T18 and 1/6067 for T13 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Harmony™ Prenatal Test/targeted free DNA based technology Country where samples were ana-lysed: USA Cut off for NIPT: not reported
Population characteristics	Maternal age in years (median [in-terquartile range]): -High-risk: 36.1 (32.1-39.5) -Intermediate-risk: 34.8 (30.8-38.4) -Low-risk: 29.9 (25.8-33.2) Gestational age in weeks (median [169]): not reported Maternal weight in Kg (median [169]): not reported Pregnancy by ART (no [% pts]): -High-risk: 16 (3.5) -Intermediate-risk: 135 (3.8) -Low-risk: 172 (2.2) Inclusion criteria: women with sin-gleton pregnancy at 11-13 week of gestation classified as high (≥1/100) or intermediate (between 1/101 and 1/2500) risk of aneuploidies by first-trimester combined test who NIPT was offered and opted for it. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 12134 Population excluded: 512

Reason for exclusion:

-not provided informed consent: 213

-Pregnant termination, miscarriage or stillbirth without karyotyping result: 169

-Lost follow-up: 60

Population included: 11692 (as-sessed by FCT, 460 samples (3.9%) classified as high risk, 3552 samples (30.4%) classified as intermediate risk and 7680 sam-ples (64.7%) classified as low risk

-with NIPT result: 3633 (3698 sam-ples with high- or intermediate risk).

-with comparator result: no inter-vention

-with reference standard result: 3633

Reference standard (% pts): CVS

Sample processing protocol

Maternal blood samples were col-lected into EDTA or Cell-Free DNA BCT tubes at enrolment centres and sent to Ariosa Diagnostic, Inc. where sequencing analysis was performed.

It was not reported if karyotyping and sequencing analysis were performed in a blinded fashion

Outcomes

Test performance

Excluding samples without cfDNA results, NIPT accuracy test were analysed in 3633 samples. NIPT test detected 41 cases of T21, 21 cases of T18 and 2 cases of T13. By invasive testing, it was confirmed 47 cases of T21, 24 cases of T18, 4 cases of T13 and 11617 cases non-trisomy.

Safety and effectiveness of NIPT for T21, T18 and T13

-Test failure (% samples): 65 samples (1.75%)

-Uncertain results rate (% samples): NA

Performance for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	43*
TN	3588
FP	1 (FP rate: 0.027)
FN	1 (FN rate: 2.3%)
S	97.7 (88.2-99.6)
Sp	99.9 (99.8-100)
PPV	97.7 (88.2-99.6)
NPV	99.9 (99.8-100)

**There were three cases the parents elected no further testing option*

Performance for T18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	21
TN	3608
FP	4 (FP rate: 0.11%)
FN	3 (FN rate: 12.5%)*
S	87.5 (69.0-95.7)
Sp	99.8 (99.7-100)
PPV	84 (65.3-93.6)
NPV	100 (99.9-100)

**The test did not provided result for three samples*

Performance for T13 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA testing
TP	2
TN	3625
FP	4 (FP rate: 0.11%)
FN	2 (FN rate: 50%)
S	50 (15.0-85.0)
Sp	99.8 (99.7-100)
PPV	33.3 (9.7-70.0)
NPV	99.9 (99.8-100)

	Additional patient relevant outcomes *Safety* -Increase the number of children born with other unconfirmed chromosomal anomalies: NA -Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA *Effectiveness* -Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA -Change in uptake of prenatal screening: NA

Table A5: Characteristics of included studies on twin pregnant population

Author(s): Fosler et al [72]	
Study characteristics	Study design: Prospective DTA study (only Cohort B included) Year of publication: 2015 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: USA Setting: routine prenatal-screening Data collection period: not reported Target population: twin pregnancies Target condition prevalence in the enrolled population: 1/81 for T21 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Verifi® Prenatal Test/SNPs Country where samples were ana-lysed: USA (Clinical Laboratory Improvement Act (CLIA)-certified Illumina Laboratory Cut off for NIPT: not reported
Population characteristics	Maternal age in years (mean±SD): 35.5±4.9 Gestational age in weeks (mean±SD): 13.7±3.9 Maternal weight (mean [169]): not reported Pregnancy by ART (% pts): not reported Chorionicity (%): not reported Inclusion criteria: women at least 18 years of age and at least 8 weeks' gestation carrying twins who meet the following criteria: advance ma-ternal age, abnormal ultrasound, previous affected pregnancy or positive serum screen Exclusion criteria: not reported
Study protocol	*Patient enrollment flow* Patient enrolled: 487 Patient included: 487 (there was 8 cancellations but not for technical reasons) -with NIPT result: 479 -with comparator result: no inter-vention -with reference standard result: 171 Reference standard (% pts): kary-otyping (amniocentesis or CVS) or ultrasound findings. It was not reported the patients percentage in which it was be used each standard reference *Sample processing protocol*

<table>
<tr><td></td><td>Maternal blood samples were collected in Streck cfDNA BCT™ tubes and processed at Illumina Laboratory. Demographic infor-mation i.e. maternal age, gesta-tional age and clinical indication for testing was provided to labora-tory.
It was not reported whether karyo-typing analysis was performed in a blinded fashion respect to ultra-sonographic results.</td></tr>
<tr><td rowspan="3">Outcomes</td><td>Performance of NIPT for T21
-Test failure (% samples): 0
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy for T21 in confirmed and unconfirmed cases (n=479)
<table><tr><th>Variable</th><th>cfDNA</th></tr><tr><td>TP</td><td>6</td></tr><tr><td>TN</td><td>472</td></tr><tr><td>FP</td><td>1 (FP rate: 0.20%)</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (61.0-100)</td></tr><tr><td>Sp</td><td>99.7 (99.8-100)</td></tr><tr><td>PPV</td><td>85.7 (48.7-97.4)</td></tr><tr><td>NPV</td><td>100 (99.2-100)</td></tr></table>Two cases were not confirmed (they were considered suspected to be concordant based on ultrasound findings). If these two cases were considered positive results, accuracy NIPT test would change as follows: S=100, E=99.7, PPV=88.9 and NPV=100. If these two cases were considered FP results (3/479, 0.6%), accuracy NIPT test would change as follows: S=100, E=99.3, PPV=66.7 and NPV=100.</td></tr>
<tr><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with other un-confirmed chromosomal anomalies: not reported cases with other anomalies
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: not report-ed cases with other anomalies
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Sarno et al [39]	
Study characteristics	In this table only were summarised population characterisctics and accuracy test results regarding to twin pregnancies Study design: prospective DTA study Year of publication: 2016 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: UK Setting: routine prenatal screening Data collection period: October 2012 to August 2015 Target population: high-risk pregnant population (singleton or twin pregnancies). Target condition prevalence in the enrolled population: Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Harmony™ Prenatal Test/CSS Country where samples were analysed: USA Cut off for NIPT: risk score for T21, 18 and T13 was ranged between >99% to <0.01%
Population characteristics	Maternal age in years (median [169]): 37.3 [34.6-40.0] Gestational age in weeks (median [169]): 11.7 [10.4-12.9] BMI in Kg/m2 (median [169]): 23.5 [21.0-26.9] Pregnancy by ART (no [% pts]): 246 [56.2] Chorionicity (%): 373 (85.2%) were dichorionic and 65 (14.8%) were monochorionic Inclusion criteria: singleton or twin pregnant women at 11+0 to 13+6 weeks' gestation who received NIPT as an option following FCT or as part of routine screening. All pregnant women were undergone FCT. Exclusion criteria: not reported
Study protocol	*Population enrollment flow* Population enrolled: 467 Population excluded: 29 Reason for exclusion: -Pregnancy ended in termination, miscarriage or stillbirth without karyotype results (n=23) -Lost to follow up (n=4) -Chromosomal anomalies other than trisomies 21, 18 or 13 (n=2) Population included: 438 -with NIPT result: 417

<table>
<tr><td></td><td>-with comparator result: no inter-vention
-with reference standard result: not reported
Reference standard (% pts): fetal karyotype (not specified method used)
Sample processing protocol
Maternal blood samples were collected at enrolment centres and sent to laboratory for its anal-ysis.</td></tr>
<tr><td rowspan="2">Outcomes</td><td>Performance of NIPT for T21, T18 and T13
-Test failure (% samples): 9.4% (41/438) after first NIPT testing. In a second testing, it was provided re-sults in 20 (51.3%) cases (test failure after 2nd test-ing=4.8% or 21/438).
-Uncertain results rate (% samples): NA</td></tr>
<tr><td>Performance for all trisomies (n=417) in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA</th></tr><tr><td>TP</td><td>11</td></tr><tr><td>TN</td><td>403</td></tr><tr><td>FP</td><td>1 (FP rate= 0.25%)</td></tr><tr><td>FN*</td><td>2 (FN rate= 15.4%)</td></tr><tr><td>S*</td><td>84.6 (57.8-95.7)</td></tr><tr><td>Sp</td><td>99.7 (98.6-100)</td></tr><tr><td>PPV</td><td>91.7 (64.6-98.5)</td></tr><tr><td>NPV</td><td>99.5 (98.2-99.9)</td></tr></table>*FN rate T21=0%, FN rate T18=25% and FN rate T13=100%; S T21= 100%, S T18= 75% and S T13= 0%</td></tr>
</table>

Author(s): Tan et al [73]	
Study characteristics	Study design: DTA study (prospec-tive cohort) Year of publication: 2016 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: China Setting: routine prenatal-screening Data collection period: January 2012-December 2013 Target population: twin pregnancy population after the treatment of assisted reproductive technology (ART)(in vitro fertilization embryo transfer (IVF-ET), ICSI or frozen embryo transfer (FET)) Target condition prevalence in the enrolled population: 1/141 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): not reported/MPS Country where samples were ana-lysed: China (Clinical Laboratories of BGI-Shenzhen) Cut off for NIPT: -If both t-score were >2.5 and the L-score was >1, the sample was in the high-risk zone -If either the t-score was >2.5 or the L-score was >1, the sample was in the warning zones -If the t-score was <2.5 and the L-score was <1, the sample was in the low-risk zone Cases falling in the "warning zone 1" were classified as affected but usually because of the presence of mosaicism or partial trisomy. Cases in "warning zone 2" were likely affected pregnancies but with inadequate fetal DNA concentration
Population characteristics	Maternal age in years (median [169]): 31 [20-43] Gestational age in weeks (median [169]): 12 [11-28] Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): 100 ART treatment (% pts): FET (25.5), ICSI (21.2), IVF-ET (52.0) and un-known (1.2) Chorionicity (% pts): dichorionic diamniotic, 96.3; monochorionic diamniotic, 1.9; monochorionic monoamniotic, 1.2; other (monochorionic monoamni-otic+ monochorionic diamniotic, tri-chorionic tri-amniotic): 0.7 Inclusion criteria: -pregnant women with twin preg-nancies after ART, -over 18 years old, -for one to two embryo transfer, confirmation of live twin pregnancy by ultrasound scan before enrolling in the study; for three embryo trans-fer, confirmation of live twin preg-nancy and no demise foetus by ultrasound scan before enrolling in the study, -voluntary received NIPT screening for fetal trisomy 21, trisomy 18 and trisomy 13, with or without prior DS screening result,

<table>
<tr><td></td><td>-gestational age>10 weeks.
Exclusion criteria: not reported</td></tr>
<tr><td>Study protocol</td><td>Patient enrollment flow
Patient enrolled: 565
Patient excluded: 5 DCDA cases
Reason for exclusion: low fetal fraction
Patient included: 560
-with NIPT result: 560
-with comparator result: no intervention
-with reference standard result: 560
Reference standard (% pts): amniocentesis (NIPT positive cases)(3.1) or follow-up (NIPT negative cases)(96.9)
Sample processing protocol
Maternal blood was collected in an EDTA tube from each participant and sent to the accredited clinical laboratory of BGI-Shenzhen.
It was not reported if sequencing karyotyping analysis was performed in a blinded fashion respect to ultrasonographic and standard screening results.</td></tr>
<tr><td>Outcomes</td><td>Performance of NIPT for T21
-Test failure (n,% samples): 5 (0.9%)
-Uncertain results rate (% samples): not reported
Diagnostic accuracy for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated basppped on study results
<table>
<tr><th>Variable</th><th>cfDNA</th></tr>
<tr><td>TP</td><td>4</td></tr>
<tr><td>TN</td><td>556</td></tr>
<tr><td>FP</td><td>0 (FP rate: 0 [0-0.70)</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (51.0-100)</td></tr>
<tr><td>Sp</td><td>100 (99.3-100)</td></tr>
<tr><td>PPV</td><td>100 (51.0-100)</td></tr>
<tr><td>NPV</td><td>100 (99.3-100)</td></tr>
</table>
Performance for T21 in pregnancies with outcome data, including test failures
Considering cases with indeterminate results as high risk of aneuploidies
<table>
<tr><th>Variable</th><th>cfDNA</th></tr>
<tr><td>TP</td><td>4</td></tr>
<tr><td>TN</td><td>556</td></tr>
<tr><td>FP</td><td>5 (add +5 indeterminate results)</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (51.0-100)</td></tr>
<tr><td>Sp</td><td>99.1 (97.9-99.6)</td></tr>
<tr><td>PPV</td><td>44.4 (18.9-73.3)</td></tr>
<tr><td>NPV</td><td>100 (99.3-100)</td></tr>
</table>
Considering cases with indeterminate results as low risk of aneuploidies
<table>
<tr><th>Variable</th><th>cfDNA</th></tr>
<tr><td>TP</td><td>4</td></tr>
<tr><td>TN</td><td>561 (add +5 indeterminate results)</td></tr>
<tr><td>FP</td><td>0</td></tr>
<tr><td>FN</td><td>0</td></tr>
<tr><td>S</td><td>100 (51.0-100)</td></tr>
<tr><td>Sp</td><td>100 (99.3-100)</td></tr>
<tr><td>PPV</td><td>100 (51.0-100)</td></tr>
<tr><td>NPV</td><td>100 (99.3-100)</td></tr>
</table>
Additional patient relevant outcomes
Safety
-Increase the number of children born with other un-confirmed chromosomal anomalies: not reported cases with other anomalies</td></tr>
</table>

	-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: not report-ed cases with other anomalies Effectiveness -Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA -Reduction in the number of miscarriage related to invasive testing: NA -Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA -Change in uptake of prenatal screening: NA

Author(s): Bevilacqua et al [74]	
Study characteristics	Study design: DTA study Year of publication: 2015 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: Belgium, United Kingdom and Spain Setting: routine prenatal-screening Data collection period: May 2013-September 2014 Target population: twin pregnant population Target condition prevalence in the enrolled population: 1/43 for T21 and 1/103 for T18 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): Harmony™ Prenatal Test/MPS Country where samples were ana-lysed: USA (Ariosa Diagnostic, Inc.) Cut off for NIPT: risk score of each trisomy with range >99% and <0.01%
Population characteristics	Maternal age in years (median [169]): 36.8 [19.0-50.3] Gestational age in weeks (median [169]): 13.6 [10.0-34.7] Maternal weight (median [169]): 64.4 [42.0-148.0] Pregnancy by ART (% pts): 52.8 Chorionicity (% pts): not reported Inclusion criteria: woman with twin pregnant at 10-28 weeks' gestation underwent to cfDNA testing either because prior high-risk of aneu-ploidies (first-trimester screening combined test or second-trimester triple/quadruple test or ultrasound examination) or as a primary screening test. Exclusion criteria: not reported
Study protocol	Patient enrollment flow Patient enrolled: 515 Patient excluded: 175 Reason for exclusion (any sam-ples had more than one exclu-sions reason): -low fetal fraction (n=16), -unknown karyotype because of pregnancy resulted in miscarriag-es or stillbirth (n=7), -outcome unknown because the pregnancies were continuing (n=19), -were lost to follow-up (n=138). Patient included: 340 -with NIPT result: 340

<table>
<tr><td></td><td colspan="2">-with comparator result: no inter-vention
-with reference standard result: 351
Reference standard (% pts): CVS, amniocentesis, neonatal blood or neonatal examination. It was not reported patient percent.
Sample processing protocol
Maternal blood was collected (Streck cfDNA BCT™ tubes) and sent to via courier to the Ariosa Diagnostic laboratory. The infor-mation provided to the laboratory was patient-unique identifier, maternal age, method of concep-tion and date of blood collection.
It was not reported if karyotyping analysis was performed in a blinded fashion respect to ultra-sonographic and standard screen-ing results.</td></tr>
<tr><td rowspan="3">Outcomes</td><td colspan="2">Performance of NIPT for T21 and T18
-Test failure (n,% samples): 3.1%
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>12</td></tr><tr><td>TN</td><td>323</td></tr><tr><td>FP</td><td>4 (FP rate: 1.22%)</td></tr><tr><td>FN</td><td>1 (FN rate: 7.7%)</td></tr><tr><td>S</td><td>92.3 (66.7-98.6)</td></tr><tr><td>Sp</td><td>98.7 (96.9-99.5)</td></tr><tr><td>PPV</td><td>75.0 (50.5-89.8)</td></tr><tr><td>NPV</td><td>99.6 (98.3-99.9)</td></tr></table></td><td>Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>cfDNA testing</th></tr><tr><td>TP</td><td>5</td></tr><tr><td>TN</td><td>323</td></tr><tr><td>FP</td><td>12 (FP rate: 3.58%)</td></tr><tr><td>FN</td><td>0</td></tr><tr><td>S</td><td>100 (56.6-100)</td></tr><tr><td>Sp</td><td>96.4 (93.8-97.9)</td></tr><tr><td>PPV</td><td>29.4 (13.3-53.1)</td></tr><tr><td>NPV</td><td>100 (98.8-100)</td></tr></table></td></tr>
<tr><td colspan="2">Additional patient relevant outcomes
Safety
-Increase the number of children born with other un-confirmed chromosomal anomalies: not reported cases with other anomalies.
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: not report-ed cases with other anomalies.
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA
-Reduction in the number of miscarriage related to invasive testing: NA
-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA
-Change in uptake of prenatal screening: NA</td></tr>
</table>

Author(s): Huang et al [75]	
Study characteristics	Study design: Prospective DTA study Year of publication: 2014 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: China Setting: routine prenatal-screening Data collection period: not reported Target population: twin pregnancy population Target condition prevalence in the enrolled population: 1/23 for T21 and 1/94 for T18 Comparator: no intervention Cut off point comparator: no inter-vention Index test (trademark/technique type): not reported/MPS Country where samples were ana-lysed: China Cut off for NIPT: -If both t-score were >2.5 and the L-score was >1, the sample was in the high-risk zone -If either the t-score was >2.5 or the L-score was >1, the sample was in the warning zones -If the t-score was <2.5 and the L-score was <1, the sample was in the low-risk zone Cases falling in the "warning zone 1" were classified as affected but usually because of the presence of mosaicism or partial trisomy Cases in "warning zone 2" were likely affected pregnancies but with inadequate fetal DNA concentration
Population characteristics	Maternal age in years (median [169]): 31 [22-44] Gestational age in weeks (median [169]): 19 [11-36] Maternal weight (median [169]): not reported Pregnancy by ART (% pts): 59.8 Chorionicity (% pts): -Monochorionic diamniotic: 16.4 -Monochrorionic monoamniotic: 1.1 -Dichrorionic diamniotic: 80.4 -Unknown: 2.1 Inclusion criteria: twin pregnancies that required invasive testing due to abnormal maternal serum screen-ing, abnormal sonographic signs or maternal anxiety. Exclusion criteria: women with intrauterine fetal demise at the time of sampling or without fetal karyo-type results.

Study protocol

Patient enrollment flow

Patient enrolled: 189

Patient excluded: 0

Reason for exclusion:

Patient included: 189

-with NIPT result: 189

-with comparator result: no inter-vention

-with reference standard result: 189

Reference standard (% pts): CVS (2.1), amniocentesis (94.2) and cordocentesis (3.7)

Sample processing protocol

Maternal blood was sampled before invasive procedures and sent to the independent clinical laboratory BGI Health, Shenzhen (ISO/IEC 17025) to its analysis. Invasive testing was performed in medical centres. Both analysis were simultaneously performed. The karyotyping and sequencing results were kept confidential until final analysis.

Outcomes

Test performance of NIPT for T21 and T18

-Test failure (% samples): not reported

-Uncertain results rate (% samples): not reported

Diagnostic accuracy measures for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA
TP	9
TN	180
FP	0
FN	0
S	100 (70.1-100)
Sp	100 (97.9-100)
PPV	100 (70.1-100)
NPV	100 97.9-100)

Diagnostic accuracy measures for T18 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)

Variable	cfDNA Testing
TP	1
TN	187
FP	0
FN	1 (FN rate: 50%)
S	50 (9.5-90.5)
Sp	100 (98.0-100)
PPV	100 (20.7-100)
NPV	99.4 (97.0-99.9)

Additional patient relevant outcomes

Safety

-Increase the number of children born with other unconfirmed chromosomal anomalies: not reported cases with other anomalies

-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: not reported cases with other anomalies

	Effectiveness *-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: NA* *-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA* *-Reduction in the number of miscarriage related to invasive testing: NA* *-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): NA* *-Change in uptake of prenatal screening: NA*

Author(s): Lau et al [76]	
Study characteristics	Study design: Prospective DTA study Year of publication: 2013 Study's registration number in clini-cal trial database: not reported Country/ies of recruitment: China Setting: routine prenatal-screening Data collection period: August 2011-April 2012 Target population: twin pregnancy population Target condition prevalence in the enrolled population: 1/12 for T21 Comparator: first or second tri-mester screening and/or first tri-mester ultrasound markers screen-ing, i.e. nuchal translucency, fetal nasal bone and Doppler assess-ment of the tricuspid valve and ductus venous Cut off point comparator: standard screening Index test (trademark/technique type): NIFTY test/MPS Country where samples were ana-lysed: laboratory where samples were analysis was previously re-ported (Lau TK, Chan MK, Lo PS, Chan HY, Chan WS, Koo TY, Ng HY, Pooh RK. Clinical utility of noninva-sive fetal trisomy (NIFTY) test--early experience. J Matern Fetal Neonatal Med. 2012 Oct;25(10):1856-9) Clinical Laboratory of BGI-Shenzhen Cut off for NIPT: -If both t-score were >2.5 and the L-score was >1, the sample was in the high-risk zone -If either the t-score was >2.5 or the L-score was >1, the sample was in the warning zones -If the t-score was <2.5 and the L-score was <1, the sample was in the low-risk zone Cases falling in the "warning zone 1" were classified as affected but usually because of the presence of mosaicism or partial trisomy Cases in "warning zone 2" were likely affected pregnancies but with inadequate fetal DNA concentration
Population characteristics	Maternal age in years (mean [169]): 36.5 [28-41] Gestational age in weeks (median [169]): 13+1 [11+6 to 20+1] Maternal weight (median [169]): not reported Pregnancy by assisted reproductive techniques (% pts): 66.7% Chorionicity (% pts): 83.3% dichrorionic and 16.7% monochrorionic Inclusion criteria: women with twin pregnancies who fulfilled the following conditions: based on standard screening they have decided not to have invasive test, request NIFTY test, fully understand that NIFTY test efficacy had not been proven, accept that this test was performed on a research base, and only a research report would be issued, and signed an informed consent Exclusion criteria: not reported

<table>
<tr><td>Study protocol</td><td>Patient enrollment flow
Patient enrolled: 12
Patient excluded: 0
Patient included: 12
-with NIPT result: 12
-with comparator result: 12
-with reference standard result: 12
Reference standard (% pts): pre-natal karyotyping or clinical exam-ination of the newborn.
Sample processing protocol
Clinical and laboratory aspects were previously reported (Lau TK, Chan MK, Lo PS, Chan HY, Chan WS, Koo TY, Ng HY, Pooh RK. Clinical utility of noninvasive fetal trisomy (NIFTY) test--early experi-ence. J Matern Fetal Neonatal Med. 2012 Oct;25(10):1856-9)
Blood samples were collected into EDTA tubes and sent to certified clinical laboratory BGI-Shenzhen (ISO/IEC 17025).
It was not reported if sequencing karyotyping analysis was per-formed in a blinded fashion re-spect to ultrasonographic and standard screening results</td></tr>
<tr><td rowspan="3">Outcomes</td><td>Test performance of NIPT for T21
-Test failure (% samples): not reported
-Uncertain results rate (% samples): not reported</td></tr>
<tr><td>Diagnostic accuracy for T21 in pregnancies with outcome data, excluding test failures and miscarriages (calculated based on study results)
<table><tr><th>Variable</th><th>Standard screening</th><th>cfDNA</th></tr><tr><td>TP</td><td>1</td><td>1</td></tr><tr><td>TN</td><td>9</td><td>11</td></tr><tr><td>FP</td><td>2 (FP rate: 18.2%)</td><td>0</td></tr><tr><td>FN</td><td>0</td><td>0</td></tr><tr><td>S</td><td>100</td><td>100 (20.7-100)</td></tr><tr><td>Sp</td><td>81.8</td><td>100 (52.3-94.9)</td></tr><tr><td>PPV</td><td>33</td><td>33.3 (6.1-79.2)</td></tr><tr><td>NPV</td><td>100</td><td>100 (70.1-100)</td></tr></table></td></tr>
<tr><td>Additional patient relevant outcomes
Safety
-Increase the number of children born with other un-confirmed chromosomal anomalies: NA
-Increase in elective pregnancy termination for other unconfirmed chromosomal anomalies: NA
Effectiveness
-Reduction in children born with undiagnosed 13, 18 and 21 trisomies: all cases of main fetal aneuplodies were detected</td></tr>
</table>

	-Reduction in the number of miscarriages or still birth of subject affected by 13, 18 and 21 trisomies: NA *-Reduction in the number of miscarriage related to invasive testing: NA* *-Reduction in uptake of invasive testing (calculated from FP cfDNA vs. standard screening, %): two less invasive testing cases in NIPT group or a reduction of 16.7%* *-Change in uptake of prenatal screening: NA*

Abbreviations: NA: not available; S: sensitivity; Sp: specificity; TP: true positive; TN: true negative; FP: false positive; FN: false negative; PPV: positive predictive value; NPV: negative predictive value.

List of ongoing and planned studies

Table A6: List of ongoing studies with NIPT

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
Comparison of false positive rates in prenatal combined screening and cell free DNA screening for trisomy 21 ISRCTN11174071	**Estimated completion date** October 2017 **Study status** Recruitment completed **Study type** Single-center randomised controlled trial	**Number of patients** 1400 **Intervention** CfDNA screening **Comparator** Combined screening (maternal age, fetal NT, PAPP-A and free beta hCG)	**Inclusion criteria** 1. Patients coming for prenatal screening for trisomy at 11-13 weeks' gestation 2. Singleton pregnancies 3. Normal ultrasound examination without increased nuchal translucency thickness >3.5mm and without fetal defects 4. Informed consent **Exclusion criteria** 1. Increased NT thickness 2. Fetal defects 3. Multiple gestations	Primary outcome: Number and proportion of false positive cases in each arm Secondary outcomes: 1. Number of cases that cannot be randomized (due to fetal defects, increased NT, multiple gestations). 2. Time interval between randomization and return of the blood results 3. Number of cases without results in each study arm. 4. Number of women who opt for invasive testing. 5. Acceptance of each screening test.
SNP-based Microdeletion and Aneuploidy RegisTry (SMART) NCT02381457	**Estimated completion date** November 2017 **Study status** Ongoing recruitment	**Number of patients** 10000 **Intervention** 22q11.2 SNP-based non-invasive prenatal screening	**Inclusion Criteria** Singleton pregnancy Receiving Panorama prenatal screening test for both microdeletions (at least 22q11.2) and aneuploidy Planned hospital delivery	Primary outcomes: test performance, including PPV, Sp and S Secondary outcomes: combined microdeletion syndrome screening test performance, failure ('no call') rate for the NATUS method for 22q11.2 detection, as well as for

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
	Study type Observational cohort study	**Comparator** NA	Gestational age of ≥ 9 weeks, 0 days based on clinical information and evaluation. Able to provide informed consent **Exclusion Criteria** Received results of the Panorama test prior to enrollment Organ transplant recipient Egg donor used	aneuploidy, low fetal fraction aneuploidy risk refinement, placental mosaicism exploration and placental complications exploration
Development of a Non-invasive Prenatal Test NCT01451684	**Estimated completion date** December 2017 **Study status** Ongoing recruitment **Study type** Observational cohort study	**Number of patients** 10000 **Intervention** **Comparator** NA	**Inclusion Criteria** Subject has singleton pregnancy confirmed via evaluation by a healthcare provider Subject is able to provide informed consent Subject is ≥ 18 years of age **Exclusion Criteria** Subject is pregnant with more than one foetus Subject is unwilling to undergo a blood draw	Primary Outcome: Absence of chromosomal anomaly
Development of a Prenatal Test for Fetal Aneuploidy Detection NCT01451671	**Estimated completion date** November 2017 **Study status** Ongoing recruitment **Study type** Observational cohort study	**Number of patients** 1500 **Intervention** Non-invasive prenatal test **Comparator** NA	**Inclusion Criteria** Subject has singleton pregnancy Subject is confirmed via invasive testing to be carrying a foetus with a chromosomal anomaly Subject is able to provide informed consent Subject is ≥ 18 years of age **Exclusion Criteria** Subject is pregnant with more than one foetus	Primary Outcome: Identification of aneuploidy

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
			Subject (mother) has a known aneuploidy	
Development of Non-invasive Prenatal Test for Microdeletion and Other Genetic Syndromes Based on Cell Free DNA (Microdel Triad) NCT02109770	**Estimated completion date** December 2018 **Study status** Ongoing recruitment **Study type** Observational cohort study	**Number of patients** 200 **Intervention** Non-invasive prenatal test **Comparator** NA	**Inclusion Criteria** Couples who have a child diagnosed with an autosomal chromosome anomaly (e.g. DS, ES, Patau syndrome). Couples who have a child diagnosed with a sex chromosome anomaly (e.g. Turner syndrome, Klinefelter syndrome, Triple X syndrome, 47, XYY). Couples who have a child diagnosed with a microdeletion/duplication syndrome (a positive microarray test). **Exclusion criteria** Not an English language or Spanish language speaker Genetics report is not available	S and Sp of the test to diagnose chromosomal microdeletions and aneuploidy in a foetus
Development of Non-invasive Prenatal Screening Test for Microdeletions Based on Fetal DNA Isolated From Maternal Blood NCT01852708	**Estimated completion date** November 2018 **Study status** Ongoing recruitment **Study type** Observational cohort study	**Number of patients** 100 **Intervention** Non-invasive prenatal test **Comparator** NA	**Inclusion Criteria** Age 18 or older at enrollment Singleton pregnancy Foetus with confirmed diagnosis of chromosomal anomaly or genetic disorder through karyotype, FISH or positive microarray results after amniocentesis or CVS The biological father of the foetus at least 18 years of age Able to provide informed consent **Exclusion Criteria** Women carrying multiples	S and Sp of the test to diagnose microdeletions (eg. 22q and 5p-) and aneuploidy in a foetus at chromosomes 13, 18, 21, X and Y

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
			Pregnancy is a result of IVF with pre-implantation genetic diagnosis Surrogate/egg or sperm donor used Previous participation in this study during a previous pregnancy	
Specimen Collection From Pregnant Women at Increased Risk for Fetal Aneuploidy NCT01429389	**Estimated completion date** March 2017 **Study status** Ongoing recruitment **Study type** Observational cohort study	**Number of patients** 2000 **Intervention** Non-invasive prenatal test **Comparator** NA	**Inclusion Criteria** pregnant between 10 and 22 weeks gestation 18 years of age or older provides signed and dated informed consent subject is at increased risk for fetal aneuploidy subject is willing to undergo a CVS and/or amniocentesis procedure for the purpose of genetic analysis subject agrees to provide the genetic results of the invasive procedure **Exclusion Criteria** Fetal demise at time of specimen sampling Previous sample donation under this protocol	Not reported
A Safer Pre-Natal Diagnosis Using Free DNA in Maternal Blood NCT01472523	**Estimated completion date** July 2019 **Study status** Ongoing recruitment **Study type**	**Number of patients** 600 **Intervention** IONA test **Comparator**	**Inclusion Criteria** Patient/subject is willing and able to give informed consent for participation in the study. Female, aged 16 years or above. Currently pregnant at time of entry to the study. Pregnancy having been identified as 'high-risk' by screening test. **Exclusion Criteria**	Primary Outcome: Validation of method of novel analysis for Aneuploidy Secondary Outcome: Optimization of existing methods for maximising cfDNA

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
	Observational cohort study	NA	The patient/subject may not enter the study if any of the following apply: The participant herself has DS or other chromosomal anomaly. Children under 16 Adults with learning disabilities, who are unconscious or very severely ill, have a terminal illness, in emergency situations, suffering from a mental illness or with dementia Prisoners Young offenders Adults who are unable to consent for themselves Any person considered to have a particularly dependent relationship with investigators	Patients to be followed up for 1 year.
A Prospective Clinical Study to Evaluate a Novel Non-invasive Prenatal Screening Method for Characterizing Fetal Whole Chromosome Aberrations and Other Major Defects and Deletions Found in the Maternal Blood NCT02317965	**Estimated completion date** January 2018 **Study status** Ongoing recruitment **Study type** Observational cohort study	**Number of patients** 340 **Intervention** Prenatal aneuploidy laboratory developed test (LDT) **Comparator** NA	**Inclusion Criteria** Subject is a pregnant woman 18-54 years of age at 8-22 weeks' gestation inclusive; Subject has additional risk indicators for fetal chromosome aneuploidy, including one or more of the following: Maternal age > 34 years at the estimated date of delivery; Positive serum screening test suggesting fetal aneuploidy; Previous positive noninvasive cfDNA test is acceptable	S, Sp, PPV and NPV of the laboratory developed test

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
			Fetal ultrasound anomaly suggesting fetal chromosomal anomaly; Personal or family history DS or other chromosomal aneuploidy. Willing to provide written informed consent Willing to be re-contacted subsequently for additional information and/or testing if necessary. **Exclusion Criteria** Subjects will not be entered into this study if they meet the following criteria: Fetal demise at the time of the blood draw; Previous specimen donation under this protocol; Unwilling or lacks the capacity to provide informed consent or to comply with study procedures; Currently under treatment for cancer Any history of autoimmune disease Any pelvic mass Previous history of radiation to pelvis Any history or current evidence of a twin demise at any gestational age.	
Expanded Noninvasive Genomic Medical Assessment: The Enigma Study	**Estimated completion date** June 2018 **Study status**	**Number of patients** 2500 **Intervention**	**Inclusion Criteria** Subject is willing to provide informed consent and comply with study procedures	Primary Outcome: Point estimates and 95% CIs for S, Sp, PPV, and NPV versus birth outcome (trisomy or Unaffected/non-trisomy) for the

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
NCT02787486	Ongoing recruitment **Study type** Observational cohort study	Noninvasive laboratory-developed tests (LDTs) Two arms: -Aneuploidy arm -TORCH arm (infectious disease arm) **Comparator** NA	Pregnant female, 18 to 54 years of age carrying a singleton foetus of 8 to 22 weeks gestational age Willing to provide a study blood sample in accordance with the protocol Willing to allow access to her medical records to collect pregnancy outcome information Willing to provide consent for release of fetal karyotype if an invasive procedure (CVS or amniocentesis) is performed during the pregnancy Subject is known to be at risk for one or more of the following: fetal gene and chromosome anomalies (e.g., T21, T18, T13, microdeletion syndromes, sex chromosome anomalies) congenital fetal infection (e.g. toxoplasmosis, syphilis, HIV, rubella, CMV, HSV) irregular blood group antigens (subject or father of the baby) other condition amenable to noninvasive prenatal testing such as a single gene disorder (e.g., CF, sickle cell, Fragile X) **Exclusion Criteria** No fetal heart activity detected Mother or father have known chromosomal anomalies (including known balanced translocations) Women with active or history of malignancy	LDT in the population of pregnancies at mixed-risk for chromosomal anomalies Secondary Outcome: To estimate FP rate of the LDT versus birth outcome (trisomy or Unaffected/non-trisomy) in a low-risk sub-population of pregnant women undergoing serum biochemical screening for fetal aneuploidy

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
Prenatal Non-invasive Aneuploidy Test Utilizing SNPs Trial (PreNATUS) NCT01545674	**Estimated completion date** December 2017 **Study status** Recruitment completed **Study type** Observational cohort study	**Number of patients** 1000 **Intervention** Non-invasive prenatal test based on SNPs **Comparator** NA	**Inclusion Criteria** Singleton pregnancy Gestational age between 8 weeks 0 days and 23 weeks, 6 days by best obstetrical estimate Mother has a high or moderate risk for trisomy Mother is planning to have or has had an amniocentesis or CVS procedure **Exclusion Criteria** Unavailability of the father to provide a genetic sample (e.g. sperm donor, non-paternity) Egg donor used Mother or father have known chromosomal anomalies (including known balanced translocations) Participation in the study in a previous pregnancy Pregnancy is a result of IVF with pre-implantation genetic diagnosis	Primary Outcome: S and Sp of the test to diagnose aneuploidy in a foetus at chromosomes 13, 18, 21, X and Y
Study of the Efficacy of New Non-invasive Prenatal Tests for Screening for Fetal Trisomies Using Maternal Blood (PEGASUS) NCT01925742	**Estimated completion date** December 2017 **Study status** Recruitment completed **Study type**	**Number of patients** 3819 **Intervention** Different screening modalities: Integrated prenatal screening for Down's syndrome Serum QUAD Assay for aneuploidy screening	**Inclusion Criteria (High risk arm)** Women 19 years or older between 10 weeks and 23 weeks 6 days gestation undergoing amniocentesis or CVS for positive prenatal screen; abnormal ultrasound; previous pregnancy with trisomy; patient or partner carrier of Robertsonian translocation involving chr 21; positive NIPT result; maternal age 40 or more **Inclusion Criteria (Low risk arm)**	Primary Outcome: Number of cases with Fetal trisomy 21, 18 or 13 Secondary Outcome: Number of women with assay failure Other Outcome:

Study title and identifier	Estimated completion date Study status Study type	Number of patients Intervention Comparator	Patient population	Endpoints
	Open label non-randomized trial	Semiconductor MPSS NIPT assay using cfDNA in maternal blood Optical-based MPSS NIPT assay using cfDNA in maternal blood Harmony™ Test (Ariosa Diagnostics) Two arms: low and high risk of aneuploidies **Comparator** NA	Women 19 years and older who are 10 and 13 weeks 6 days gestation based on dating ultrasound (CRL) and are undergoing screening for DS (FCT, SIPS or IPS) **Exclusion Criteria** Women with multiple gestations; women with twin demise (spontaneous or elective) at any gestational age; women with active or history of malignancy	Overall costs of screening algorithm

Abbreviations: NA – not available

Systematic review/meta-analysis and HTA reports

Table A7: Overview of systematics reviews/meta-analysis and HTA reports

Author(s) Year of publication Type of study Country	Methodological issues Aim, study criteria selection,	Description of included studies Outcomes assessed	Summary of conclusion and/or recommendations related to NIPT
Iwarson et al [170] 2017 Systematic review/meta-analysis Sweden	To review the performance of NIPT for detection of trisomy 21, 18 and 13 in a general pregnant population as well as to update the data on high-risk pregnancies. Mainly, it was included primary studies in English or Scandinavian languages in singlenton pregnancies published in 1998-2015 and reached moderate or high quality using QUADAS tool.	Finally, it was included 32 studies, i.e. 23 prospective cohort studies and 9 case-control studies. Among these, five investigated a general preganant population, two included both high-risk and average-risk population and the remaining 25 studies analysed a high-risk pregnant population Outcome measures were S, Sp, number of TP, FP, TN and FN	For general pregnant population With a moderate level of evidence, 21 trisomy pooled S=0.993[95% CI 0.955-0.999] and Sp=0.999[95% CI 0.998-0.999]. Pooled S and Sp for trisomy 18 and 13 was not calculated due to insufficient number of studies. For high-risk pregnant population With a moderate level of evidence, 21 and 18 trisomy pooled S=0.988[95% CI 0.981-0.999] and 0.977[95% CI 0.958-0.987] respectively. For T13 with a low level of evidence, S=0.975[95% CI 0.819-0.997]. The pooled Sp for all trisomies was 0.999[95% CI 0.998-0.999] Authors concluded that NIPT perform well as a screen for trisomy 21 in a general population. Moreover due to false positive rate of NIPT, positive results should be confirmed by invasive testing.
Mackie et al [171] 2017 Systematic review/meta-analysis UK	Determine accuracy of cfDNA-based NIPT for main trisomies i.e. 21, 18 and 13 and other conditions (fetal sex, monosomy, rhesus D/C/E, 47XXX, 47XXY, 47XYY, trisomy 16, congenital adrenal hyperplasia, deletion-duplication syndromes, sickle cell anaemia, thalassaemia, human platelet antigen 1a and KEL1). Evaluate influence of other factors on test performance The authors only considered prospective studies in women with a singleton	A total of 117 cohort studies were included that analysed 18 conditions. Summary measures including S, Sp, DOR, LR+, LR- were calculated when there were more than five studies per condition (fetal sex, rhesus D, trisomy 21, trisomy 18, monosomy X and trisomy 13)	For conditions meta-analysed, S and Sp reached 90-100%. The included studies reported an inconclusive result rate of 0.32-5.30%. For fetal sex and rhesus D, NIPT can be considered diagnostic. However, for trisomy 21, 18 and 13 due to its lower S, Sp and disease prevalence combined with the biological influence of confined placental mosaicism designates it a screening test. The authors concluded that this work demonstrates that there is a sufficient body of evidence for the accuracy and reproducibility of cfDNA-based NIPT to allow. its introduction into routine clinical practice within the UK

Author(s) Year of publication Type of study Country	Methodological issues Aim, study criteria selection,	Description of included studies Outcomes assessed	Summary of conclusion and/or recommendations related to NIPT
	pregnancy and different level of aneuploidies risk; and therefore excluded pre-implantation testing, fetal cel testing, case-control studies and case series with fewer than five participants		
Taylor-Phillips et al [172] 2016 Systematic review/meta-anlysis UK	To measure test accuracy of NIPT for Ds, ES and Patau syndromes using cfDNA and identify factors affecting accuracy. In this review, it was included english language journal articles describing case-control studies with ≥15 trisomy cases or cohort studies with ≥50 pregnant women who had been given NIPT and a reference standard.	According to study selection criteria, the authors selected 41 studies for the meta-analysis Outcome measures were S, Sp, number of TP, FP, TN, FN, PPV and probability of FP	Pooled sensitivity was 97-99% and specificity was 99% for three trisomies. Sensitivity was lower in twin than singleton pregnancies. The authors concluded that NIPT test has high sensitivity and specificity for T21 and slightly lower S for T18 and T13. Moreover, pooled sensitivity was lower in the first trimester of pregnancy evaluated in general obstetric population or cohort studies with consecutive enrolment. Due to NIPT accuracy is not 100%, it should not be used as a final diagnosis for positive cases and therefore an invasive diagnostic test would be recommended on these cases. Finally, the authors pointed out that test performance in clinical practice could differ from those results showed in the published evidence due to high risk of bias of studies of cfDNA and unexplained heterogenity
García-Pérez et al [173] 2016 HTA report Spain	To review the validity and cost-effectiveness of NIPT for the detection of trisomy 21, 18 and 13 To assess the cost-effectiveness of screening strategy that includes NIPT for the detection of trisomy 21, 18 and 13 from the National Heath System (NHS) perpective in Spain To report aspects related to ethical, organizational, legal and other domains	Finally it was retrieved 49 articles about diagnostic accuracy of NIPT and 12 full economic evaluations Outcome measures were S, Sp, PPV, NPV, cost of each alternative and ICER	According to meta-analysis performed, S and Sp of NIPT was over 95% for all trisomies. The systematic review of economic evaluations found contradictory results. In the economic evaluation, the authors calculated a estimated ICER of €234.596 per correctly diagnosed case. The prenatal screening with contingent NIPT has the advantage of a reduced number of invasive test related fetal losses.

Author(s) Year of publication Type of study Country	Methodological issues Aim, study criteria selection,	Description of included studies Outcomes assessed	Summary of conclusion and/or recommendations related to NIPT
	with the aim of the informing the decision making For NIPT validity assessment it was included case-control studies with more than 15 cases and cohort studies with more than 500 cases all of these published in Spanish or English. In the systematic review of cost-effectiveness were considered full economic evaluations that compared a screening strategy that included NIPT to detect T21, T18 and T13 in the foetus with a screening strategy not including NIPT or the alternative “no screening” In the economic analysis were compared detection rate of trisomy 21, 18 and 13 and cost of two screening strategies: first or second-trimester screening versus contingent NIPT strategy		
Juvet et al [174] 2016 HTA report Norway	To analyze the diagnostic test accuracy of NIPT and health economic implications and highlight ethical consequences related to the national introduction of NIPT for detection of trisomy (21, 18 and 13) in pregnant women In this HTA report searched systematic reviews published 2010 to 2015. For health economic analysis it was considered three scenarios involving NIPT as both primary or secondary test	Finally, it was included two systematic reviews, one from Sweden and one from UK. For test accuracy analysis were investigated outcomes as diagnostic test accuracy, predictive values, likehood ratios and inconclusive results. For health economic analysis number of correctly identified cases of trisomy 21, 18 and 13, number of undetected cases (FN), number of invasive test performed, total programme costs, costs per	Acccording to two systematic reviews identified, S and Sp of NIPT for trisomy is near to 100% and therefore it was not recommended as a replacement for an invasive diagnostic test. The reports authors concluded that NIPT is a more accurate test for detecting trisomy than combination of blood test and ultrasound (CUB). Moreover, the number of invasive test is considerably reduced in all alternative health economic scenarios involving NIPT compared with CUB screening practice

Author(s) Year of publication Type of study Country	Methodological issues Aim, study criteria selection,	Description of included studies Outcomes assessed	Summary of conclusion and/or recommendations related to NIPT
		diagnosis and incremental costs per additional case of trisomy detected compared with current screening practice	
Taylor-Phillip et al [175] 2015 HTA report UK	See above Moreover systematic review and meta-analysis, the authors made an economic model to compare three options for implementation of NIPT in the NHS: -current NHS screening programme using combined test and invasive testing offered to women with risk greater than 1/150 -combined test and cfDNA offered to women with risk greater than 1/150 and if they tested positive offered an invasive test use cfDNA test as first-line instead of the combined test	See above	See above By economic analysis, the authors reported that the second option resulted in similar numbers of trisomies detected, 43 fewer miscarriages of healthy pregnancies and may cost approximately the same as currently. The third option would cost an extra £105 million to the NHS and result in more invasive tests than the second option
Yang et al [176] 2015 Systematic review China	To evaluate the system accuracy of noninvasive prenatal diagnosis for abnormal chromosome genetic diseases using cfDNA in maternal plasma In this review, it were included studies published in English or Chinese which determine the accuracy of noninvasive prenatal diagnosis and/or compare it to traditional standards, i.e. karyotyping or FISH.	In this review were included four studies that analysed accuracy test for T21 and T18 Outcomes measures were S and Sp	S for T21 was 100% and 97.4% for T18. Sp was similar for both trisomies (99%). Therefore, the authors concluded that noninvasive prenatal diagnosis can be used to identify abnormal chromosomes with high accuracy using free fetal DNA in the maternal plasma. The method used to diagnose T21 has become more advanced, while the application for T18 diagnosis requires additional research
Hulstaert et al [157] 2014 HTA report	To evaluate the economic impact of introducing NIPT in the prenatal testing process for DS whereby a systematic	By systematic review, it was identified 7 full economic evaluations on the cost-effectiveness of NIPT.	According to modelling exercise, average cost for detection of a case of trisomy 21 was about €87 000. The authors concluded that use NIPT after a positive current screening was cost saving but limiting its use to the

Author(s) Year of publication Type of study Country	Methodological issues Aim, study criteria selection,	Description of included studies Outcomes assessed	Summary of conclusion and/or recommendations related to NIPT
Belgium	review and modelling exercise for the introduction of NIPT in Belgium. For systematic review, it were included full economic evaluations that compared at least two alternative treatments in terms of costs and outcomes for diagnosis of DS in singleton pregnant women. For economic evaluation, it was modelled two scenarios: NIPT as primary screening test and NIPT as second line test for triage after the current test.	It was reported different economic outcomes as ICER, cost of screening, cost of DS, average cost for detection of a case of trisomy 21, etc.	5% screen positives (risk cut-off 1:300). Moreover, a possible introduction of NIPT into the health insurance should be accompanied by an obligatory registration of the NIPT result and the final diagnosis after invasive testing and the pregnancy outcomes.
Gil et al [177] 2015 Systematic review/meta-analysis UK	To review clinical validation or implementation studies of maternal blood cfDNA analysis in screening for aneuploidies (trisomy 21/18/13, monosomy X and SCAs other than monosomy X) and to explore the potential use of this method in clinical practice. The inclusion criteria were studies reporting on clinical validation or implementation of maternal cfDNA testing in screening for aneuploidies, in which the laboratory scientists carrying out the test were not aware of the fetal karyotype or pregnancy outcomes.	A total of 33 studies were included in the review. Among these, 28 studies were used for meta-analysis of test performance (only two in general population) and the remaining five were assessed in order to evaluate clinical implementation of NIPT in aneuploidies screening. Outcome measures were detection rate (DR) FP rate for each trisomy and SCA.	For singleton pregnancies group, DR was over 90% for all trisomies, except for monosomy X that only reached 88.6%. And for twin pregnancies, the DR was 94.4% for T21. The overall FP rate for three trisomies was 0.4%, although for T21 showed the lowest FP rate (0.08%) in comparison to trimosy 18 and 13 (0.15-0.20%) The authors concluded that cfDNA in maternal plasma blood provides effective screening for trisomies. Moreover in this review were proposed two options for the clinical implementation of cfDNA, i.e. as routine screening of th whole population (first-line method) or as contingent screening based on results of first-line screening by another method (first or second-trimester serum test). This last option retains the major advantages of cfDNA testing in increasing DR and decreasing FP rate, but at considerably lower cost than offering the test to the whole population
Baños et al [178] 2012	To evaluate test accuracy of NIPT test based on cfDNA for diagnosis of aneuploidies.	By bibliographic search, it were identified 3 primary studies, i.e. two DTA studies and one case-control	The main conclusion of report were: -S was 79-100% and Sp 97.9-99.7%

Author(s) Year of publication Type of study Country	Methodological issues Aim, study criteria selection,	Description of included studies Outcomes assessed	Summary of conclusion and/or recommendations related to NIPT
HTA report Spain	In this review were considered studies which reported NIPT test accuracy in comparison with invasive diagnostic test (amniocentesis or CVS). Only it were excluded letters to director, editorials or narrative reviews.	study that reported pregnant women at high risk of trisomy 21. Outcome measures were S, Sp, PPV and NPV.	-In pregnancies at high-risk, NIPT could avoid invasive diagnostic testing reducing the discomfort and the anxiety they generate and the possible birth complications caused by instrumentation and even the rsik of fetal loss

Abbreviations: S-sensitivity, Sp-Specificity, TP-true positive, FP-false positive, TN-true negative, FN-false negative, LR+ positive likehood ratio, LR- negative likehood ratio, DOR-diagnostic odds ratio, DR-detection rate, FPR-false positive rate, PPV-positive predictive value, NPV-negative predictive value, CVS-chorionic villus sampling

Risk of bias tables

Table A8: Risk of bias – study level (DTA study or cross-sectional study)

NIPT in replacement of combined test: General pregnant population

Author/year: Sarno et al. [39], 2016	Judgment	Explanation
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Insufficient information to establish if the study includes unselected population
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT was carried out prior to invasive testing
If a threshold was used, was it pre-specified?	YES	Cut-off point reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	According to following signalling questions, reference standard condunct could have introduced bias
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Fetal karyotype method was not reported
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Interpretation or conduction of reference standard matched the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	According to following signalling questions, there is high probability of bias
Was there an appropriate interval between index test(s) and reference standard?	YES	Interval between index test and reference standard was appropriate
Did all patients receive a reference standard?	NO	
Did patients receive the same reference standard?	UNCLEAR	Reference standard method was not reported
Were all patients included in the analysis?	NO	168/10698 cases were excluded from the analysis
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Norton et al. [31], 2015	Judgment	Explanation
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	LOW	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	YES	Exclusion aligned with indications
Applicability: Is there concern that the included patients do not match the review question?	LOW	Unselected pregnant population
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the re sults of the reference standard?	YES	The analyses and interpretation of cfDNA data were performed in a blinded fashion
If a threshold was used, was it pre-specified?	YES	1/100 or higher was classified as high risk
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Newborn outcomes determined by medical record review of the physical examination at birth and any genetic testing
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	The target condition defined by the reference standard did match the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Miscarriage and termination results not always confirmed
Did patients receive the same reference standard?	NO	Positive NIPT cases were confirmed by invasive testing and negative cases by neonatal examination
Were all patients included in the analysis?	NO	Exclusion of 16% of cases (women without standard screening, reference standard results, NIPT failures and missed cases, including miscarriages and stillbirths without verification results
OVERALL JUDGMENT	At risk of bias	

Author/year: Pérez-Pedregosa et al. [41], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Insufficient information to establish if the study includes a general unselected obstetric populatio
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	UNCLEAR	
Were the index test results interpreted without knowledge of the results of the reference standard?	UNCLEAR	It was not reported if result interpretation was performed in a blinded fashion
If a threshold was used, was it pre-specified?	YES	Cut-off point for NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Follow up carried out by clinical examination or telephone contact
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	The target condition defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	It was reported that 100% cases received a reference standard
Did patients receive the same reference standard?	NO	CVS or amniocentesis for positive NIPT or standard screening cases and telephome contact for rest of cases
Were all patients included in the analysis?	NO	There were three cases excluded due to test failure
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Quezada et al. [42], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Lack of information regarding inclusion/exclusion criteria
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Insufficient information to establish if the study includes general unselected obstetric population
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT performed prior to invasive testing
If a threshold was used, was it pre-specified?	YES	Cut-off point reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Insufficient information regarding verification of negative cases (information obtained from obstetrician, general practitioner or patient)
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	UNCLEAR	Target condition as defined by the reference standard does not differ from the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT performed prior to invasive testing
Did all patients receive a reference standard?	NO	No fetal karyotyping in miscarriages or stillbirths
Did patients receive the same reference standard?	NO	Karyotyping was used only in positive cases
Were all patients included in the analysis?	NO	Women without reference standard results, miscarriages and stillbirths were excluded from the analysis
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Zhang et al. [43], 2015	Judgment	Explanation
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Unclear inclusion/exclusion criteria
Applicability: Is there concern that the included patients do not match the review question?	HIGH	Study included participants offered primary or secondary screening
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	Cell free DNA analysis was blinded to sample karyotype
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT was not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Telephone interviews were performed to obtain information on clinical outcomes
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	HIGH	Important uncertainty regarding the classification of the target population
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT was prior to reference standard
Did all patients receive a reference standard?	NO	Outcome data was only available for 77% of the population
Did patients receive the same reference standard?	NO	Information was obtained by neonatal outcome, physical examination or cytogenetic testing
Were all patients included in the analysis?	NO	Inappropriate samples, test failures and samples with no results on clinical outcomes (elective terinations and pregnancies) were excluded from the analyiss
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Comas et al. [40], 2015	Judgment	Explanation
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	The study excluded cases of ultrasound anomalies and those at high risk of other genetic conditions
Applicability: Is there concern that the included patients do not match the review question?	HIGH	Study did not include general unselected population
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	UNCLEAR	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT was performed before invasive testing
If a threshold was used, was it pre-specified?	NO	Test cut off values were not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation is in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Insufficient information regarding on neonatal examination of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard did match the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT performed prior invasive testing
Did all patients receive a reference standard?	NO	All patients included present complete follow up but information is lacking regarding miscarriages and stillbirths
Did patients receive the same reference standard?	NO	Positive NIPT cases were confirmed by invasive testing and negative cases by neonatal examination
Were all patients included in the analysis?	NO	Information regarding test failures, lost cases, miscarriages and stillbirths is missing
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: **Bianchi et al. [30], 2014**	Judgment	Explanation
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Lack of information regarding population enrollment
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Exclusion of women without accessibility to pregnancy and delivery records
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Unclear if the study includes a general unselected obstetric population
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	LOW	Authors report that all personnel were unaware of clinical data and outcomes
If a threshold was used, was it pre-specified?	YES	Cut-off point reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Outcome determined on the basis of the newborn physical examination in 97% of cases
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Cytogeneticists were unaware of the results of cfDNA testing
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by reference standard did match the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	Invasive testing performed after NIPT or at least 2 weeks before plasma sampling
Did all patients receive a reference standard?	NO	Patients without clinical outcomes or karyotyping results excluded
Did patients receive the same reference standard?	NO	Both karyotyping or neonatal examination was used as reference standard
Were all patients included in the analysis?	NO	128 patients were excluded of analysis
OVERALL JUDGMENT	At risk of bias	

Author/year: Pergament et al. [44], 2014	**Judgment**	**Explanation**
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	HIGH	
Was a consecutive or random sample of patients enrolled?	NO	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Lack of information regarding inclusion/exclusion criteria
Applicability: Is there concern that the included patients do not match the review question?	YES	Study did not include general non unselected population
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	Algorithm blinded to sample karyotype
If a threshold was used, was it pre-specified?	YES	Cut off-point reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing was used for confirmation in all cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported but considered irelevant as invasive testing is an objective test)
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	No concern regarding the target condition
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	UNCLEAR	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard in majority of cases; in few cases at least 4 days after invasive procedure
Did all patients receive a reference standard?	YES	All verified by standard invasive testing
Did patients receive the same reference standard?	YES	All verified by standard invasive testing
Were all patients included in the analysis?	NO	Exclusion of patients with sex chromosome anomalies, confirmed triploidy, fetal mosaicism
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Song et al. [45], 2013		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	HIGH	Only women < 35 yrs
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	Results analysis was performed in a blinded fashion.
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT was provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding the follow up of birth outcomes
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Results analysis was performed in a blinded fashion.
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	The target condition defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Cases excluded did not receive reference standard
Did patients receive the same reference standard?	NO	Pregnant women received karyotyping (amniocentesis or CVS) or neonatal follow-up
Were all patients included in the analysis?	NO	175 cases were excluded from the analysis
OVERALL JUDGMENT	At risk of bias	

NIPT in add-on to combined test: High-risk pregnant population

Persico et al. [49], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	HIGH	
Was a consecutive or random sample of patients enrolled?	YES	Consecutive enrolment of pregnant women
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Inclusion of women undergoing invasive testing
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not only defined by a FCT threshold)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT was provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered adequate for classifying target condition
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All pregnant women had results confirmed
Did patients receive the same reference standard?	YES	Pregnant women received amniocentesis or CVS as refere rence standard
Were all patients included in the analysis?	NO	Lack of information regarding miscarriages, still births
OVERALL JUDGMENT	Unclear risk of bias Concern regarding applicability	

Author/year: Oepkes et al. [48], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	LOW	
Was a consecutive or random sample of patients enrolled?	YES	All pregnant women who chose NIPT involved
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	YES	Exclusion criteria aligned with indications
Applicability: Is there concern that the included patients do not match the review question?	LOW	Inclusion aligned with review question (high risk women based on FCT or medical history), though risk threshold was not defined
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	This information was reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Follow up of consisted in a return form filled out by the women after pregnancy/birth
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	Complete follow up in 99% pregnancies
Did patients receive the same reference standard?	NO	53 samples confirmed by invasive testing and 1376 confirmed by neonatal follow-up
Were all patients included in the analysis?	YES	No cases were excluded from the analysis
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Zhang et al. [50], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	YES	Exclusion criteria aligned with indications
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by FCT) Pregnant women received NIPT test from the first to third trimester
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	This information was provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding the neonatal examination
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All results confirmed by karyotyping, follow up examination by neonatologists or blood karyotyping
Did patients receive the same reference standard?	NO	Pregnant women received amniocentesis (24.1%), neonatal blood karyotyping (42.3%) or follow-up examination of newborn.
Were all patients included in the analysis?	UNCLEAR	Lack of information regarding the exclusion of patients
OVERALL JUDGMENT	At risk of bias Unclear concern regarding applicability	

Author/year: Ma et al. [47], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not clearly reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by FCT) NIPT test was provided to 19 weeks of gestation
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	UNCLEAR	
Were the index test results interpreted without knowledge of the results of the reference standard?	UNCLEAR	Sequencing analysis was performed in a blinded fashion
If a threshold was used, was it pre-specified?	YES	Cut-off point reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding postnatal follow up
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	UNCLEAR	Unclear information
Did patients receive the same reference standard?	NO	Pregnant women received karyotype testing or postnatal follow-up as reference standard
Were all patients included in the analysis?	NO	Exclusion of patients without karyotyping confirmation or follow up results
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Kim et al. [46], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	No information regarding exclusion criteria
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Inclusion aligned with review question (high risk women based on FCT or medical history), though risk threshold was not defined. Around 30% cases received NIPT test at second trimester of pregnancy
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT results interpretation was blinded to karyotyping information
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Reference standard used i.e. amniocentesis, CVS and newborn examination are considered adequate for classifying the target condition
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	LOW	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard i.e. amniocentesis
Did patients receive the same reference standard?	YES	All cases received reference standard i.e. amniocentesis
Were all patients included in the analysis?	YES	All cases were included in the analysis
OVERALL JUDGMENT	Unclear concern regarding applicability	

Author/year: Wang et al. [58], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Consecutive women
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by FCT)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	HIGH	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	This information was not provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	UNCLEAR	Unclear information regarding the conduct and interpretation of NIPT
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	YES	The clinical follow up (once/month) from birth to 6 months of negative cases was considered appropriate
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	LOW	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard
Did patients receive the same reference standard?	NO	Positive cases verified by conventional karyotyping analysis or fluorescence *in situ* hybridization-FISH and negative cases by neonatal follow-up
Were all patients included in the analysis?	YES	No cases were excluded from the analysis
OVERALL JUDGMENT	At risk of bias	

Author/year: Sánchez-Usabiaga et al. [56], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	YES	Exclusion criteria aligned with indications
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding follow up of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	NIPT failure cases did not receive reference standard
Did patients receive the same reference standard?	NO	Cases received CVS, amniocentesis or follow up as reference standard
Were all patients included in the analysis?	NO	Exclusion of test failures and lack of information on miscarriages amd stilL births
OVERALL JUDGMENT	At risk of bias	

Author/year: Benachi et al. [51], 2015	Judgment	Explanation
DOMAIN 1: PATIENT SELECTION		
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT was performed in a blinded fashion respect to the fetal karyotype.
If a threshold was used, was it pre-specified?	YES	NIPT cut-off point reported.
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Reference standard used i.e. amniocentesis or CVS are considered adequate for classifying the target condition
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question.
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Lack of information of women who did not undergoe invasive testing
Did patients receive the same reference standard?	YES	All received invasive testing (amniocentesis or CVS)
Were all patients included in the analysis?	NO	Only cases with a karyotype and cell-free DNA included in the analysis
OVERALL JUDGMENT	At risk of bias	

Author/year: Ke et al. [53], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold). Women received NIPT test mainly in second trimester
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	HIGH	
Were the index test results interpreted without knowledge of the results of the reference standard?	UNCLEAR	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut off point not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	UNCLEAR	Unclear information regarding conduct and interpretation
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding follow up of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	LOW	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard
Did patients receive the same reference standard?	NO	Positive NIPT cases received amniocentesis and negative test cases received neonatal follow-up
Were all patients included in the analysis?	YES	All cases were included in the analysis
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Lee et al. [54], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold).
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	All clinical data and NIPT results were blinded to the laboratory investigators.
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Cordocentesis was used to confirm NIPT results in some cases (number not reported)
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	All clinical data and NIPT results were blinded to the laboratory investigators
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All included cases received invasive testing
Did patients receive the same reference standard?	NO	Different reference standard i.e. amniocentesis, CVS, cordocentesis or neonatal peripheral blood or products of conception
Were all patients included in the analysis?	NO	Analysis was restricted to pregnant women who underwent invasive testing
OVERALL JUDGMENT	At risk of bias	

Author/year: Song et al. [57], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (inclusion criteria:≥ 35 yrs).
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT testing was performed in a double-blinded manner
If a threshold was used, was it pre-specified?	YES	Cut-off point provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Neonatal examination by patient´s pediatricians
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Karyotyping analysis was performed in a double-blinded manner
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Non verification of quality control failures, miscarriages or still births
Did patients receive the same reference standard?	NO	Pregnant women received karyotyping testing (CVS or amniocentesis) in positive NIPT cases and were followed to birth and assessed clinically by paediatrician in negative NIPT test
Were all patients included in the analysis?	NO	Exclusion of women who presented quality control failures, miscarriages or still births
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Sago et al. [55], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold).
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	UNCLEAR	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT not provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	.
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding follow up of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Data exists for only 1638 of the 7740 negative cases. Most of miscarriages and still births did not receive a reference standard
Did patients receive the same reference standard?	NO	Positive cases underwent invasive testing (chorionic villus sampling or amniocentesis) and negative cases were followed up
Were all patients included in the analysis?	NO	Exclusion of test failures and patients without outcomes
OVERALL JUDGMENT	At risk of bias	

Author/year: Hernández-Gómez et al. [52], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold).
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Unclear information regarding verification of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	UNCLEAR	Unclear information regarding verification of negative cases
Did patients receive the same reference standard?	NO	Four cases received amnioncentesis and 37 cases neonatal follow-up
Were all patients included in the analysis?	YES	All cases included
OVERALL JUDGMENT	At risk of bias	.

Author/year: Korostelev et al. [60], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Inclusion criteria aligned with review question (high risk women based on FCT or medical history), though risk threshold was not defined. Around 30% cases received NIPT test at second trimester of pregnancy
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	UNCLEAR	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding follow up of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	It was not reported if reference standard was performed in a blinded fashion
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matched the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	423/1968 cases did not receive a reference standard
Did patients receive the same reference standard?	NO	Positive NIPT cases received karyotyping or chromosomal microarray analysis and negative NIPT cases were followed up
Were all patients included in the analysis?	NO	Cases without reference standard result were excluded from analysis
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Stumm et al. [62], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Consecutive enrolled women
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold).
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	NIPT conduct and interpretation in accordance with standard procedure
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT test was blinded to patient clinical information including karyotyping.
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	The conduct or interpretation of NIPT did not refer from the review question
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matched the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Samples which failed quality control or did not undergoe invasive testing did not receive a reference standard
Did patients receive the same reference standard?	NO	Pregnant women received amniocentesis (69.1), chorionic villus sampling (30.3) or cordocentesis (0.6)
Were all patients included in the analysis?	NO	Patients with no outcome results were excluded from the analysis
OVERALL JUDGMENT	At risk of bias	

Author/year: Jeon et al. [59], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	HIGH	
Was a consecutive or random sample of patients enrolled?	YES	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Study only included pregnant women who were scheduled for invasive testing
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold).First and second trimester pregnancies included.
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	It is unknown if reference standard results were analysed in a blinded fashion
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	LOW	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard
Did patients receive the same reference standard?	YES	All cases received amniocentesis
Were all patients included in the analysis?	YES	All cases were included in the analysis
OVERALL JUDGMENT	At risk of bias	

Author/year: Porreco et al. [61], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	HIGH	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Only women who had decided to undergone invasive testing considered
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold). First and second trimester pregnancies included.
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	Blinded analysis
If a threshold was used, was it pre-specified?	YES	Cut off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Blinded analysis
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All patients received invasive testing
Did patients receive the same reference standard?	YES	Pregnant women received CVS (25%) or amniocentesis (75%)
Were all patients included in the analysis?	NO	Exclusion of cases which failed quality control, did not undergoe invasive testing or were fetal demises (740/4170 cases excluded)
OVERALL JUDGMENT	At risk of bias	

Zhou et al. [64], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	LOW	
Was a consecutive or random sample of patients enrolled?	YES	Offered to all women integrated in the workflow
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	HIGH	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT not provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	UNCLEAR	Unclear information regarding the conduct and interpretation of NIPT
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding neonatal follow up
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Fetal karyotyping was performed blindly following NIPT
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Outcome data only available for 3894/7705 pregnancies
Did patients receive the same reference standard?	NO	Pregnant women received amniocentesis (n=54) or neonatal follow-up (n=3894)
Were all patients included in the analysis?	NO	Only patients with outcome data included in the analysis
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Willems et al. [63], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	LOW	
Was a consecutive or random sample of patients enrolled?	YES	Consecutive sampling
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	HIGH	Only 660/994 who had FCT showed elevated risk
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Lack of information on follow up of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matched the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	Terminations and miscarriages did not receive a reference standard
Did patients receive the same reference standard?	NO	Only positive cases received amniocentesis or chorionic villus sampling
Were all patients included in the analysis?	NO	Exclusion of test failures, terminations and miscarriages
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Liang et al. [65], 2013		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold. Mostcases received NIPT test in the 2º trimester of pregnancy
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT test was performed in a blinded fashion
If a threshold was used, was it pre-specified?	YES	Cut off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Karyotyping information were conducted in a blinded fashion
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	UNCLEAR	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received a reference standard
Did patients receive the same reference standard?	YES	All women underwent invasive diagnosis
Were all patients included in the analysis?	NO	Exclusion of samples that failed quality control
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Auhtor/year: Verweij et al. [67], 2013		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	YES	Consecutive sampling
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Exclusion of failed samples, women with other trisomies, amongst others
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (women requesting NIPT for anxiety reasons also included)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	The laboratory personnel were blinded to the clinical information.
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	The conduct or interpretation have not introduced bias
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Independent analysis
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases included received invasive testing
Did patients receive the same reference standard?	YES	All cases received CVS (n=280) or amniocentesis (n=240)
Were all patients included in the analysis?	NO	Exclusion of subjects with low fetal fraction and test failures.
OVERALL JUDGMENT	At risk of bias	

Author/year: Nicolaides et al. [66], 2013		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	LOW	Most of the included patients matched the review question (high risk based on FCT)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is consided the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Blinded to karyotype
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received CVS
Did patients receive the same reference standard?	UNCLEAR	This information was not clearly reported
Were all patients included in the analysis?	NO	Exclusion of cases which failed quality controls. No information regarding miscarriages and still births.
OVERALL JUDGMENT	At risk of bias	

Author/year: Norton et al. [69], 2012		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	HIGH	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Unclear elegibility criteria
Applicability: Is there concern that the included patients do not match the review question?	HIGH	All women planning to undergoe invasive testing included for any indication
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	Cut off point of NIPT not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference test
Did all patients receive a reference standard?	YES	All cases received invasive testing
Did patients receive the same reference standard?	YES	All received invasive testing (amniocentesis or CVS)
Were all patients included in the analysis?	NO	Exclusion of subjects with fetal fraction and assay failures (774/4002). Lack of information on miiscarriages and still births
OVERALL JUDGMENT	At risk of bias	

Author/year: Lau et al. [68], 2012		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria do not exactly match the review question (only 47% were due to positive FCT)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	Blinded to karyotyping information
If a threshold was used, was it pre-specified?	YES	Cut-off point NIPT was reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	Risk of bias related to reference standard performance was not considered relevant.
Is the reference standard likely to correctly classify the target condition?	YES	Reference standard are considered adequate for classifying the target condition
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	LOW	There is low risk of bias due to patient flow
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard
Did patients receive the same reference standard?	NO	94.4% cases received chorionic villus sampling and amniocentesis was used in 5.6% of cases
Were all patients included in the analysis?	YES	All cases were included in the analysis
OVERALL JUDGMENT	Unclear risk of bias and concern regarding applicability	

Author/year: Ehrich et al. [70], 2011		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	LOW	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	LOW	The inclusion criteria do not exactly match the review question (risk is not defined by a FCT threshold
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	Blinded to reference standard
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Invasive testing is considered the gold standard
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	Independent analysis
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard
Did patients receive the same reference standard?	YES	80.9% cases received amniocentesis and 19% CVS
Were all patients included in the analysis?	NO	Cases with sampling or quality test failure were excluded from analysis (n=31)
OVERALL JUDGMENT	Low risk of bias and concern regarding applicability	

NIPT add-on to combined test: High or intermediate risk pregnant population

Author/year: Gil et al. [71], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not reported
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	NO	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	The inclusion criteria match the review question, though a slightly higher FCT risk threshold is used (1:100)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Unclear information regarding the follow up of negative cases
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	It was not reported if karyotyping was performed in a blinded fashion
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	UNCLEAR	Unclear whether reference standard was used in all pregnant women
Did patients receive the same reference standard?	NO	Invasive testing for positive NIPT cases and follow up for negative cases
Were all patients included in the analysis?	NO	Exclusion of miscarriages, stillbirths and women lost to follow up (1.78%)
OVERALL JUDGMENT	At risk of bias	

NIPT add-on to combined test: Twin pregnant population

Author/year: Fosler et al. [72], 2017		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	UNCLEAR	Unclear information regarding the enrolment of pregnant women
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	The interpretation of NIPT results could have introduced bias
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	NO	Cut-off point of NIPT not provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Ultrasound findings is not considered a reference standard for diagnosing prenatal aneuploidies.
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by ultrasound findings matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	NO	Interval between index test and reference standard might not have been appropriate in all cases
Did all patients receive a reference standard?	NO	Aneuploidy outcome information was only available for 35.7% of patients
Did patients receive the same reference standard?	NO	Pregnant women received karyotyping (amniocentesis or CVS) or ultrasound findings
Were all patients included in the analysis?	NO	Exclusion of patients without outcomes
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Tan et al. [73], 2016		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	LOW	
Was a consecutive or random sample of patients enrolled?	YES	All women receiving NIPT included
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	HIGH	100% women included had a pregnant by ART (double or multiple pregnancies)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT not reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	HIGH	
Is the reference standard likely to correctly classify the target condition?	NO	Pregnant outcome was surveyed by telephone interview
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	NO	No information regarding miscarriages or stillbirths
Did patients receive the same reference standard?	NO	Pregnant women received amniocentesis (NIPT positive cases) (3.1%) or follow-up (NIPT negative cases) (96.9%)
Were all patients included in the analysis?	NO	Exclusion of cases of miscarriages and stillbirths
OVERALL JUDGMENT	At risk of bias Concern regarding applicability	

Author/year: Bevilacqua et al. [74], 2015		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	LOW	The inclusion criteria match the review question, although FCT risk threshold was not specified
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to reference standard
If a threshold was used, was it pre-specified?	YES	NIPT cut off point reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Lack of information regarding neonatal examination
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard does not differ the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to referene standard
Did all patients receive a reference standard?	NO	Lack of information regarding miscarriages and stillbirths
Did patients receive the same reference standard?	NO	Pregnant women CVS, amniocentesis, neonatal blood or neonatal examination.
Were all patients included in the analysis?	NO	175/515 cases were excluded from the analysis
OVERALL JUDGMENT	At risk of bias	

Author/year: Huang et al. [75], 2014		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	UNCLEAR	
Was a consecutive or random sample of patients enrolled?	UNCLEAR	Not specified
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	HIGH	Patients who opted to have invasive testing included (abnormal screening, sonographic signs aswell as anxiety)
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	The sequencing results were kept confidential until final analysis.
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT provided
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	LOW	
Is the reference standard likely to correctly classify the target condition?	YES	Reference standard used are considered adequate for classifying the target condition
Were the reference standard results interpreted without knowledge of the results of the index test?	YES	The karyotyping results were kept confidential until final analysis.
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	LOW	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received invasive testing
Did patients receive the same reference standard?	YES	All received invasive testing as reference standard
Were all patients included in the analysis?	YES	All cases included in the analysis
OVERALL JUDGMENT	Low risk of bias or concern regarding applicability	

Author/year: Lau et al. [76], 2013		
DOMAIN 1: PATIENT SELECTION	**Judgment**	**Explanation**
Risk of bias*:* Could the selection of patients have introduced bias?	HIGH	
Was a consecutive or random sample of patients enrolled?	NO	Only some twin pregnancies considered
Was a case-control design avoided?	YES	Prospective DTA trial
Did the study avoid inappropriate exclusion?	UNCLEAR	Exclusion criteria not reported
Applicability: Is there concern that the included patients do not match the review question?	HIGH	NIPT indications do not match the review question
DOMAIN 2: INDEX TEST(S)		
Risk of bias: Could the conduct or interpretation of the index test have introduced bias?	LOW	
Were the index test results interpreted without knowledge of the results of the reference standard?	YES	NIPT prior to FCT
If a threshold was used, was it pre-specified?	YES	Cut-off point of NIPT reported
Applicability: Is there concern that the index test, its conduct, or interpretation differ from the review question	LOW	NIPT conduct and interpretation in accordance with standard procedure
DOMAIN 3: REFERENCE STANDARD		
Risk of bias: Could the reference standard, its conduct, or its interpretation have introduced bias?	UNCLEAR	
Is the reference standard likely to correctly classify the target condition?	UNCLEAR	Unclear information regarding clinical examination of the newborn
Were the reference standard results interpreted without knowledge of the results of the index test?	UNCLEAR	Not reported
Applicability: Is there concern that the target condition as defined by the reference standard does not match the review question?	LOW	Target condition as defined by the reference standard matches the review question
DOMAIN 4: FLOW AND TIMING		
Risk of bias: Could the patient flow have introduced bias?	HIGH	
Was there an appropriate interval between index test(s) and reference standard?	YES	NIPT prior to reference standard
Did all patients receive a reference standard?	YES	All cases received reference standard
Did patients receive the same reference standard?	NO	Pregnant women received prenatal karyotyping or clinical examination of the newborn
Were all patients included in the analysis?	YES	All cases included
OVERALL JUDGMENT	At risk of bias	

Table A9: GRADE assessment of diagnostic test accuracy outcomes. NIPT as an add-on to combined testing in general pregnant population

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
21 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	8 studies 136544 pregnant women	DTA studies (5 comparative)	Serious[a]	Not serious[b]	Not serious	Not serious	Unlikely[c]	⊕⊕⊕◯ MODERATE	Critical
True negative (patients without 21 trisomy) **False positive** (patients incorrectly classified as having 21 trisomy)	8 studies 136544 pregnant women	DTA studies (5 comparative)	Very serious[a]	Not serious[b]	Not serious	Not serious	Unlikely[c]	⊕⊕◯◯ LOW	Critical
18 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	7 studies 135639 pregnant women	DTA studies (5 comparative)	Serious[d]	Not serious[b]	Not serious	Very serious[e]	Unlikely[c]	⊕◯◯◯ VERY LOW	Critical
True negative (patients without 21 trisomy) **False positive** (patients incorrectly	7 studies 135639 pregnant women	DTA studies (5 comparative)	Very serious[d]	Not serious[b]	Not serious	Not serious	Unlikely[c]	⊕⊕◯◯ LOW	Critical

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
classified as having 21 trisomy)									
13 trisomy									
True positives (patients with 21 trisomy)	6 studies 130203 pregnant women	DTA studies (4 comparative)	Serious[f]	Not serious[b]	Not serious	Very serious[g]	Unlikely[c]	⊕◯◯◯ VERY LOW	Critical
False negatives (patients incorrectly classified as not having 21 trisomy)									
True negative (patients without 21 trisomy)	6 studies 130203 pregnant women	DTA studies (4 comparative)	Very serious[f]	Not serious[b]	Not serious	Not serious	Unlikely[c]	⊕⊕◯◯ LOW	Critical
False positive (patients incorrectly classified as having 21 trisomy)									

a. Many studies presented a high or unclear risk of bias regarding reference standard and index test due to performance of both test could have not blinded; for sentivity estimations, RoB is very serious as some studies did not confirm negative NIPT cases.
b. Although included studies used different NIPT platform, it is considered that it is not relevant to rate down quality of evidence for indirectness.
c. Possibility of publication bias not excluded but not considered sufficient to downgrade quality of evidence.
d. Many studies presented a high or unclear risk of bias regarding reference standard and index test due to performance of both test could have not blinded; moreover some of them did not confirm negative NIPT cases.
e. Four studies (Bianchi et al 2014, Norton et al 2015, Quezada et al 2015 and Song et al 2013) showed wide confidence intervals of sensitivity.
f. Many studies presented a high or unclear risk of bias regarding reference standard and index test due to performance of both test could have not blinded; moreover some of them did not confirm negative NIPT cases.
g. Four studies (Bianchi et al 2014, Norton et al 2015, Song et al 2013 and Pérez-Pedregosa et al 2015) showed wide confidence intervals of sensitivity

Table A10: GRADE assessment of diagnostic test accuracy outcomes. NIPT as an add-on to combined testing in high-risk pregnant population

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
21 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	23 studies 28188 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	⊕⊕⊕◯ MODERATE	Critical
True negative (patients without 21 trisomy) **False positive** (patients incorrectly classified as having 21 trisomy)	23 studies 28188 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	⊕⊕◯◯ LOW	Critical
18 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	20 studies 25972 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely[b]	⊕◯◯◯ VERY LOW	Critical
True negative (patients without 21 trisomy) **False positive** (patients incorrectly	20 studies 25972 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	⊕⊕◯◯ LOW	Critical

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
classified as having 21 trisomy)									
13 trisomy									
True positives (patients with 21 trisomy)	15 studies 22650 pregnant women	DTA studies	Serious[a]	Not serious	Not serious	Very serious[c]	Unlikely[b]	⊕◯◯◯ VERY LOW	Critical
False negatives (patients incorrectly classified as not having 21 trisomy)									
True negative (patients without 21 trisomy)	15 studies 22650 pregnant women	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely[b]	⊕⊕◯◯ LOW	Critical
False positive (patients incorrectly classified as having 21 trisomy)									

a. 44% of studies presented a high risk of bias regarding flow and timing domain; 50-60% of studies showed a high or unclear risk of bias regarding reference standard or index test domains; moreover for sensitivity estimations, RoB is very low as some studies did not confirm negative NIPT cases.
b. Although Deeks's Funnel Plot Asymmetry Test reached statistical significance (p=0.00) for publication bias, it is considered that it is not relevant to downgrade quality of evidence.
c. 17/20 studies reported wide confidence intervals around estimates of sensitivity.

Table A11: GRADE assessment of diagnostic test accuracy outcomes. NIPT as an add-on to combined testing in high or intermediate risk pregnant population

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
21 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	⊕⊕⊕◯ MODERATE	Critical
True negative (patients without 21 trisomy) **False positive** (patients incorrectly classified as having 21 trisomy)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	⊕⊕⊕◯ MODERATE	Critical
18 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Very serious[b]	Unlikely	⊕⊕◯◯ LOW	Critical

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
True negative (patients without 21 trisomy) **False positive** (patients incorrectly classified as having 21 trisomy)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	⊕⊕⊕◯ MODERATE	Critical
13 trisomy									
True positives (patients with 21 trisomy) **False negatives** (patients incorrectly classified as not having 21 trisomy)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Very[b] serious	Unlikely	⊕⊕◯◯ LOW	Critical
True negative (patients without 21 trisomy) **False positive** (patients incorrectly classified as having 21 trisomy)	1 study 3633 pregnant women	DTA study	Serious[a]	Not serious	Not serious	Not serious	Unlikely	⊕⊕⊕◯ MODERATE	Critical

a. This study showed a high risk of bias regarding of flow and timing domain and there was an unclear risk due to reference standard and index test domains as both test was not performed in a blinded fashion.
b. Study reported wide confidence intervals around estimates of sensitivity.

Table A12: GRADE assessment of diagnostic test accuracy outcomes. NIPT as an add-on to combined testing in twin pregnant population

Outcome	Nº of studies (Nº of patients	Study design	Factors that may decrease quality of evidence					Test accuracy QoE	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
21 trisomy									
True positives (patients with 21 trisomy)	6 studies 1985 patients	DTA studies	Serious[a]	Not serious	Not serious	Serious[b]	Unlikely	⨁◯◯◯ VERY LOW	Critical
False negatives (patients incorrectly classified as not having 21 trisomy)									
True negative (patients without 21 trisomy)	6 studies 1985 patients	DTA studies	Very serious[a]	Not serious	Not serious	Not serious	Unlikely	⨁⨁◯◯ LOW	Critical
False positive (patients incorrectly classified as having 21 trisomy)									

a. 60-80% of studies showed a high or unclear risk of bias regarding of some of the domains.
b. All studies performed in twin pregnant population reported wide confidence intervals around estimates of sensitivity.

Applicability tables

Table A13: Summary table characterising the applicability of a body of studies

Domain	Description of applicability of evidence
Population	As mentioned in the PICO question, three target populations were considered i.e. general pregnant population, that is without any pre-defined fetal aneuploidy risk factor, high-risk population (>1:300 from first or second serum combined testing) and intermediate risk population (1:300-1:1000 from first or second serum combined testing) which all included pregnant women at least 8-9 weeks' gestation. With regards to general pregnant population, although all retrieved studies enrolled women who were undergoing routine screening, some of them included patients defined as high risk by first or second combined test (16.5-64%) or used a narrow patient selection criteria, that is women who had elegibility to health records, with known results, NIPT results verified by invasive testing, only included women <35 years old or excluded twin pregnancies or in vitro fertilisations with user donor oocytes. Moreover, the prevalence of most common trisomies reported in these studies is quite higher than values described in literature. Therefore, pregnant women included in these studies do not match completely the general population and reducing the applicability of evidence. The studies performed in high risk pregnant womenalso present important applicability concerns as included population do not match to the review question. Twenty-five percent of studies on high risk women reported that advanced maternal age was the main or only indication for NIPT or included ≤15% of women with no risk indication, that is due to anxiety, not suitable for invasive testing or other reasons not specified. The only DTA study on intermediate-risk population included women with risk ≥2500. Finally, the evidence reported in the studies performed in twin pregnant women could maybe be applicable to the review question raised. However, it should be taken into account that one study exclusively enrolled women who conceived after assisted reproductive technology.
Intervention	With regard to the five types of intervention proposed, the retrieved evidence only assessed NIPT as a primary testing method in general pregnant population and studies on NIPT as an add-on to combined test and other factors for women estimated to be at high/intermediate risk by combined screening. Therefore, there is no evidence available regarding the second and fifth type of intervention (NIPT as a part of combined test and NIPT in replacement of invasive testing respectively).
Reference standard	The appropriate reference standard of NIPT would have to be a confirmation method of screening results, i.e. fetal karyoptype for positive NIPT results or clinical examination for negative NIPT results. All studies used these reference standards as appropiate, so there is no concern about the applicability of evidence regarding this aspect.
Comparators	The comparator of NIPT is a standard screening based on first or second serum combined testing followed by invasive diagnostic test. Although only four studies performed on general population compared NIPT vs. standard screening, all of these used an appropriate comparator. Therefore, retrieved evidence would seem to match the review question in relation to this aspect.
Outcomes	Effectiveness of NIPT was evaluated not only in terms of accuracy of test, that is FP, FN, S, Sp, PPV and PNV but also in terms of assessing the impact of NIPT in a prenatal screening pathway, i.e. reduction of miscarriages related to invasive testing or reduction of children born with undiagnosed trisomy. However, most studies only reported the accuracy of NIPT. Therefore, it was not possible to provide conclusions about safety or effectiveness of prenatal screening with NIPT.
Setting	Mainly, retrieved studies were performed at routine prenatal-screening in medical centers from different European countries, USA, China, Republic of Korea, Japan, México and Russia. Thus, it is considered that there is no concern about the applicability of evidence related to this aspect.

Abbreviations: NIPT, Non-Invasive, Prenatal Test; DTA, Diagnostic Test Accuracy; USA, United States of America; FP, False Positive, FN, False Negative, S, Sensitivity; Sp, Specificity, PPV, Predictive Positive Value; PNV, Predictive Negative Value.

Sources: Evidence retrieved for effectiveness and safety domains

APPENDIX 2: REGULATORY AND REIMBURSEMENT STATUS

Table A14: Regulatory status

Country	Institution issuing approval	Authorisation status yes/no/ongoing	Verbatim wording of the (anticipated) indication(s)	Specified contra-indications	Date of approval (include expiry date for country of assessment)	Launched yes/no If no include date of launch	Approval number (if available)
USA[1]	Food and Drug Administration (FDA)	Not applicable NIPT is considered LDTs (laboratory developed tests), a subset of IVDs devices that have not to comply FDA requirements for IVDs	-	-	-	Yes	-
Australia[2]	Australian Therapeutic Good Administration (TGA)	Not applicable If NIPT are IVDs developed "in house", these only required to have approval and yearly validation If sequencing analysis is performed in overseas countries, only equipment to collect and transport samples have to be registered	-	-	-	Yes (nearly established)	-
Canada[3]	Ministry of Health and Long-Term Care (MOHLTC) in Ontario region British Columbia (BC) Ministry of Health in British Columbia region	Yes (reimbursement only for women who fullfil indications in Ontario and British Columbia)	NIPT is available for trisomy 21, 18, 13 and sex aneuploidy These test are approved for women with: -a positive prenatal screening and ultrasound results	Microdelection testing is not funded NIPT is not recommended for multiple gestations	-	Yes	-

Country	Institution issuing approval	Authorisation status yes/no/ongoing	Verbatim wording of the (anticipated) indication(s)	Specified contra-indications	Date of approval (include expiry date for country of assessment)	Launched yes/no If no include date of launch	Approval number (if available)
			-a previous trisomic pregnancy or history of aneuploidy -advanced age (≥40 years) -other reasons i.e. fetal congenital anomalies or risk of a sex-limited disorder				
European countries[4,5]	Organization in charge of giving CE mark in each country	Yes EC-IVD certification (annexe III- IV) for different NIPT trademark (VeriSeq, MateriT21 PLUS, IONA Test, Panorama, Harmony, etc. see Tables 3-4, TEC domain)	This information is not clearly reported in EC-IVD certification According to manufacturers information, NIPT test detected trisomy 21, 18, 13 and sex chromosome aneuploidies. And moreover, some of them could be used to identify microdeletions in all pregnant women (single or twin pregnancies)(see Table 3, TEC domain)	Information not available in EC-IVD certification	2013-2017	Yes	-
China[6]	China Food and Drug Administration (CFDA)	Yes BGISEQ-100, NextSeq CN500, NFTY	Pregnant women with high risk of aneuploidies (Trisomy 21, 18 and 13)	-	-	-	-

Abbreviations: NIPT, Non-Invasive Prenatal Test; IVDs, "in vitro" diagnostic; LDTs, Laboratory Diagnostic Tests; CE, Conformité Européenne.
Sources: [1]Oversight of Laboratory Developed Test, Food and Drug Administration (FDA), available from: https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Reports/UCM472777.pdf; [2]Non-invasive prenatal testing (NIPT), HealthPACT, available from: https://www.health.qld.gov.au/_data/assets/pdf_file/0033/426993/wp142.pdf; [3]Non-invasive prenatal testing, GECKO, available from: http://geneticseducation.ca/educational-resources/gec-ko-on-the-run/non-invasive-prenatal-testing/; [4]Companies web pages; [5]Submission file/information provided by company; [6]China Food and Drug Administration webpage (http://eng.sfda.gov.cn/WS03/CL0755/).

Table A15: Summary of recommendations and level of reimbursement of NIPT in European countries

Country and issuing organisation e.g. G-BA, NICE	Summary of reimbursement recommendations and restrictions	Level of reimbursement
UK, National Screening Commitee	Evaluative implementation of NIPT to assess the impact on the existing NHS fetal anomaly screening programms (combined test risk score for T21 greater than 1 in 150 and combined test risk score for T18 and T13 greater than or equal to 1 in 15) UK National Screening Committee – GOV.UK	Implementation starting 2018 for a 2 year study. Fully reimbursed on contingent with risk >1:150 with FCT.
France, HAS	Recommended for T21 in women with trisomy high and intermediate risk by contingent screening approach" https://www.has-sante.fr/portail/jcms/c_2768510/fr/place-des-tests-adn-libre-circulant-dans-le-sang-maternel-dans-le-depistage-de-la-trisomie-21-foetale	Fully reimbursed on contingent with risk >1:1000 with FCT
Belgium, Ministry of Health	Recommended for T21 as first line screening test for all pregnant women". http://www.deblock.belgium.be/fr/maggie-de-block-rembourse-le-test-dpni-pour-le-syndrome-de-down-%C3%A0-toutes-les-femmes-enceintes-qui	Implementation started 2017. All women, 8 euro out of pocket payment
Germany, G-BA	Ongoing	No reimbursement at present Offered Privately
Spain, Ministry of Health	Ongoing	Reimbursed in some regions Offered Privately
Netherlands, Health Council	Recommended as a first line screening test for T21, T18 and T13 instead of the combined test" (ref. Prenatale screening, Health Council of the Netherlands, December 2016 https://www.gezondheidsraad.nl/nl/taak-werkwijze/werkterrein/preventie/prenatale-screening)	Implementation started 2017 for a 3 year study for all women, choice between primary FCT or primary NIPT (for T21, 13 and 18 or for all autosomes). Fully reimbursed for women with risk >1:200 with FCT, 170 euro out of pocket for risk < 1:200.
Denmark	NIPT is recommended for screening of fetal aneuploidies in women with high risk (1:300) by contingent screening strategy	Fully reimbursed on contingent with risk >1:300 from FCT

Country and issuing organisation e.g. G-BA, NICE	Summary of reimbursement recommendations and restrictions	Level of reimbursement
Norway, Health Directorate	No reimbursement recommendation available	Not yet released
Sweden, Swedish National Health Board Investigation application of NIPT	SBU published a recommendation on NIPT in 2015 and endorsed NIPT offering for high risk (Analys av foster-DNA i kvinnans blod: icke-invasiv fosterdiagnostik (NIPT) for trisomy 21, 18 and 13 http://www.sbu.se/sv/publikationer/SBU-utvarderar/analys-av-foster-dna-i-kvinnans-blod-icke-invasiv-fosterdiagnostik-nipt-for-trisomi-13-18-och-21/	Offered privately, no reimbursement
Italy, Ministry of Health (Consiglio Superiore di Sanitá)	NIPT introduction recommended as a first or second line test. (http://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=2381)	No reimbursement at present Offered Privately
Greece	No national prenatal screening program NIPT offered privately	Private
Poland	No national prenatal screening program NIPT offered privately	Private
Ireland	No national prenatal screening program NIPT offered privately	Private
Switzerland, BAG	NIPT is recommended for women with intermediate or high risk of aneuploidies (>1:1000) assessed by FCT https://www.bag.admin.ch/bag/de/home/themen/versicherungen/krankenversicherung/krankenversicherung-leistungen-tarife/Analysenliste.html	Fully reimbursed on contingent with risk>1:1000 from FCT with NGS based technologies only (Analysenliste 1.7.2017 BAG)

Abbreviations: FCT, first-trimester combined test
Sources: Companies web pages, Submission file/information provided by company.

APPENDIX 3: CHECKLIST FOR POTENTIAL ETHICAL, ORGANISATIONAL, PATIENT AND SOCIAL AND LEGAL ASPECTS

1	**Ethical**	
1.1	Does the introduction of the new technology and its potential use/non-use instead of the defined, existing comparator(s) give rise to any new ethical issues?	Yes
	Routine introduction of NIPT for prenatal genetic screening could lead to changes in the risk managing approach of pregnant women, which may cause ethical issues for the couple as well as for the health-care provider, as benefits/risks could be substantially different and must be carefully explained.	
1.2	Does comparing the new technology to the defined, existing comparators point to any differences that may be ethically relevant?	Yes
	Prenatal genetic screening NIPT testing can be offered to women with different risks of developing fetal aneuploides, leading to important ethical considerations, NIPT testing could create a great demand that is probably not justified on health grounds in some risk groups.	
2	**Organisational**	
2.1	Does the introduction of the new technology and its potential use/non-use instead of the defined, existing comparator(s) require organisational changes?	Yes
	The new intervention could require important organisational changes if NIPT is implemented in hospital premises and centralised to tertiary care units. Even if the samples are sent to external clinical labs, organisational changes might be required to ensure that there are no delays and an important budget impact can be expected.	
2.2	Does comparing the new technology to the defined, existing comparator(s) point to any differences that may be organisationally relevant?	Yes
	NIPT could replace other screening tests and lead to a change in the current pathways of care, affecting the work load at different levels (reduce imaging, amniocentesis, etc.).	
3	**Social**	
3.1	Does the introduction of the new technology and its potential use/non-use instead of the defined, existing comparator(s) give rise to any new social issues?	Yes
	NIPT are being offered as accurate tests which could avoid invasive testing, and this could have led to great expectations regarding their application, leading to a non-justified demand in some groups. Pressure can also be imposed on parents to avoid a child with anormalities and lead to possible discrimination of people with anomalities.	
3.2	Does comparing the new technology to the defined, existing comparator(s) point to any differences that may be socially relevant?	No
4	**Legal**	
4.1	Does the introduction of the new technology and its potential use/non-use instead of the defined, existing comparator(s) give rise to any legal issues?	No
4.2	Does comparing the new technology to the defined, existing comparator(s) point to any differences that may be legally relevant?	No

APPENDIX 4: ADDITIONAL TABLES AND FIGURES

Table A16: Alternative measures of diagnostic accuracy: positive and negative likelihood ratios (LR+/LR-) and diagnostic odds ratio (DOR)*

Condition	Population	Measure	Mean	95% confidence interval (CI)	
				Lower bound	Upper bound
21 trisomy	General risk	LR+	1521.47	698.79	3312.69
		LR-	0.0066	0.0019	0.0219
		DOR	229941.4	76247.02	693443.9
	High risk	LR+	2021.187	1418.76	2879.4
		LR-	0.0078	0.0023	0.2149
		DOR	258584.9	134179.3	498334.2
	Twin pregnant	LR+	399.54	95.04	1679.54
		LR-	0.0080	0.00005	1.1817
		DOR	49358.04	187.94	1.30 e+07
18 trisomy	General risk	LR+	1853.03	787.01	4362.97
		LR-	0.0256	0.0116	0.0565
		DOR	72245.4	22213.85	234961.4
	High risk	LR+	3319.87	1583.59	6959.85
		LR-	0.0313	0.0080	0.1221
		DOR	106012.2	23475.54	478736.2
13 trisomy	General risk	LR+	2448.46	1788.67	3351.64
		LR-	0.0018	5.01 e-08	68.2318
		DOR	1324686	32.9292	5.33 e+10
	High risk	LR+	5530.72	1347.24	22074.7
		LR-	0.0232	0.0008	0.6104
		DOR	238300.4	7852.63	7231599

Figure A1: Hierarchical summary ROC for general pregnant population

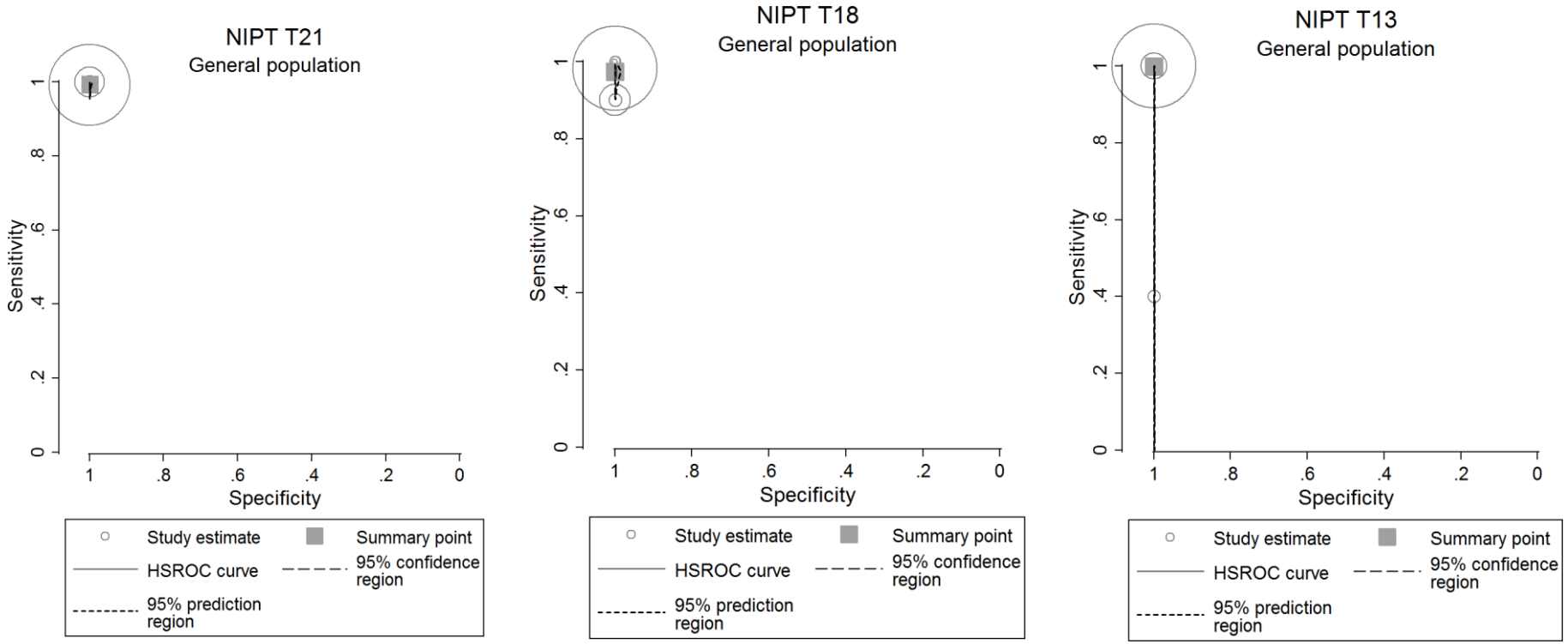

Figure A2: Hierarchical summary ROC for high risk pregnant population

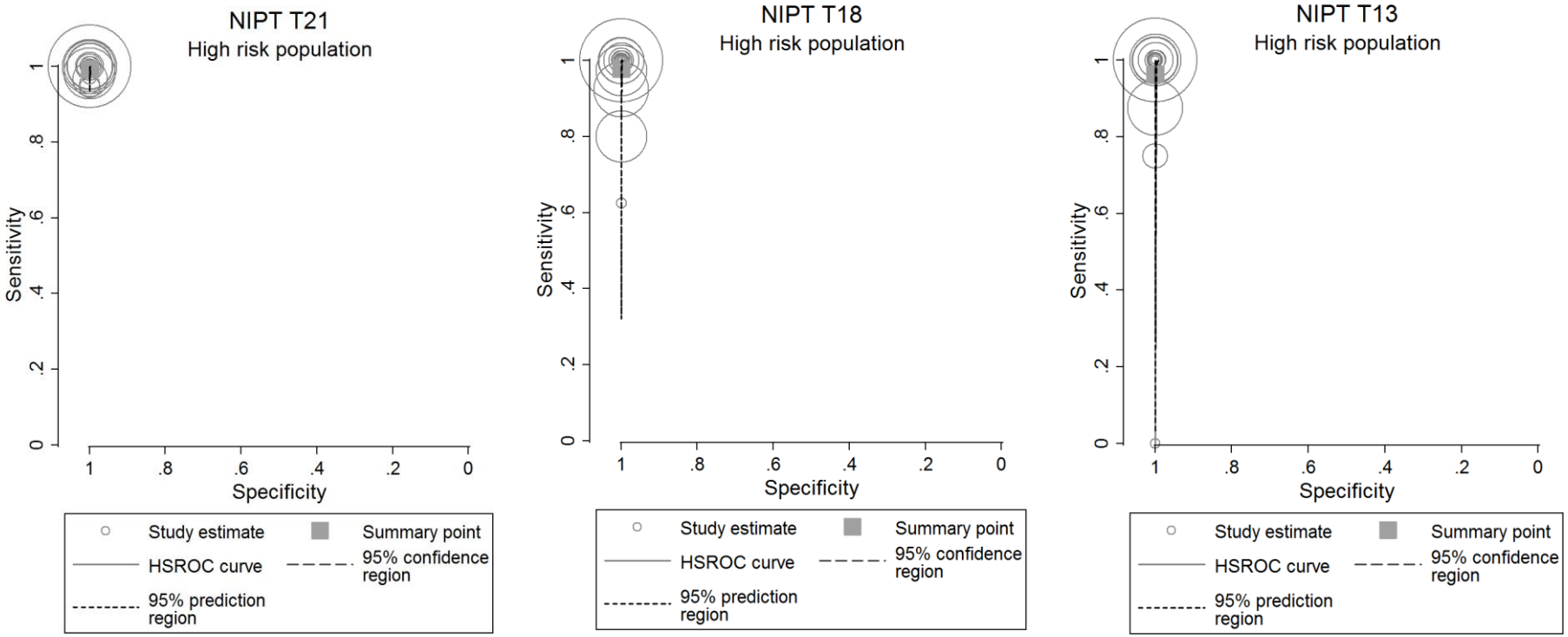

Figure A3: Hierarchical summary ROC for twin pregnant population