Second trimester serum tests for Down's Syndrome screening (Review)

Alldred SK, Deeks JJ, Guo B, Neilson JP, Alfirevic Z

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[Diagnostic Test Accuracy Review]

Second trimester serum tests for Down's Syndrome screening

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ABSTRACT

Background

Down's syndrome occurs when a person has three copies of chromosome 21 - or the specific area of chromosome 21 implicated in causing Down's syndrome - rather than two. It is the commonest congenital cause of mental retardation. Noninvasive screening based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allows estimates of the risk of a pregnancy being affected and provides information to guide decisions about definitive testing.

Objectives

To estimate and compare the accuracy of second trimester serum markers for the detection of Down's syndrome.

Search methods

We carried out a sensitive and comprehensive literature search of MEDLINE (1980 to May 2007), EMBASE (1980 to 18 May 2007), BIOSIS via EDINA (1985 to 18 May 2007), CINAHL via OVID (1982 to 18 May 2007), The Database of Abstracts of Reviews of Effectiveness (*The Cochrane Library* 2007, Issue 1), MEDION (May 2007), The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (May 2007), The National Research Register (May 2007), Health Services Research Projects in Progress database (May 2007). We studied reference lists and published review articles.

Selection criteria

Studies evaluating tests of maternal serum in women at 14-24 weeks of gestation for Down's syndrome, compared with a reference standard, either chromosomal verification or macroscopic postnatal inspection.

Data collection and analysis

Data were extracted as test positive/test negative results for Down's and non-Down's pregnancies allowing estimation of detection rates (sensitivity) and false positive rates (1-specificity). We performed quality assessment according to QUADAS criteria. We used hierarchical summary ROC meta-analytical methods to analyse test performance and compare test accuracy. Analysis of studies allowing direct comparison between tests was undertaken. We investigated the impact of maternal age on test performance in subgroup analyses.

Main results

Fifty-nine studies involving 341,261 pregnancies (including 1,994 with Down's syndrome) were included. Studies were generally high quality, although differential verification was common with invasive testing of only high-risk pregnancies. Seventeen studies made direct comparisons between tests. Fifty-four test combinations were evaluated formed from combinations of 12 different tests and maternal age; alpha-fetoprotein (AFP), unconjugated oestriol (uE3), total human chorionic gonadotrophin (hCG), free beta human chorionic gonadotrophin (βhCG), free alpha human chorionic gonadotrophin (αhCG), Inhibin A, SP2, CA125, troponin, pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PGF) and proform of eosinophil major basic protein (ProMBP).

Meta-analysis of 12 best performing or frequently evaluated test combinations showed double and triple tests (involving AFP, uE3, total hCG, free βhCG) significantly outperform individual markers, detecting six to seven out of every 10 Down's syndrome pregnancies at a 5% false positive rate. Tests additionally involving inhibin performed best (eight out of every 10 Down's syndrome pregnancies) but were not shown to be significantly better than standard triple tests in direct comparisons. Significantly lower sensitivity occurred in women over the age of 35 years. Women who miscarried in the over 35 group were more likely to have been offered an invasive test to verify a negative screening results, whereas those under 35 were usually not offered invasive testing for a negative screening result. Pregnancy loss in women under 35 therefore leads to under ascertainment of screening results, potentially missing a proportion of affected pregnancies and affecting the accuracy of the sensitivity.

Authors' conclusions

Tests involving two or more markers in combination with maternal age are significantly more sensitive than those involving one marker. The value of combining four or more tests or including inhibin have not been proven to show statistically significant improvement. Further study is required to investigate reduced test performance in women aged over 35 and the impact of differential pregnancy loss on study findings.

BACKGROUND

This is one of a series of reviews on antenatal screening for Down's syndrome following a generic protocol (Alldred 2010) - see Published notes for more details.

Target condition being diagnosed

Down's syndrome

Down's syndrome affects approximately 1 in 800 live-born babies (Cuckle 1987a). It results from a person having three, rather than two, copies of chromosome 21 - or the specific area of chromosome 21 implicated in causing Down's syndrome - as a result of trisomy or translocation. If not all cells are affected, the pattern is described as 'mosaic'. Down's syndrome can cause a wide range of physical and mental problems. It is the commonest cause of mental retardation, and is also associated with a number of congenital malformations, notably affecting the heart. There is also an increased risk of cancers such as leukaemia, and numerous metabolic problems including diabetes and thyroid disease. Some of these problems may be life threatening, or lead to considerable ill health, while some individuals with Down's syndrome have only mild problems and can lead a relatively normal life.

There is no cure for Down's syndrome, and antenatal diagnosis allows for preparation for the birth and subsequent care of a baby with Down's syndrome, or for the offer of a termination of pregnancy. Having a baby with Down's syndrome is likely to have a significant impact on family and social life, relationships and parents' work. Special provisions may need to be made for education and care of the child, as well as accommodating the possibility of periods of hospitalisation.

Definitive invasive tests (amniocentesis and chorionic villus sampling (CVS)) exist that allow the diagnosis of Down's syndrome before birth but carry a risk of miscarriage. No test can predict the severity of problems a person with Down's syndrome will have. Noninvasive screening tests based on biochemical analysis of maternal serum or urine, or fetal ultrasound measurements, allow an estimate of the risk of a pregnancy being affected and provide parents with information to enable them to make choices about definitive testing. Such screening tests are used during the first and second trimester of pregnancy.

Initially, screening was determined solely by using maternal age to classify a pregnancy as high or low risk for trisomy 21, as it was known that older women had a higher chance of carrying a baby with Down's syndrome (Penrose 1933).

Further advances in screening were made in the early 1980s, when Merkatz et al investigated the possibility that low maternal serum alpha-fetoprotein (AFP), obtained from maternal blood in the second trimester of pregnancy could be associated with chromosomal abnormalities in the fetus. Their retrospective case-control study showed a statistically significant relationship between fetal trisomy, such as Down's syndrome, and lowered maternal serum AFP (Merkatz 1984). This was further explored by Cuckle et al in a larger retrospective trial using data collected as part of a neural tube defect (NTD) screening project (Cuckle 1984). This work was followed by calculation of risk estimates using maternal serum AFP values and maternal age, which ultimately led to the introduction of the two screening parameters in combination (Alfirevic 2004).

In 1987 in a small case-control study of women carrying fetuses with known chromosomal abnormalities, Bogart and colleagues investigated maternal serum levels of human chorionic gonadotrophin (hCG) as a possible screening tool for chromosomal abnormalities in the second trimester (Bogart 1987). This followed the observations that low hCG levels were associated with miscarriages, which are commonly associated with fetal chromosomal abnormalities. They concluded that high hCG levels were associated with Down's syndrome and because hCG levels plateau at 18-24 weeks, that this would be the most appropriate time for screening. Later work suggested that the ß sub-unit of hCG was a more effective marker than total hCG (Macri 1990; Macri 1993).

Second trimester unconjugated oestriol (uE3), produced by the fetal adrenals and the placenta, was also evaluated as a potential screening marker. In another retrospective case-control study, uE3 was shown to be lower in Down's syndrome pregnancies compared with unaffected pregnancies. When used in combination with AFP and maternal age, it appeared to identify more pregnancies affected by Down's syndrome than AFP and age alone (Canick 1988).

Further work suggested that all three serum markers (AFP, hCG and uE3) showed even higher detection rates when combined with maternal age (Wald 1988a; Wald 1988b) and appeared to be a cost-effective screening strategy (Wald 1992a).

Two other serum markers, produced by the placenta, have been linked with Down's syndrome, namely pregnancy-associated plasma protein A or PAPP-A, and Inhibin A. PAPP-A has been shown to be reduced in the first trimester of Down's syndrome pregnancies, with its most marked reduction in the early first trimester (Bersinger 1995). Inhibin A is high in the second trimester in pregnancies affected by Down's syndrome (Cuckle 1995; Wallace 1995). There are some issues concerning the biological stability and hence reliability of this marker, and the effect this will have on individual risk.

Antenatal screening is used for several reasons (Alfirevic 2004), but the most important is to enable parental choice regarding pregnancy management and outcome. Before a woman and her partner opt to have a screening test, they need to be fully informed about the risks, benefits and possible consequences of such a test. This includes the choices they may have to face should the result show that the woman has a high risk of carrying a baby with Down's syndrome and implications of both false positive and false negative screening tests. They need to be informed of the risk of a miscarriage due to invasive diagnostic testing, and the possibility that a miscarried fetus may be chromosomally normal. If, follow-

ing invasive diagnostic testing, the fetus is shown to have Down's syndrome, further decisions need to be made about continuation or termination of the pregnancy, the possibility of adoption and finally, preparation for parenthood. Equally, if a woman has a test that shows she is at a low risk of carrying a fetus with Down's syndrome, it does not necessarily mean that the baby will be born with a normal chromosomal make up. This possibility can only be excluded by an invasive diagnostic test (Alfirevic 2003).The decisions that may be faced by expectant parents inevitably engender a high level of anxiety at all stages of the screening process, and the outcomes of screening can be associated with considerable physical and psychological morbidity.

Index test(s)

This review examines serum screening tests used in the second trimester of pregnancy (14 to 24 weeks gestation) comprised of the following individual markers; Alpha feto-protein (AFP), unconjugated oestriol (uE3), total human chorionic gonadotropin (total hCG), free ß human chorionic gonadotropin (free β hCG), free alpha human chorionic gonadotropin (free α hCG), Inhibin A, SP2, CA125, troponin, pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PGF), and proform of eosinophil major basic protein (ProMBP). These markers can be used individually, in combination with age, and can also be used in combination with each other. The risks are calculated by comparing a woman's test result for each marker with values for an unaffected population, and multiplying this with her age-related risk. Where several markers are combined, risks are computed using risk equations (often implemented in commercial software) that take into account the correlational relationships between the different markers and marker distributions in affected and unaffected populations.

Alternative test(s)

Down's syndrome can be detected during pregnancy with invasive diagnostic tests such as amniocentesis or CVS, with or without prior screening. These tests are considered to be reference tests rather than index or screening tests. The ability to determine fetal chromosomal make up (also known as a karyotype) from amniotic fluid samples was demonstrated in 1966 by Steele and Breg (Steele 1966), and the first antenatal diagnosis of Down's syndrome was made in 1968 (Vaklenti 1968). Amniocentesis is an invasive procedure which involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks gestation. Chorionic Villus Sampling involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks gestation. Amniocentesis and CVS are both methods of obtaining fetal chromosomes material which are then used to diagnose Down's syndrome. Both tests use ultrasound scans to guide placement of the needle. Amniocentesis carries a risk of miscarriage in the order of 1%; transabdominal CVS may carry a similar risk (Alfirevic 2003).
There are many different screening tests which are available and offered which will be the subject of additional Cochrane reviews (currently in preparation) and there are other reviews looking at this area. Tests to be assessed in Cochrane reviews include first trimester serum tests; urine tests; first trimester ultrasound markers; tests that involve combine serum and ultrasound markers; and tests that combine markers from the first trimester with markers from the second trimester. Second trimester ultrasound markers have been assessed in a previous systematic review (Smith-Bindman 2001).

Rationale

This is one of a suite of Cochrane reviews, the aim of which is to identify all screening tests for Down's syndrome used in clinical practice, or evaluated in the research setting, in order to try to identify the most accurate test(s) available, and to provide clinicians, policy-makers and women with robust and balanced evidence on which to base decisions about interpreting test results and implementing screening policies to triage the use of invasive diagnostic testing. The full set of reviews is described in the generic protocol (Alldred 2010).
The topic has been split into several different reviews to allow for greater ease of reading and greater accessibility of data, and also to allow the reader to focus on separate groups of tests, for example, first trimester serum tests alone, first trimester ultrasound alone, first trimester serum and ultrasound, second trimester serum alone, first and second trimester serum, combinations of serum and ultrasound markers and urine markers alone. An overview review will compare the best tests, focusing on commonly used strategies, from each of these groups to give an comparative results between the best tests in the different categories. This review is written with the global perspective in mind, rather than to conform with any specific local or national policy, as not all tests will be available in all areas where screening for Down's syndrome is carried out.
A systematic review of second trimester ultrasound markers in the detection of Down's syndrome fetuses was published in 2001 which concluded that nuchal fold thickening may be useful in detecting Down's syndrome, but that it was not sensitive enough to use as a screening test. The review concluded that the other second trimester ultrasound markers did not usefully distinguish between Down's syndrome and pregnancies without Down's syndrome (Smith-Bindman 2001). There has yet to be a systematic review and meta-analysis of the observed data on serum, urine and first trimester ultrasound markers, in order to draw rigorous and robust conclusions about the diagnostic accuracy of available Down's syndrome screening tests.

OBJECTIVES

The aim of this review was to estimate and compare the accuracy of second trimester serum markers for the detection of Down's syndrome in the antenatal period, both as individual markers and as combinations of markers. Accuracy is described by the proportion of fetuses with Down's syndrome detected by screening before birth (sensitivity or detection rate) and the proportion of women with a low risk (normal) screening test result who subsequently had a baby unaffected by Down's syndrome (specificity). We grouped our analyses to focus on investigating the value of adding increasing numbers of markers (comparing single, dual, triple and quadruple tests), and of including Inhibin A, the most recent routine addition to serum marker combinations.

Investigation of sources of heterogeneity

We investigated whether a uniform screening test is suitable for all women, or whether different screening methods are more applicable to different groups, defined by advanced maternal age, ethnic groups and aspects of the pregnancy and medical history such as multiple pregnancy, diabetes and family history of Down's syndrome. We also considered whether there existed evidence of overestimation of test accuracy in studies evaluating risk equations in the derivation sample rather than in a separate validation sample.

METHODS

Criteria for considering studies for this review

Types of studies

We included studies in which all women from a given population had one or more index test(s) compared to a reference standard. Both consecutive series and diagnostic case-control study designs were included. Randomised trials where individuals were randomised to different screening strategies and all verified using a reference standard were also eligible for inclusion. Studies in which test strategies were compared head-to-head either in the same women, or between randomised groups were identified for inclusion in separate comparisons of test strategies. Studies were excluded if they included less than five Down's syndrome cases, or > 20% of participants were not followed up.

Participants

Pregnant women at between 14 and less than 24 weeks gestation confirmed by ultrasound, who had not undergone previous testing for Down's syndrome in their pregnancy were eligible. Studies were included if the pregnant women were unselected, or if they represented groups with increased risk of Down's syndrome, or difficulty with conventional screening tests including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

Index tests

The following index tests were examined; Alpha feto-protein (AFP), unconjugated oestriol (uE3), total human chorionic gonadotropin (total hCG), free ß human chorionic gonadotropin (free β hCG), free alpha human chorionic gonadotropin (free α hCG), Inhibin A, SP2, CA125, Troponin, pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PGF) and proform of eosinophil major basic protein (ProMBP), and combinations of these markers combined with maternal age. Combinations without maternal age were not analysed, however, information on such test combinations is provided.

We looked at comparisons of tests in isolation and in various combinations. These included single (one marker), double (two markers), triple (three markers), quadruple (four markers) and quintuple (five markers) test strategies, all maternal age-adjusted. We also looked at combinations that included Inhibin A as this has been the most recently routinely introduced marker.

Where tests were used in comparison, we looked at the performance of test comparisons according to predicted probabilities computed using risk equations and dichotomised into high risk and low risk.

Target conditions

Down's syndrome in the fetus due to trisomy, translocation or mosaicism.

Reference standards

We considered several reference standards, involving chromosomal verification and postnatal macroscopic inspection.

Amniocentesis and CVS are invasive chromosomal verification tests undertaken during pregnancy. They are highly accurate, but the process carries a 1% miscarriage rate, and therefore they are only used in pregnancies considered to be at high risk of Down's syndrome, or on the mother's request. All other types of testing (postnatal examination, postnatal karyotyping, birth registers and Down's syndrome registers) are based on information available at the end of pregnancy. The greatest concern is not their accuracy, but the loss of the pregnancy to miscarriage between the serum test and the reference standard. Miscarriage with cytogenetic testing of the fetus is included in the reference standard where available.

We anticipated that older studies, and studies undertaken in older women are more likely to have used invasive chromosomal verification tests in all women.

Studies undertaken in younger women and more recent studies were likely to use differential verification as they often only used prenatal karyotypic testing on fetuses considered screen positive/high risk according to the screening test; the reference standard for most unaffected infants being observing a phenotypically normal baby. Although the accuracy of this combined reference standard is considered high, it is methodologically a weaker approach as pregnancies that miscarry between the index test and birth are likely to be lost from the analysis, and miscarriage is more likely to occur in Down's than normal pregnancies. We investigated the impact of the likely missing false negative results in sensitivity analyses.

Search methods for identification of studies

Electronic searches

We applied a sensitive search strategy to search the following databases. We used one generic search strategy to identify studies for all reviews in this series.

Databases searched included;

- MEDLINE via OVID (1980 to 18 May 2007)
- EMBASE via Dialog Datastar (1980 to 18 May 2007)
- BIOSIS via EDINA (1985 to 18 May 2007)
- CINAHL via OVID (1982 to 18 May 2007)
- The Database of Abstracts of Reviews of Effectiveness (*The Cochrane Library* 2007, Issue 1)
- MEDION
- The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine (www.ifcc.org/)
- The National Research Register (May 2007)
- Health Services Research Projects in Progress database (HSRPROJ)

The search strategy combined three sets of search terms (*see:* Appendix 1). The first set was made up of named tests, general terms used for screening/diagnostic tests and statistical terms. Note that the statistical terms were used to increase sensitivity and were not used as a methodological filter to increase specificity. The second set was made up of terms that encompass Down's syndrome and the third set made up of terms to limit the testing to pregnant women. All terms within each set were combined with the Boolean operator OR and then the three sets were combined using AND. The terms used were a combination of subject headings and free text terms. The search strategy was adapted to suit each database searched.

We attempted to identify cumulative papers which reported data from the same data set, and contacted authors to obtain clarification of the overlap between data presented in these papers, in order to prevent data from the same women being analysed more than once.

Searching other resources

In addition, we examined references cited in studies identified as being potentially relevant, and those cited by previous reviews. We contacted authors of studies where further information was required. We did not apply a diagnostic test filter, and we did not apply language restrictions to the search.

We carried out forward citation searching of relevant items, using the search strategy in ISI citation indices, Google scholar and PubMed 'related articles'.

Data collection and analysis

Selection of studies

Two review authors screened the titles and abstracts (where available) of all studies identified by the search strategy. Full text versions of studies identified as being potentially relevant were obtained and independently assessed by two review authors for inclusion, using a study eligibility screening pro forma according to the pre-specified inclusion criteria. Any disagreement between the two authors was settled by consensus, or where necessary, by a third party.

Data extraction and management

A data extraction form was developed and piloted using a subset of 20 identified studies.Two review authors independently extracted data, and where disagreement or uncertainty existed, a third author validated the information extracted.

Data on each marker were extracted as binary test positive/test negative results for Down's and non-Down's pregnancies, with a high-risk result - as defined by each individual study - being regarded as test positive (suggestive or diagnostic of Down's syndrome), and a low risk result being regarded as test negative (suggestive of absence of Down's Syndrome). Where results were reported at several thresholds data were extracted at each.

Note was made of those in special groups which posed either increased risk of Down's syndrome or difficulty with conventional screening tests including maternal age greater than 35 years old, multiple pregnancy, diabetes mellitus and family history of Down's syndrome.

Assessment of methodological quality

We used a modified version of the QUADAS tool (Whiting 2003), a quality assessment tool for use in systematic reviews of diagnostic accuracy studies, to assess the methodological quality of included studies. We anticipated that a key methodological issue would be

the potential for differential verification bias arising from the use of different invasive tests (amniocentesis versus CVS). Further bias was likely to arise from follow-up for the reference standard according to index test results. Finally, we expected to find bias as a result of miscarriage, where karyotyping was not performed, as this could potentially influence the false negative and true negative rates. We chose to code this issue as originating from differential verification in the QUADAS tool: we are aware that it could also be coded under delay in obtaining the reference standard, and reporting of withdrawals. We omitted the QUADAS item assessing quality according to length of time between index and reference tests, as Down's syndrome is either present or absent rather than a condition that evolves and resolves, and disregarding the differential reference standard issue any length of delay is acceptable. Two review authors assessed each included study separately. Any disagreement between the two review authors was settled by consensus, or where necessary, by a third party. Each item in the QUADAS tool was be marked as 'yes', 'no' or 'unclear', and scores are presented graphically and in tables. We have not used a summary quality score.

QUADAS criteria included the following ten questions:

1. Was the spectrum of women representative of the women who will receive the test in practice? (Criteria met if the sample was selected from a wide range of childbearing ages, or selected from a specified 'high-risk' group such as over 35s, family history of Down's Syndrome, multiple pregnancy or diabetes mellitus, provided all affected and unaffected fetuses were included that could be tested at the time point when the screening test would be applied; criteria not met if the sample taken from a select or unrepresentative group of women (i.e. private practice), was an atypical screening population or recruited at a later time point when selection could be affected by selective fetal loss.)
2. Is the reference standard likely to correctly classify the target condition? (amniocentesis, CVS, postnatal karyotyping, miscarriage with cytogenetic testing of the fetus, a phenotypically normal baby or birth registers are all regarded as meeting this criteria.)
3. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?
4. Did women receive the same reference standard regardless of the index test result?
5. Was the reference standard independent of the index test result (i.e. the index test did not form part of the reference standard)?
6. Were the index test results interpreted without knowledge of the results of the reference standard?
7. Were the reference standard results interpreted without knowledge of the results of the index test?
8. Were the same clinical data (i.e. maternal age and weight, ethnic origin, gestational age) available when test results were interpreted as would be available when the test is used in practice?
9. Were uninterpretable/intermediate test results reported?
10. Were withdrawals from the study explained?

Statistical analysis and data synthesis

Results of all tests evaluated across all common risk thresholds for screen positive result were initially examined using forest plots and plotting study results in ROC space. Test strategies were selected for further investigation if they were evaluated in four or more studies or, if there were three or fewer studies, but the individual study results indicated performance likely to be superior to a sensitivity of 70% and specificity of 90%.

Estimation of average sensitivity and specificity

The analysis for each test strategy was undertaken first restricting to studies which reported a common threshold to estimate average sensitivity and specificity rates for each test at each threshold. Although data on all thresholds were extracted, we present only key common thresholds close to risks of 1:384, 1:250 and the 5% FPR, unless other thresholds were more commonly reported. The thresholds chosen are those most commonly quoted in studies. There have been recent moves to reduced the FPR from 5% to 3% in some countries, in order to reduce the number of invasive tests performed, but this has only recently become commonplace in the literature. Where combinations of tests were used in a risk score, we extracted the result for the test combination using the risk score and not the individual components that made up the test.

Meta-analyses were undertaken using hierarchical summary ROC curve methods which included estimation of random effects in accuracy and threshold parameters when there were four or more studies. Otherwise, average sensitivity and specificity values were computed using separate univariate random-effects meta-analysis, averaging the logit sensitivity and logit specificity as inadequate data would be available to estimate all parameters in the HSROC model. It is common in this field for studies to report sensitivity for a fixed specificity (usually a 5% false positive rate). This removes the requirement to use a bivariate meta-analytical method for analysis at this threshold as all specificity rates are the same, hence logit sensitivity values were also pooled using a univariate random-effects method. All analyses were undertaken using the METADAS macro for SAS.

Comparisons between tests

Comparisons between tests were first made utilising all available studies, selecting one threshold from each study to estimate a summary ROC curve without restricting to a common threshold. The threshold was chosen for each study according to the following order of preference a) the risk threshold closest to 1 in 250; b) a multiples of the median (MoM) or presence/absence threshold; c) the performance closest to a 5% false positive rate (FPR) or 95th percentile. The 5% false positive rate was chosen as a cut-off point

as this is the cut-off most commonly reported in the literature. The analysis including data from all studies fitted a single HSROC model including the 12 selected test strategies, including two indicator terms for each test to allow for differences in accuracy and threshold. As there was limited evidence of differing SROC curve shapes between tests, a single SROC shape parameter was included in the model such that the fitted SROC curves did not cross. An estimate of the sensitivity of each test for a 5% false positive rate was derived from the summary ROC curve, and associated confidence interval obtained using the delta method.

Direct comparisons between tests were based on results of very few studies, and were analysed using a fixed-effect HSROC model with symmetrical underlying SROC curves as there were inadequate data to estimate between study heterogeneity in accuracy and threshold or asymmetric shape. A separate model was used to make each pair wise comparison. Comparisons between tests were assessed by the significance of differences in accuracy, and expressed as relative diagnostic odds ratios reported with 95% confidence intervals. As studies rarely reported data cross-classified by both tests for Down's and normal pregnancies, the analytical method did not take full account of the pairing of test results, but the restriction to direct head-to-head comparisons should have removed the potential confounding of test comparisons with other features of the studies. The strength of evidence for differences in performance of test strategies relied on evidence from both the direct and indirect comparisons.

Investigations of heterogeneity

Investigations of heterogeneity were only undertaken when there were 10 or more studies available for a test. Subgroup analyses were undertaken by adding covariates for differences in accuracy and threshold to the HSROC meta-analytical model.

Sensitivity analyses

In many of the included studies, mothers with pregnancies identified as high risk for Down's syndrome by the serum testing were offered immediate definitive testing by amniocentesis, whereas, the remainder were assessed for Down's syndrome by inspection at birth. Such delayed and differential verification will introduce bias most likely through there being greater loss to miscarriage in the Down's syndrome pregnancies that were not detected by the serum testing (the false negative diagnoses). Testing and detection of miscarriages is impractical in many situations, and no clear data are available on the magnitude of these miscarriage rates.

To account for the possible bias introduced by such a mechanism, we undertook sensitivity analyses where we inflated the false negative count in studies where delayed verification in test negatives occurred (Mol 1999). This was undertaken for two analyses - the main comparison of the 12 key test combinations, and the investigation of the impact of maternal age on test sensitivity. For both analyses, we increased the percentage of false negatives in each study incrementally from 10% to 50%, the final value representing a scenario where a third more Down's pregnancies than normal pregnancies were likely to miscarry, thought to be higher than the likely value.

RESULTS

Results of the search

The search for the whole suite of reviews identified a total of 13,403 papers, once the results from each bibliographic database were combined and duplicates were removed. After screening out obviously inappropriate papers based on their title and abstract 904 papers remained and copies were obtained for formal assessment of eligibility. From these a total of 239 studies were deemed eligible and included in the suite of reviews. A total of 59 studies (reported in 72 publications) were included in this review of second trimester serum screening, involving 341,261 pregnancies including 1994 Down's syndrome pregnancies.

A total of 54 different test strategies combinations were evaluated in the 59 studies. These tests are produced from combinations of 12 different tests, with and without maternal age; AFP, uE3, total hCG, free β hCG, free α hCG, Inhibin A, SP2, CA125, Troponin, PAPP-A, PGF and ProMBP. Strategies evaluated included three quintuple tests, five quadruple, 12 triple, 14 doubles and nine single tests in combination with age; the remaining 11 assessed single tests without age. Forty-two of the 59 studies only evaluated the performance of a single second trimester serum test or test strategy, seven compared two, a further seven compared between three and six, one compared 11 (Bartels 1994a), one compared 20 (Wald 2003a) and one compared 21 (Forest 1995).

The following combinations evaluated included four or more studies:

Quadruple tests

- Total hCG, uE3, AFP, Inhibin A and maternal age (five studies, 38,342 women, including 232 Down's syndrome pregnancies)

Triple tests

- Total hCG, uE3, AFP and maternal age (24 studies, 89,047 women, including 648 Down's syndrome pregnancies)
- Free β hCG, AFP and uE3 and maternal age (seven studies, 10,541 women, including 249 Down's syndrome pregnancies)

Double Tests

- Total hCG, AFP and maternal age (15 studies, 133,783 women, including 473 Down's syndrome pregnancies)
- Free β hCG, AFP and maternal age (12 studies, 45,597 women including 341 Down's syndrome pregnancies)

Single tests

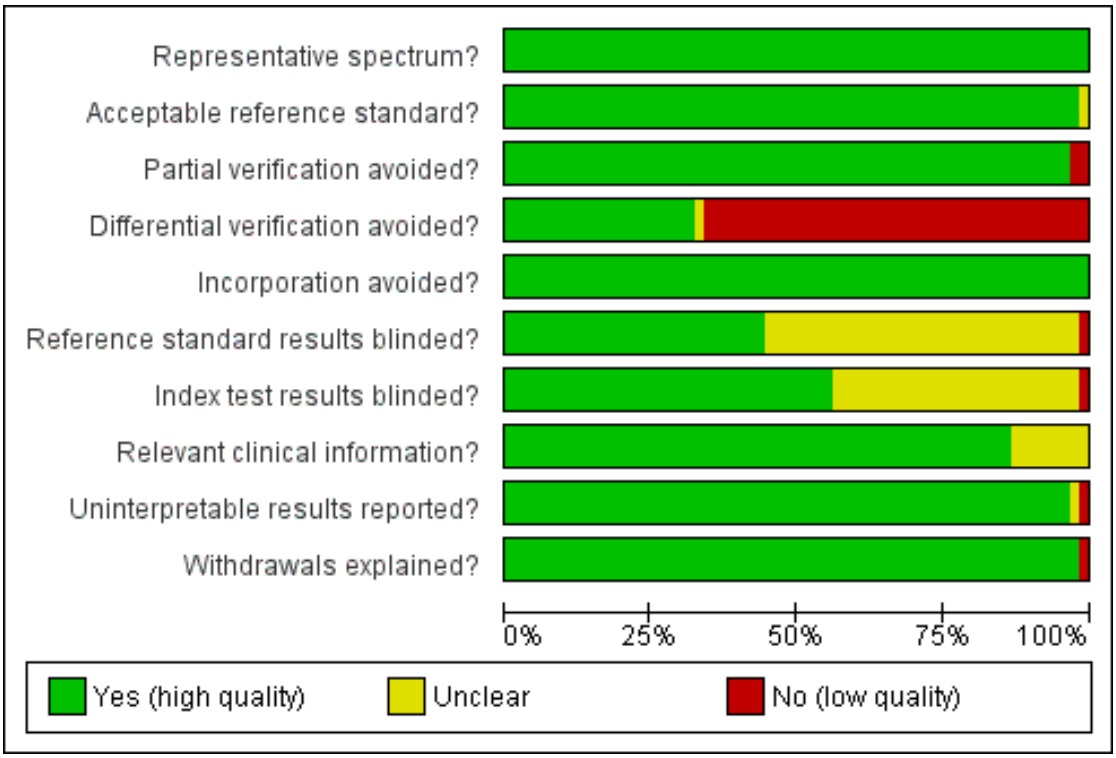
- Total hCG and maternal age (four studies, 57,768 women including 280 Down's syndrome)
- AFP and maternal age (four studies, 13,764 women, including 173 Down's syndrome pregnancies)
- Free β hCG and maternal age (four studies, 14,985 women, including 192 Down's syndrome pregnancies)

Of the remaining test combinations, seven were evaluated in two studies and the remaining 28 in single studies only.

Methodological quality of included studies

Methodological quality of the studies was judged to be high in most categories (Figure 1). Due to the nature of testing for Down's syndrome screening and the potential side effects of invasive testing, differential verification is almost universal in the general screening population, as most women whose screening test result is defined as low risk will have their screening test verified at birth, rather than by invasive diagnosis in the antenatal period. Additionally, it was not always possible to ascertain from the included studies whether or not the results of index tests and reference standards were blinded. It would be difficult to blind clinicians performing invasive diagnostic tests (reference standards) to the index test result, unless all women received the same reference standard, which would not be appropriate in most scenarios. However, any biases secondary to a lack of clinician blinding are likely to be minimal.

Figure 1. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.



Most studies reported 100% follow-up, however, there will inevitably be losses to follow-up due to women moving out of area, for example. Studies usually accounted for these and it is unlikely to have introduced significant bias. There was definitely under ascertainment of miscarriage, and very few papers accounted for miscarriage or performed tissue karyotyping in pregnancies resulting in miscarriage. Some studies attempted to adjust for predicted miscarriage rate and the incidence of Down's syndrome in this specific population, but most did not. We have not attempted to ad-

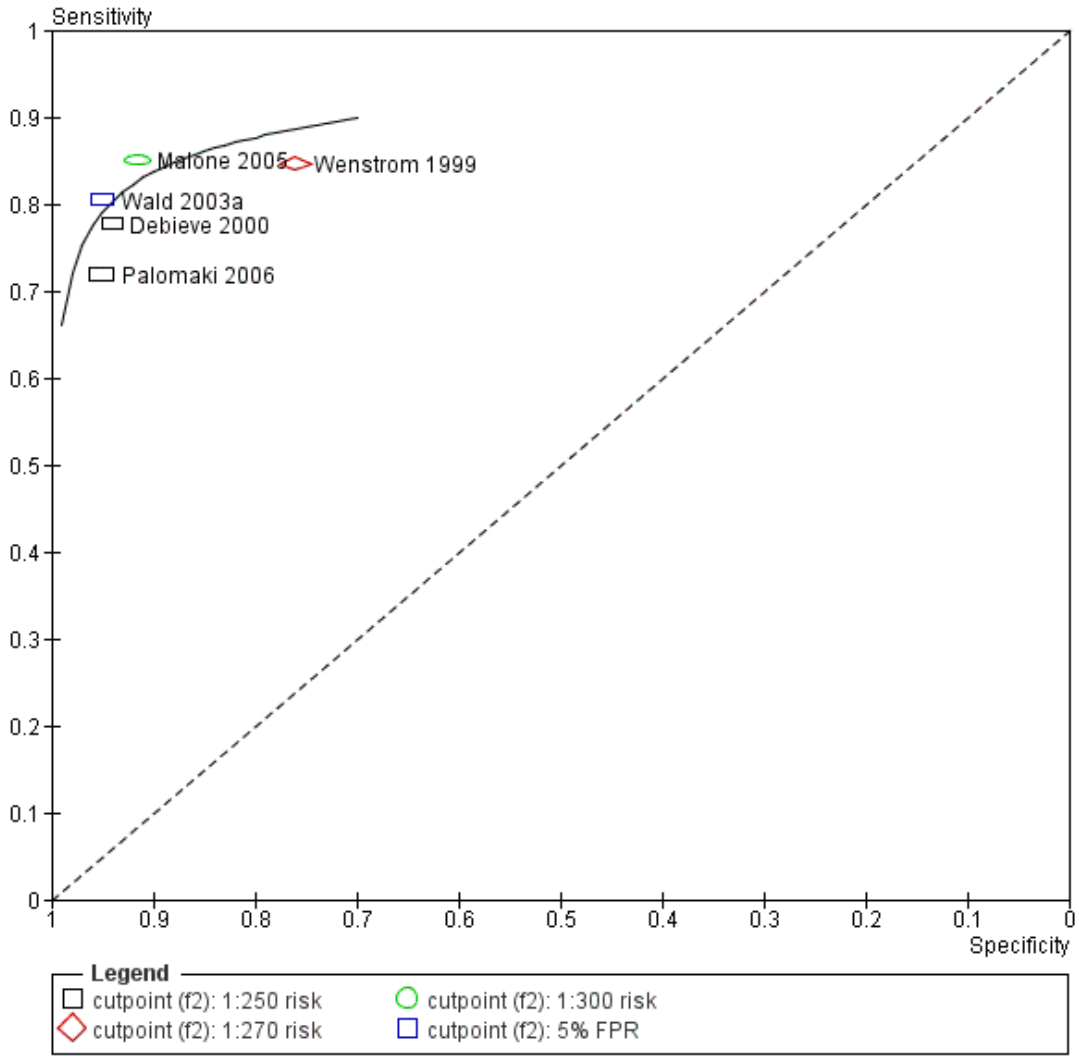
just for expected miscarriage rate in this review but have explored the impact in a sensitivity analysis. This issue has the potential to have more influence with first trimester testing due to a higher miscarriage rate per se in this trimester.

Some studies which provided estimates of risk using multivariable equations used the same data set to evaluate performance of the risk equation as was used to derive the equation. This is often thought to lead to over-estimation of test performance. The impact of inclusion of these studies was investigated in subgroup analyses, reported below.

Findings

1) Total hCG, AFP, uE3, Inhibin A and maternal age (Quadruple test) (Figure 2)

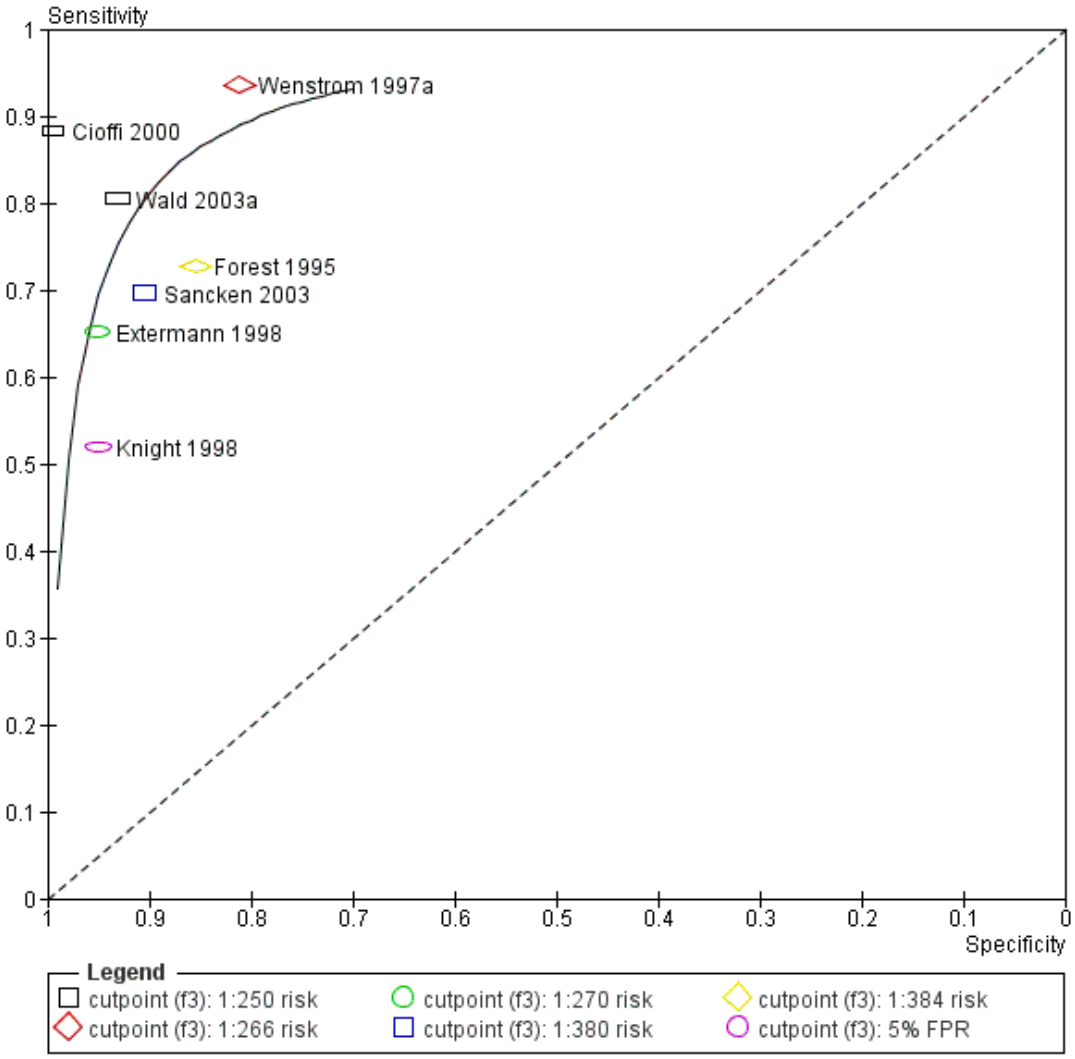
Figure 2. Studies evaluating combination of maternal age, Total hCG, AFP, uE3 and Inhibin showing summary ROC curve



Results for this quadruple test were derived from five studies (Debieve 2000; Malone 2005; Palomaki 2006; Wald 2003a; Wenstrom 1999), and included 38,342 women in whom 150 pregnancies were known to be affected by Down's syndrome. Thirty-five thousand two hundred and thirty-six (95% of total pregnancies) including 87 Down's cases (58%) originated from the FASTER study (Malone 2005). Wald 2003a and Wenstrom 1999 contributed over 1,000 pregnancies each to the data. Studies presented data for cut-points of 5% FPR (Wald 2003a), 1;150 (Debieve 2000; Palomaki 2006; Wenstrom 1999), 1:250 (Debieve 2000; Palomaki 2006), 1:270 (Wenstrom 1999) and 1:300 (Malone 2005). At a cut-point of 5% FPR, Wald estimated a sensitivity of 80.5% (95% confidence interval (CI) 70.3 to 88.4). At a cut-point of 1:250 (two studies), the sensitivity is estimated at 73.9% (95% CI 60.0 to 84.2) and the specificity is 94.8% (CI 92.8 to 96.2); at a cut-point of 1:300 Malone estimated the sensitivity at 85.0% (CI 75.8 to 91.8) and the specificity at 91.5% (CI 91.2 to 91.8).

2) Free βhCG, AFP, uE3 and maternal age (Triple test) (Figure 3)

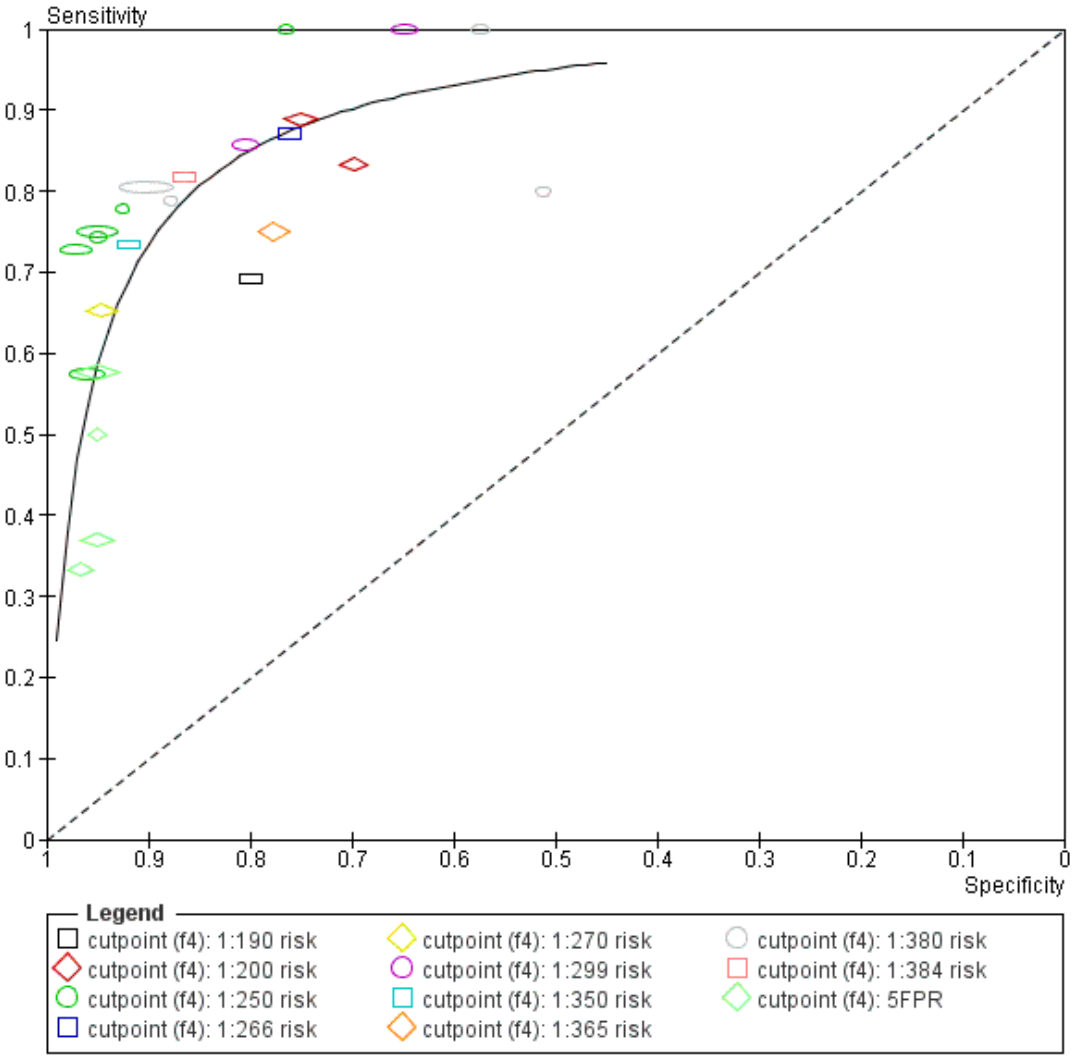
Figure 3. Studies evaluating combination of maternal age, Free ßhCG, AFP and uE3 showing summary ROC curve



Results for this triple test were derived from seven studies (Cioffi 2000; Extermann 1998; Forest 1995; Knight 1998; Sancken 2003; Wald 2003a; Wenstrom 1997a), including 10,541 women, in whom 249 pregnancies were known to be affected by Down's syndrome. Over half of the women were derived from Knight's study. Studies presented data from cut-points of 5% FPR (Knight 1998; Sancken 2003; Wald 2003a), 1:250 (Cioffi 2000; Wald 2003a), 1:384 (Forest 1995) and 1:380 (Extermann 1998). At a cut-point of 5% FPR, the estimated sensitivity was 65.1% (95% CI 46.4 to 80.1). At the cut-point of 1:250, the estimated sensitivity was 81.5% (95% CI 72.5 to 88.1) for an estimated specificity of 97.9% (95% CI 87.7 to 99.7).

3) Total hCG, AFP, uE3 and maternal age (Triple test)(Figure 4)

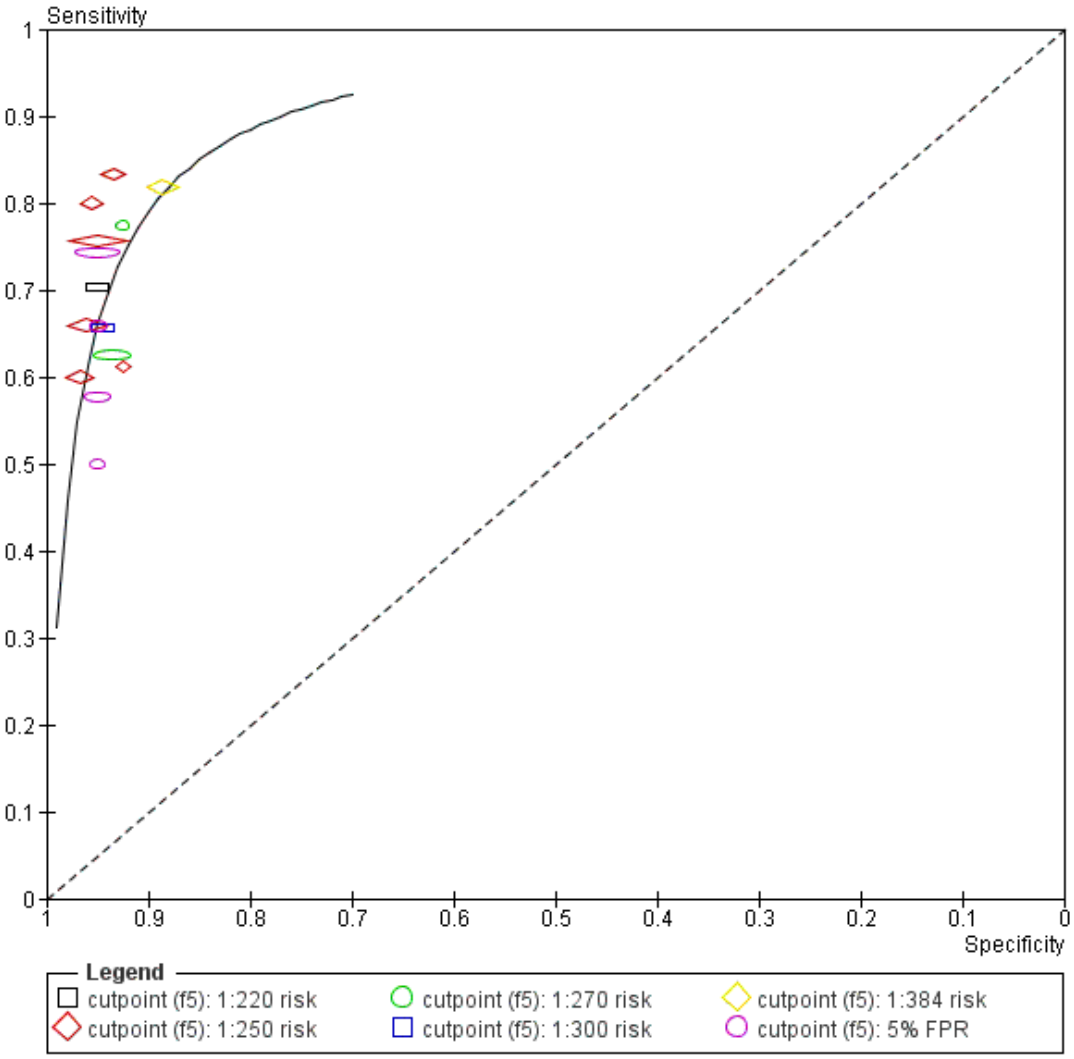
Figure 4. Studies evaluating combination of maternal age, Total hCG, AFP and uE3 showing summary ROC curve



Results for this triple test were derived from 24 studies (Bahado-Singh 1999a; Bahado-Singh 2000; Bartels 1994a (divided into Bartels 1994a and Bartels 1994b); David 1996; Debieve 2000; Extermann 1998; Forest 1995; Haddow 1994; Heyl 1990; Huderer-Duric 2000; Kishida 2000; Knight 1998; Mancini 1991; Perona 1997; Piggott 1994;Rosen 2002; Sancken 2003; Suzumori 1997; Verloes 1995; Wald 2003a Ward 1999; Wenstrom 1997a; Wenstrom 1999) and included 89,047 women, in whom 648 were known to be affected by Down's syndrome. Of the 24 studies, there are four with a sample size of over 5,000 (David 1996; Haddow 1994; Knight 1998; Piggott 1994), two over 10,000 (Verloes 1995; Ward 1999) and one over 20,000 (Perona 1997). Seven studies evaluated sensitivity at 5% FPR (Bahado-Singh 1999a; Bahado-Singh 2000; Bartels 1994a; Haddow 1994; Knight 1998; Sancken 2003; Wald 2003a), five evaluated a cut-point of 1:250 (David 1996; Debieve 2000; Mancini 1991; Piggott 1994; Ward 1999). At a cut-point of 5% FPR, the estimated sensitivity was 53.5% (95% CI 43.0 to 63.7). At the cut-point of 1:250, the estimated sensitivity was 76.9% (95% CI 52.7 to 90.9) for an estimated specificity of 93.6% (95% CI 87.7 to 96.8).

4) Total hCG, AFP and maternal age (Double test) (Figure 5)

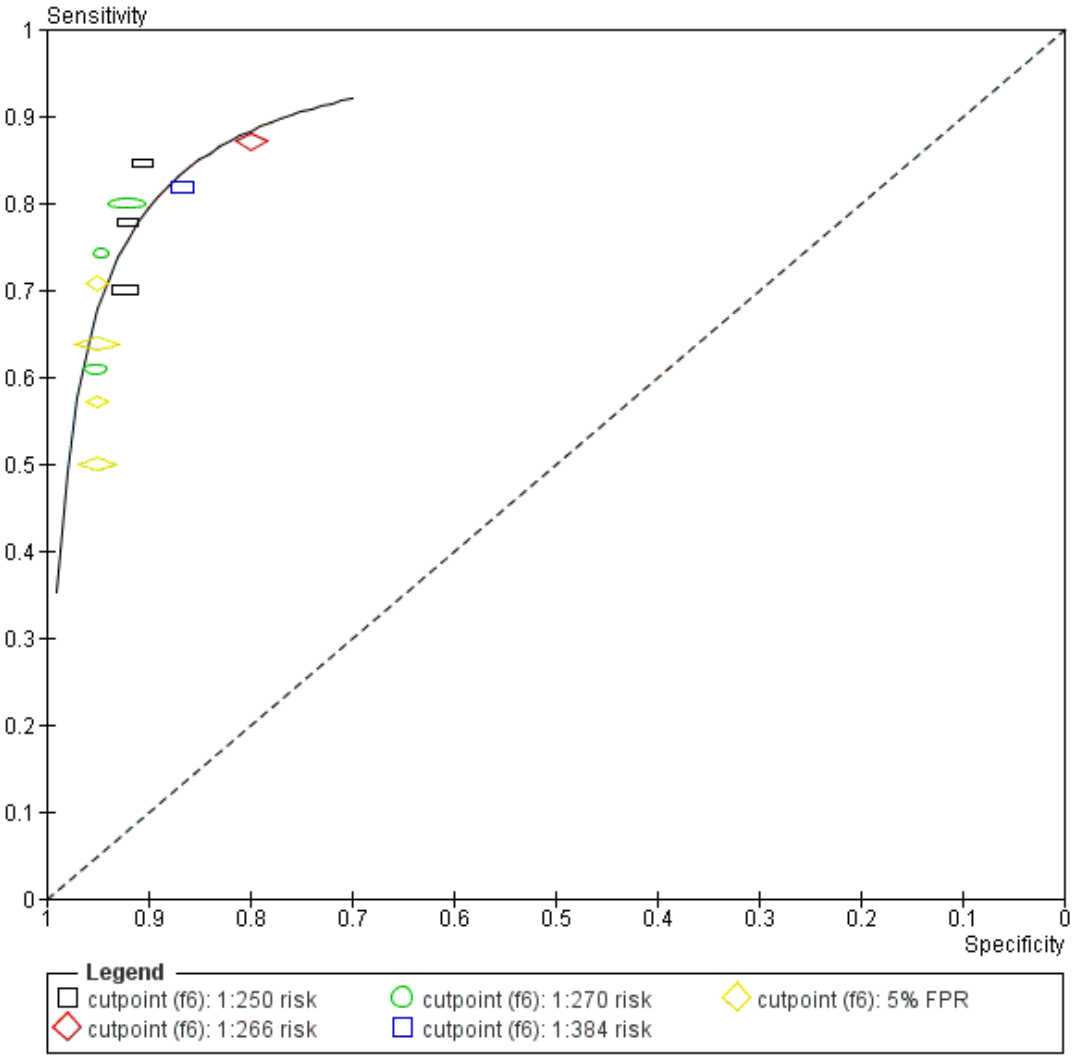
Figure 5. Studies evaluating combination of maternal age, Total hCG and AFP showing summary ROC curve



Results for this double test were derived from 15 studies (Audibert 2001a; Bartels 1994a; Beekhuis 1993; Benattar 1999; Crossley 1994; David 1996; Debieve 2000; Forest 1995; Jou 2000; Knight 1998; Lam 2002; Lemay 1995; Milunsky 1993; Roberts 2000; Wald 2003a) and included 133,783 women, in whom 473 pregnancies were known to be affected by Down's syndrome. Of the 15 studies, four presented data on with sample sizes of greater than 15,000 (Jou 2000; Lam 2002; Lemay 1995; Roberts 2000) and one with greater than 30,000 (Crossley 1994). Four studies gave data for a cut-point of 5% FPR (Bartels 1994a; Knight 1998; Lam 2002; Wald 2003a), six studies gave data for a cut-point of 1:250 (Audibert 2001a; Beekhuis 1993; Benattar 1999; David 1996; Debieve 2000; Roberts 2000). At a cut-point of 5% FPR, the estimated sensitivity was 61.7% (95% CI 53.5 to 69.2), and at the cut-point of 1:250, the estimated sensitivity was 69.9% (95% CI 60.3 to 78.1) for a specificity of 95.3% (95% CI 94.3 to 96.2) .

5) Free β hCG, AFP and maternal age (Double test) (Figure 6)

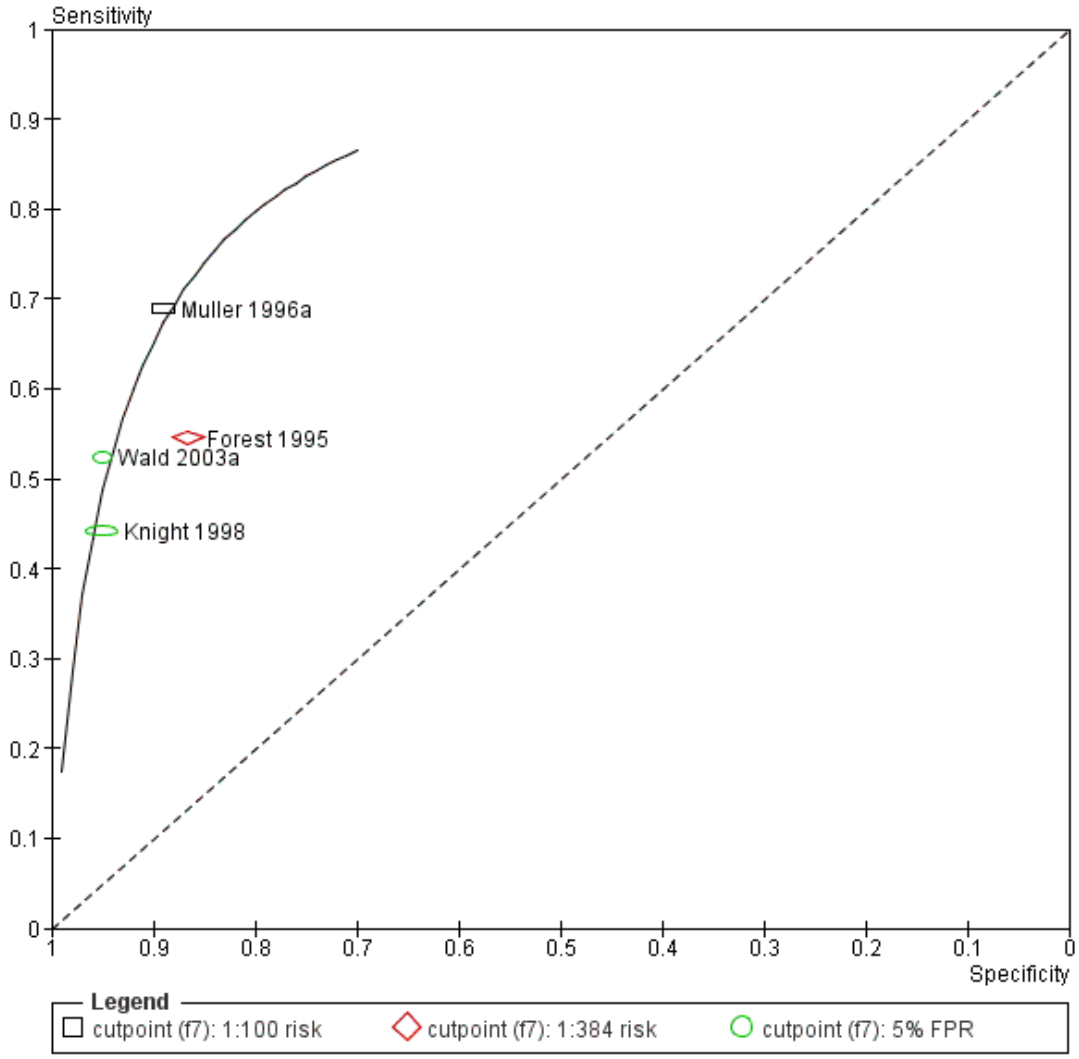
Figure 6. Studies evaluating combination of maternal age, Free ßhCG and AFP showing summary ROC curve



Results for this double test were derived from 12 studies (Anandakumar 1999; Brajenovic 1998; Chao 1999; Extermann 1998; Forest 1995; Hsu 1997a; Kadir 1999; Knight 1998; Milunsky 1993; Rozenberg 2002; Wald 2003a; Wenstrom 1997a) including 45,597 women, of which 341 were affected by Down's syndrome. Of the 12 studies, four presented data on more than 5,000 women (Chao 1999; Hsu 1997a; Knight 1998; Rozenberg 2002). Five studies gave data for a 5% FPR (Anandakumar 1999; Hsu 1997a; Knight 1998; Rozenberg 2002; Wald 2003a) and three for a cut-point of 1:250 (Brajenovic 1998; Kadir 1999; Rozenberg 2002). At a cut-point of 5% FPR, the estimated sensitivity was 61.7% (95% CI 52.7 to 69.9), and at the cut-point of 1:250, the estimated sensitivity was 75.5% (95% CI 60.1 to 86.4) for a specificity of 91.6% (95% CI 90.5 to 92.6) .

6) Total hCG and maternal age (Single test) (Figure 7)

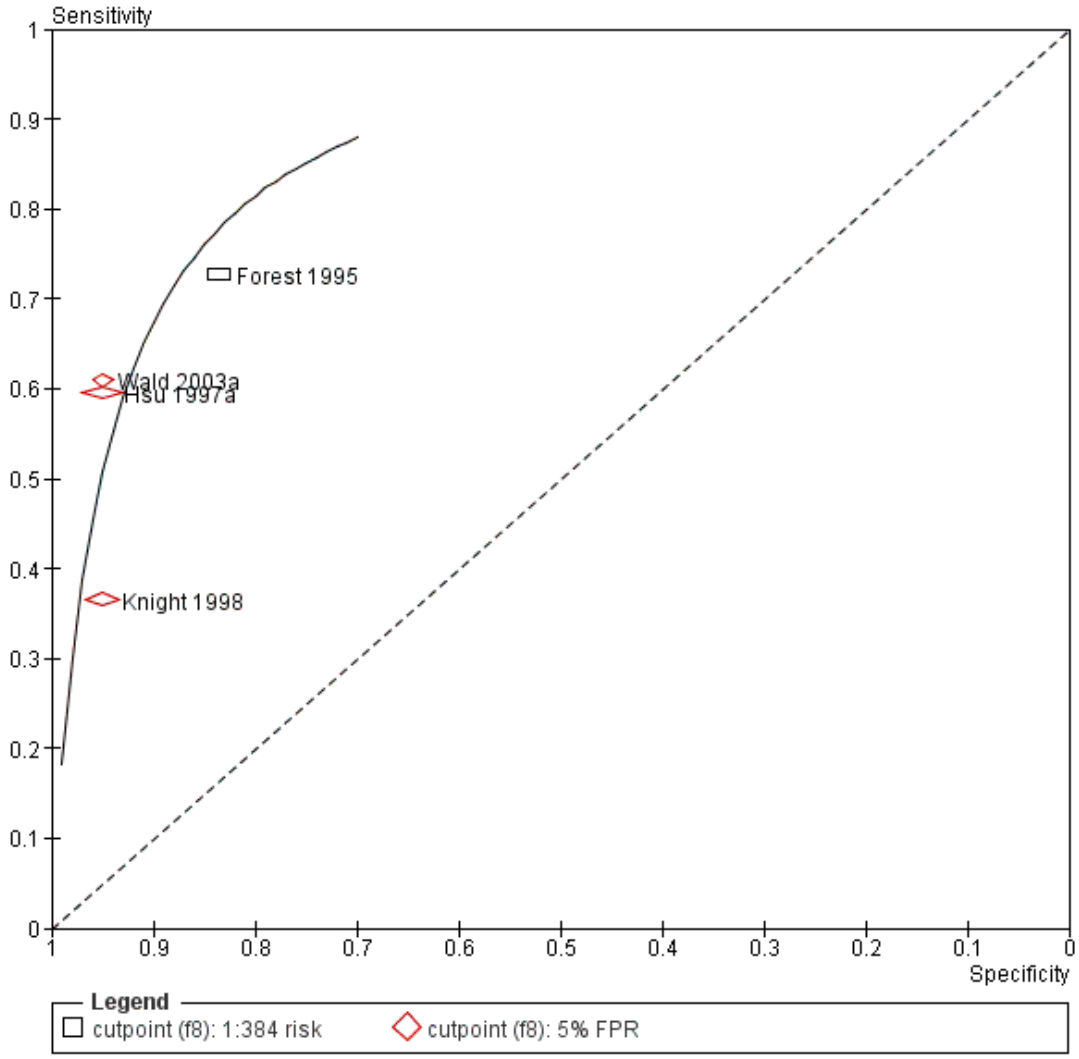
Figure 7. Studies evaluating combination of maternal age and Total hCG showing summary ROC curve



Results for this single test were derived from four studies (Forest 1995; Knight 1998; Muller 1996a; Wald 2003a) including 57,668 pregnancies of which 280 were known to be affected by Down's syndrome. Of the four studies, two presented data on more than 5,000 women (Knight 1998; Muller 1996a). Three studies gave data for a 5% FPR cut-point (Knight 1998; Muller 1996a; Wald 2003a). At this cut-point the sensitivity was estimated at 56.1% (95% CI 41.0 to 70.2). The cut-point for Forest 1995 was 1:384 and is included on the figure.

7) Free β hCG and maternal age (Single test) (Figure 8)

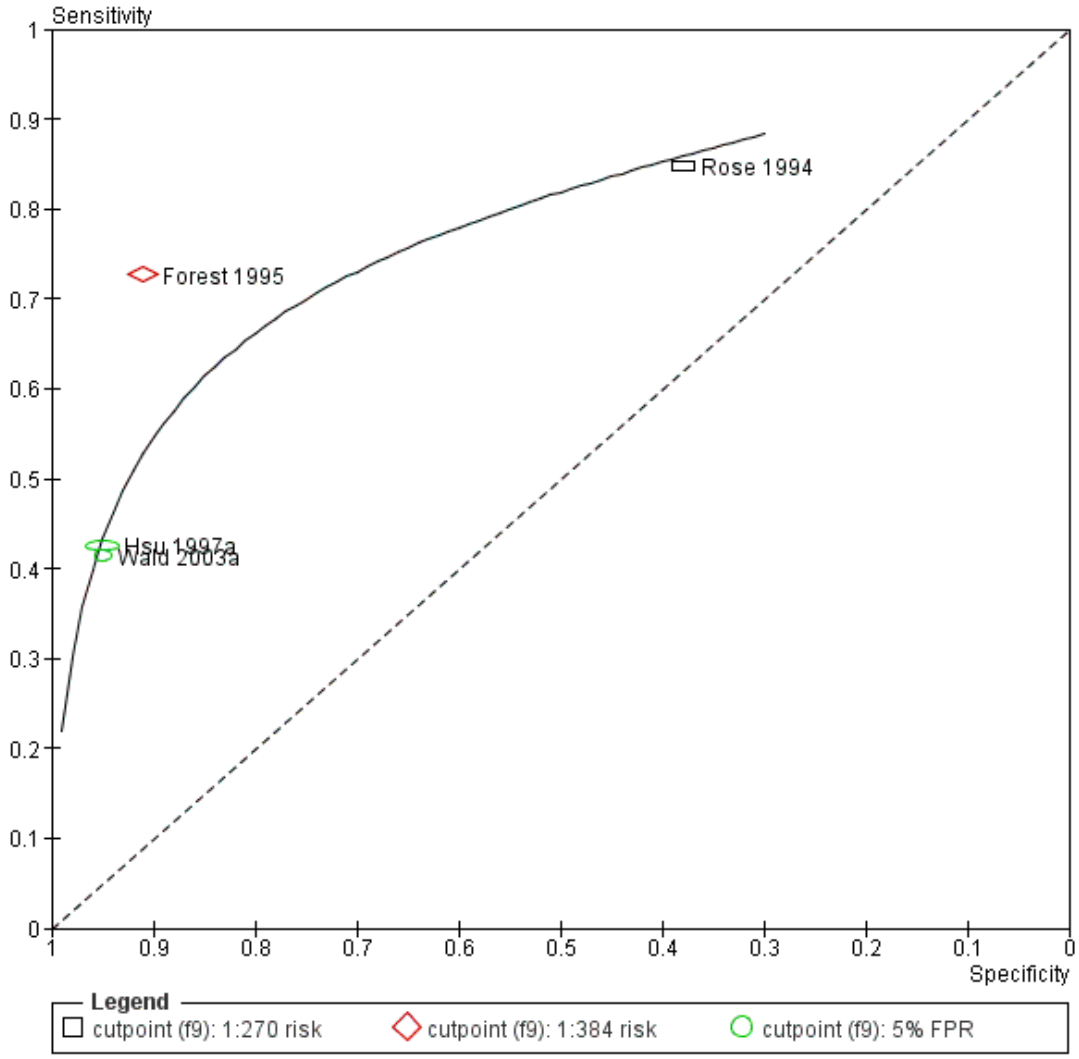
Figure 8. Studies evaluating combination of maternal age and Free ßhCG showing summary ROC curve



Results for this single test were derived from four studies (Forest 1995; Hsu 1997a; Knight 1998; Wald 2003a) including 14,985 pregnancies, of which 192 were known to be affected by Down's syndrome. Of the four studies, Hsu 1997a was the largest, presenting data on more than 9,000 pregnancies.Three studies gave data for a cut-point of 5% FPR (Hsu 1997a; Knight 1998; Wald 2003a). At this cut-point the sensitivity was estimated at 52.6% (95% CI 37.4 to 67.4). The cut-point for Forest 1995 was 1:384 and is included on the figure.

8) AFP and maternal age (Single test) (Figure 9)

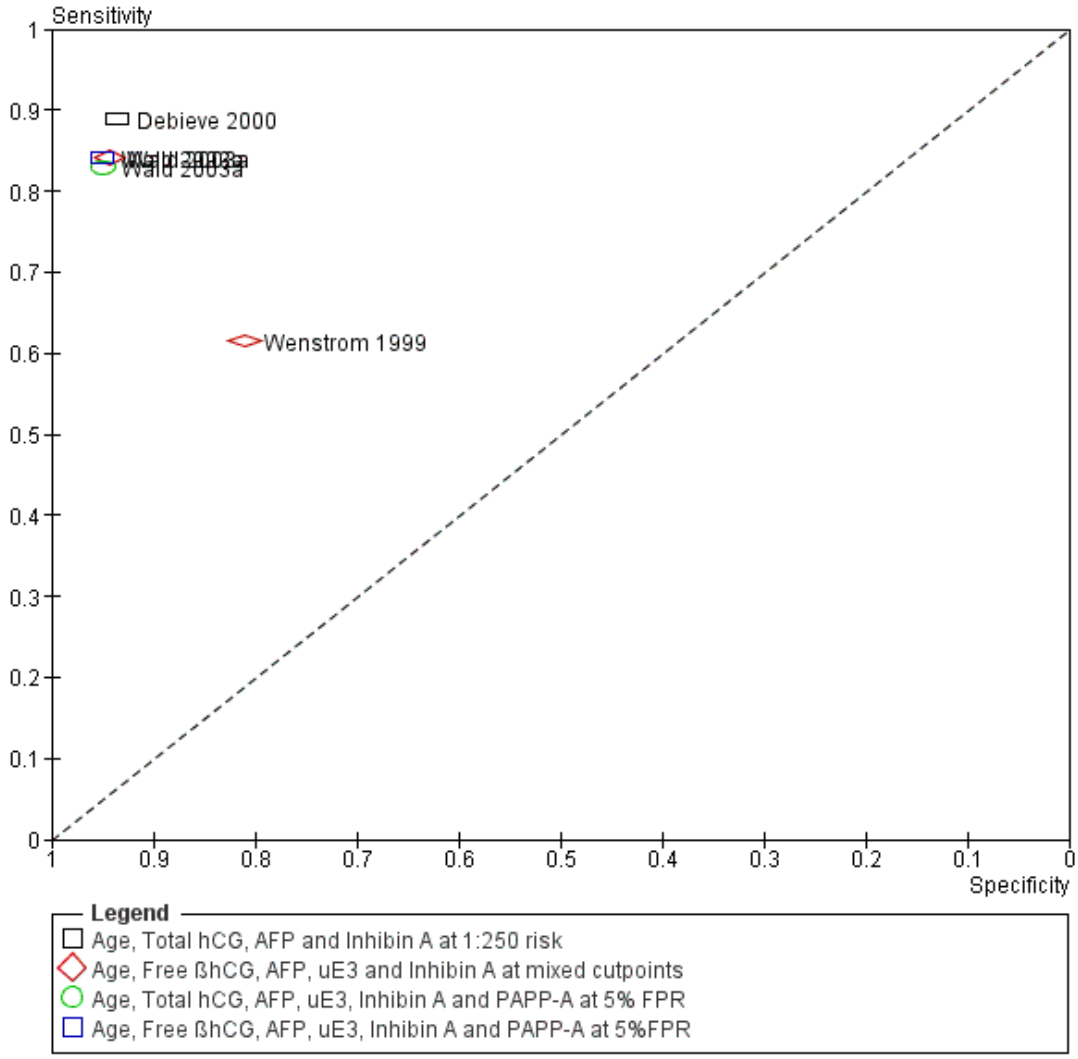
Figure 9. Studies evaluating combination of maternal age and AFP showing summary ROC curve



Results for this single test were derived from four studies of 13,764 pregnancies including 173 Down's syndrome pregnancies (Forest 1995; Hsu 1997a; Rose 1994; Wald 2003a). Of the four studies, (Hsu 1997a) was the largest presenting data on 8,265 (48%) of pregnancies including 47 Down's syndrome pregnancies. Studies presented data for cut-points of 5% FPR (Hsu 1997a; Wald 2003a), 1:270 (Rose 1994) and 1:384 (Forest 1995). Two studies gave data for a cut-off of 5% FPR estimating a sensitivity of 41.9% (95% CI 33.7 to 50.5) .

9) Other test combinations (Figure 10)

Figure 10. Studies of four promising test combinations evaluated in in only one or two studies



Of the 36 test combinations evaluated in one or two studies, only four test combinations demonstrated estimated sensitivities of more than 70% and estimated specificities of more than 90%.

- A **quintuple** test of **total hCG, AFP, uE3, Inhibin A, PAPP-A and maternal age** evaluated in a single study (Wald 2003a) estimated a sensitivity of 82.9% (CI 73.0 to 90.3%) at a cut-point of 5% FPR.
- A **quintuple** test of **free βhCG, AFP, uE3, Inhibin A, PAPP-A and maternal age** evaluated in a single study (Wald 2003a) estimated a sensitivity of 84.1% (CI 74.4 to 91.3%) at a cut-point of 5% FPR.
- A **quadruple** test of **free βhCG, uE3, AFP, Inhibin A and maternal age** evaluated in Wald 2003a estimated a sensitivity of 84.1% (CI 74.4 to 91.3) and specificity of 94.3% (CI 92.6 to 95.6) at a cut-point of 1 in 250. However, a second evaluation in Wenstrom 1999 estimated much lower values.
- A **triple** test of **total hCG, Inhibin A, AFP and maternal age** evaluated in a single study (Debieve 2000) estimated a sensitivity of 88.9% (CI 65.3 to 98.6) for a specificity of 93.5% (CI 89.1 to 96.5) at a cut-point of 1:250.

10) Individual markers

There were data available on 10 individual markers, not combined

with maternal age, the results of which are presented in the forest plots available in the full review report. There was substantial heterogeneity noted in the sensitivities of Inhibin A and SP2 in these studies.

Comparative analysis of the eleven selected test strategies

Formal statistical comparison of the 12 test strategies listed above was made using HSROC meta-analytical models, firstly to quantify the difference in test performance (expressed with 95% confidence intervals), and secondly to assess the strength of evidence of real differences in performance between the strategies. Comparative analysis was undertaken by comparing summary ROC curves estimated by first making pair wise comparisons pooling studies which made compared tests in the same mothers, and then by pooling all available studies for the 12 test strategies listed above. Estimates of the differences in accuracy obtained from the HSROC models are expressed as relative DORs which are not easy to interpret. To provide more accessible estimates of performance, we have also computed the detection rate (sensitivity) for a fixed false positive rate (specificity), a metric which is commonly used in Down's syndrome screening to describe performance. We chose to estimate detection rates at a 5% FPR, in common with much of the literature.

Figure 11 shows point estimates of detection rates for a 5% FPR based on all available data for all 12 test combinations described above, and the confidence intervals at a fixed 5% FPR. For example, the plot shows that for the triple test with a marker combination of free βhCG, AFP, uE3 and maternal age the estimated detection rate at a 5% FPR is 70.1% (95% CI 61.8 to 77.3) based on data from seven studies with 249 affected cases and 10,541 total participants.The test combinations in the Figure are ordered according to decreasing detection rates. The three single test strategies (AFP with maternal age; total hCG with maternal age and free βhCG with maternal age) have the worst performance, whereas, the five triple, quadruple and quintuple strategies containing inhibin have the highest performance. In between lie the standard triple tests (total hCG, AFP, uE3 and maternal age; free-βhCG, AFP, uE3 and maternal age) and double tests (total hCG, AFP and maternal age; free βhCG, AFP and maternal age). However, it is noted that the confidence intervals on these estimates are wide (particularly for the inhibin-based combinations) and overlap for the first six strategies, suggesting that any of the differences observed may be explicable by chance.

Figure 11. Detection rates (% sensitivity) at a false positive rate of 5% for the 12 selected test combinations (estimates from summary ROC curves)

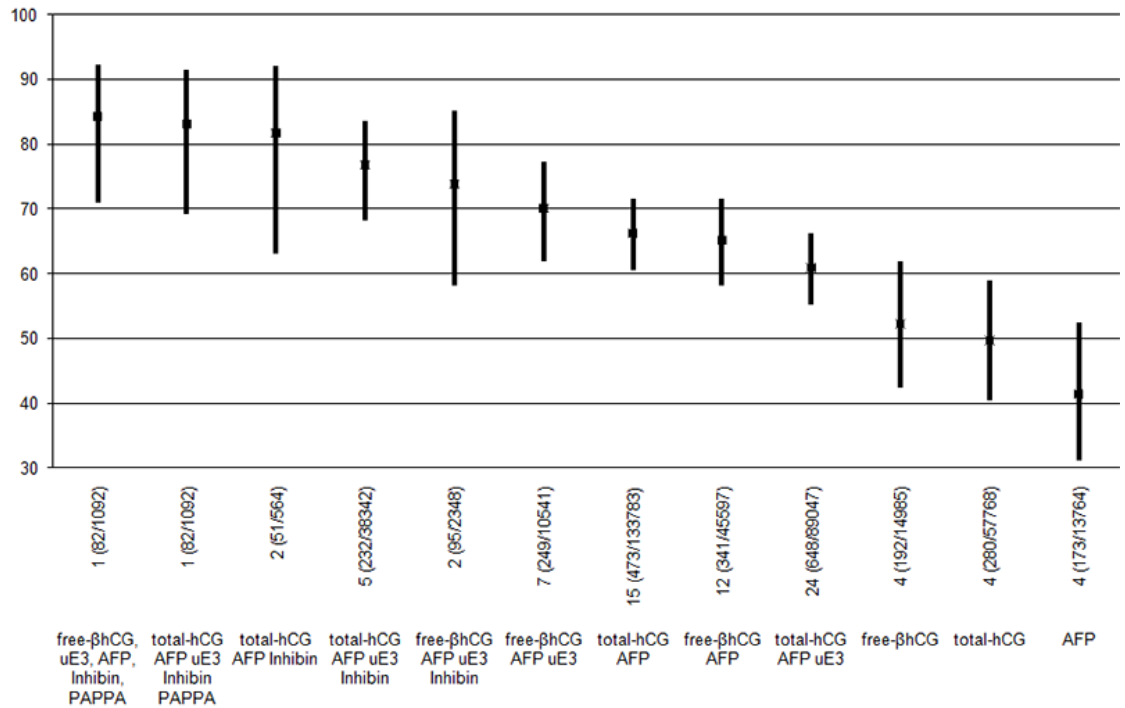


Table 1 shows pair wise direct comparisons (head-to-head) where studies were available. Such comparisons are regarded as providing the strongest evidence as they are unconfounded. The table shows the ratios of DOR with 95% CI and P values for each test combination, the number of studies (K) for which data were available. The table shows that the diagnostic accuracy of the single test combinations (AFP and maternal age, total hCG and maternal age and free βhCG and maternal age) tends to be significantly worse ($P < 0.05$) than the double, triple, quadruple and quintuple tests where data are available. The double test comprised of total hCG, AFP and maternal age also appears to have significantly worse ($P < 0.05$) test accuracy than quadruple and quintuple test combinations containing inhibin. Otherwise, there was no strong evidence of significant differences in test accuracy between triple, quadruple and quintuple tests containing inhibin and the standard double (total hCG, AFP and maternal age; free βhCG, AFP and maternal age) and triple tests (total hCG, AFP, uE3 and maternal age; free-βhCG, AFP, uE3 and maternal age). However, most comparisons in this table are based on only single studies and are unlikely to be powered to detect differences in detection rates.

Table 2 shows the same comparisons made using all available data (as used to create Figure 10). Results are in agreement with the direct comparisons, and in addition, showed some statistically significance differences ($P < 0.05$) suggesting that quintuple and quadruple tests containing inhibin and total hCG outperform standard double tests (total hCG, AFP and maternal age; free βhCG, AFP and maternal age) and one standard triple test (total hCG, AFP, uE3 and maternal age). However, these comparisons are potentially confounded by differences between the studies.

Investigations of heterogeneity and subgroup analysis

Three test combinations included 10 or more studies allowing investigation of sources of heterogeneity (two double tests (free β hCG, AFP and maternal age; total hCG, AFP and maternal age) and one triple test (total hCG, AFP, uE3, and maternal age)). Adequate data were only available to consider the impact of two potential sources: advanced maternal age and the use of the same data set for deriving and evaluating the risk equation (derivation versus validation). The results of these two comparisons for each of the three tests are presented in Table 3.

There is a significant difference in sensitivity for women over the age of 35 years for two test combinations. The double test comprised of free β hCG, AFP and maternal age showed a significant decrease in sensitivity in women over 35 years of age when compared to a standard screening population (51.7% sensitivity versus 66.4% for a fixed 5% FPR ($P = 0.03$)) with a larger decrease being observed for the triple test comprised of total hCG, AFP, uE3 and maternal age (48.4% versus 68.6% for a fixed 5% FPR ($P < 0.0001$)). A non-significant difference of the same magnitude was noted for the double test comprised of total hCG, AFP and maternal age.

No significant differences or consistent effects were noted when comparing evaluations undertaken in the same data sets used for derivation of the risk equation rather than separate validation data sets for any of the three test combinations.

Results of sensitivity analysis investigating the impact of possible pregnancy loss through delayed verification of test negatives

Figure 11 shows the results of the sensitivity analysis comparing test combinations when the number of false negatives are inflated by 50% in studies with delayed verification of test negatives. The estimate of the sensitivity decreases for all test combinations, with a small degree of variability in magnitude, but not large enough to cause any reordering of the performance of the tests. Thus it appears that the ranking of tests is not affected by delayed verification of test negatives in studies which ascertained Down's syndrome at birth in those at low risk.

Table 4 reports results of the investigation of the effect of maternal age, with similar inflations of false negatives from 10% to 50% in studies with delayed verification of test negatives. Delayed verification was not common in studies undertaken entirely in women aged 35 or over as they tended to be offered amniocentesis on the basis of the increased risk associated with advanced maternal age alone, and the corrections to the false negatives made very little difference to the estimates of sensitivity. However, in younger mothers the correction reduced sensitivity, and consequently reduced the apparent relationship between maternal age and test performance, observed through the ratio of diagnostic odds ratios approaching one. But even with an increase of 50% in the false negatives cells, the difference in sensitivity between age-groups for the triple test comprised of total hCG, AFP, uE3, and maternal age remained statistically significant, although its magnitude nearly halved from 20% to 12%. The effect seen for the double test comprised of free β hCG, AFP and maternal age combination lost its borderline significance with even the smallest increase in false negatives.

Summary of results

Summary of results 1. Performance of the 12 most evaluated and best performing second trimester serum strategies

	Studies	Women (cases)	Sensitivity* (95% CI) at a 5% FPR	Tests shown inferior in direct comparisons ($P < 0.05$)	Tests shown inferior in indirect comparisons ($P < 0.05$)

Summary of results 1. Performance of the 12 most evaluated and best performing second trimester serum strategies (*Continued*)

Quintuple Tests (with maternal age)					
free ßhCG AFP uE3 Inhibin A and PAPP-A	1	1,092 (82)	84 (71,92)	All single tests; total-hCG+AFP	All single tests; All double tests; total-hCG+AFP+uE3
Quadruple Tests (with maternal age)					
total hCG AFP uE3 Inhibin PAPP-A	1	1,092 (82)	83 (69, 92)	All single tests; total-hCG+AFP	All single tests; All double tests; total-hCG+AFP+uE3
total hCG AFP uE3 Inhibin	5	38,342 (232)	77 (68, 84)	All single tests; total-hCG+AFP	All single tests; All double tests; total-hCG+AFP+uE3
free ßhCG AFP uE3 Inhibin	2	2,348 (95)	74 (58, 85)	All single tests; total-hCG+AFP	All single tests
Triple Tests (with maternal age)					
total hCG AFP Inhibin	2	564 (51)	82 (63, 92)		All single tests; total-hCG+AFP+uE3
total hCG AFP uE3	24	89,047 (648)	61 (55, 66)	All single tests	total-hCG, AFP
free ßhCG AFP uE3	7	10,541 (249)	70 (62, 77)	All single tests	All single tests
Double Tests (with maternal age)					
total hCG AFP	15	133,783 (473)	66 (60, 72)	All single tests	All single tests
free ßhCG AFP	12	45,597 (341)	65 (58, 72)	total-hCG; AFP	All single tests
Single Tests (with maternal age)					
free ßhCG	4	14,985 (192)	52 (42, 62)	AFP	
total hCG	4	57,768 (280)	50 (40, 59)		
AFP	4	13,764 (173)	41 (31, 53)		

* these figures for sensitivity can be interpreted as the number of women out of every hundred carrying a Down's syndrome fetus who would be detected when the test is used at a cut off point corresponding to a 5% false positive rate. They have been calculated based on all available data using a single meta-analytical model.

Summary of results 2. Performance of the remaining 31 second trimester serum strategies (all involving maternal age)

	Studies	Women (cases)	Sensitivity* (95% CI)	Specificity* (95% CI)	Threshold
Single tests (with maternal age)					
uE3	2	1603 (93)	65 (33, 87)	92 (81, 97)	mixed
Free ßhCG to AFP ratio	1	8265 (47)	62 (46,75)	95	5% FPR
Inhibin	2	1117 (87)	59 (48, 68)	95	5% FPR
PAPP-A	2	1117 (87)	38 (20, 59)	95	5% FPR
ProMBP	1	256 (105)	49 (39,59)	95 (90,98)	1:250 risk
Free ßhCG	1	511 (11)	73 (39,94)	89 (86,91)	1:384 risk
Double tests (with maternal age)					
Total hCG and free ßhCG	1	511 (11)	55 (23,83)	82 (79,86)	1:384 risk
Total hCG and uE3	2	881 (61)	63 (27,89)	92 (79,97)	mixed
Total hCG and SP1	1	370 (50)	44 (30,59)	95	5% FPR
Total hCG and free ßhCG	1	511 (11)	82 (48,98)	86 (83,89)	1:384 risk
Free ßhCG and uE3	1	511 (11)	73 (39,94)	83 (79,86)	1:384 risk
Free ßhCG and free αhCG	1	511 (11)	73 (39, 94)	87 (84,90)	1:384 risk
AFP and uE3	2	881 (61)	49 (14,85)	92 (81,97)	mixed
uE3 and free ßhCG	1	511 (11)	82 (48,98)	85 (82,88)	1:384 risk
uE3 and SP1	1	370 (50)	36 (23,51)	95	5% FPR
AFP and SP1	1	370 (50)	34 (21,49)	95	5% FPR
AFP and Hyperglycosylated hCG	1	328 (50)	54 (39,68)	95	5% FPR

Summary of results 2. Performance of the remaining 31 second trimester serum strategies (all involving maternal age) (*Continued*)

AFP and free ßhCG	1	511 (11)	82 (48,98)	85 (82,88)	1:384 risk
Triple tests (with maternal age)					
Total hCG, free ßhCG and AFP	1	344 (31)	87 (70,96)	82 (77,86)	1:266 risk
Total hCG, uE3 and SP1	1	370 (50)	44 (30,59)	95	5% FPR
Total hCG, AFP and SP1	1	370 (50)	50 (36,64)	95	5% FPR
Total hCG, AFP and CA125	1	328 (22)	82 (60,95)	84 (80,88)	1:190 risk
Free ßhCG, AFP and Inhibin A	1	1256 (13)	62 (32,86)	80 (78,82)	1:190 risk
Free ßhCG, AFP and ProMBP	1	334 (107)	60 (50,69)	95	5% FPR
Free ßhCG, AFP and uE3	1	511 (11)	100 (72,100)	78 (74,81)	1:384 risk
AFP, uE3 and Inhibin A	1	346 (33)	88 (72,97)	79 (74,83)	1:233 risk
AFP, uE3 and SP1	1	370 (50)	38 (25,53)	95	5% FPR
Quadruple tests (with maternal age)					
Total hCG, free ßhCG, AFP and uE3	1	511 (11)	64 (31,89)	85 (82,88)	1:384 risk
Total hCG, AFP, uE3 and free αhCG	1	511 (11)	91 (59,100)	85 (81,88)	1:384 risk
Total hCG, AFP, uE3 and SP1	1	370 (50)	50 (36, 64)	95	5% FPR
Quintuple tests (with maternal age)					

Summary of results 2. Performance of the remaining 31 second trimester serum strategies (all involving maternal age) (*Continued*)

Total hCG, free ßhCG, AFP, uE3, and free αhCG	1	511 (1)	91 (59,100)	86 (82,89)	1:384 risk

* Sensitivity and specificity values obtained by separate pooling of sensitivities and specificities where there are two studies.

DISCUSSION

Summary of main results

The systematic review found a large number of studies evaluating second trimester Down's syndrome serum screening tests, including studies evaluating the commonly used double and triple tests. Fewer studies were available to evaluate the performance of test strategies involving inhibin, which have been more recently developed, and few studies provided unconfounded comparisons of test strategies by applying and comparing several strategies using the same serum sample, the majority of studies only evaluating a single test combination. A summary of results for the 12 most common and best performing strategies is given in this Summary of results 1, briefer details for the remaining 31 strategies are given in Summary of results 2.

Six key findings were noted.

1. Double and triple tests significantly outperform the use of single tests. Single tests (total hCG with maternal age, free βhCG with maternal age and AFP with maternal age) detect only between four and five out of every 10 Down's syndrome pregnancies when used at a threshold corresponding to a 5% false positive rate. Standard triple tests (total hCG, AFP, uE3 and maternal age; free βhCG, AFP, uE3 and maternal age) and double tests (total hCG, AFP and maternal age; free βhCG, AFP and maternal age) detect between six and seven out of every 10 Down's syndrome pregnancies at the same threshold.

2. Whilst the four quintuple, quadruple and triple test combinations including inhibin show the highest detection rates (total hCG, AFP, uE3, inhibin and PAPP-A with maternal age; total hCG, AFP, uE3 and inhibin with maternal age; free βhCG, AFP, uE3 and Inhibin A with maternal age; and total hCG, AFP and inhibin with maternal age), they were not shown to be statistically superior to double and triple tests that do not include inhibin in the direct comparisons. Whilst some significant differences between these categories of tests were noted in the indirect comparisons, the potential for confounding (particularly related to study year) is of concern. Estimates suggest that inhibin-based combinations may detect between seven and eight out of every 10 Down's syndrome pregnancies at a 5% false positive rate. With the exception of the quadruple test comprised of free βhCG, uE3, AFP and inhibin with maternal age (n = 2348), the number of pregnancies studied for these combinations was markedly smaller than for test combinations excluding inhibin. It is therefore difficult to make strong recommendations on the use or exclusion of inhibin as a marker in combined tests, as we cannot conclude there are no differences as there is limited power to detect them.

3. The evidence that quintuple tests are significantly better at detecting Down's syndrome than quadruple tests or triple tests is not strong, and similarly quadruple tests are not shown to be significantly better than triple tests. Whilst the trend suggests that the more markers used in a test, the higher the diagnostic accuracy, the amount of evidence, particularly available for direct comparisons, is insufficient to make strong recommendations.

4. There was no obvious benefit in using free βhCG over total hCG. Six studies made direct comparisons between the two alternative triple tests with no obvious difference in test accuracy (ratio of DOR 1.0; 95%CI (0.7 to 1.6); P = 0.93); four studies made direct comparisons between the two alternative double tests also with no obvious difference (ratio of DOR 1.0; 95%CI (0.6 to 1.6); P = 0.91).

5. The sensitivity of tests in women over the age of 35 years is markedly reduced. Evidence was available for three tests at a fixed 5% FPR showing reductions in detection rates of between 10% and 20%. Part of this effect may be explained by studies in younger mothers missing false negative cases lost through increased miscarriage in Down's pregnancies, but this does not fully explain the full effect. We are unable to draw any conclusions as to why this may be the case. There was no obvious difference in algorithms used to calculate risk, the marker assays used, nor was there any obvious difference in the dates of the studies involved. There may be differences in placental function in women over 35 years of age that explains the differences in performance of markers, however, this is conjecture.

Strengths and weaknesses of the review

This review is the first comprehensive review of second trimester serum screening. It has examined papers from around the world, covering a wide cross section of women in varying populations. We have contacted authors to verify data where necessary to give as complete a picture as possible while trying to avoid replication of data.

There were a number of factors which have made meta-analysis of the data difficult, which we have tried to adapt for in order to allow for comparability of data presented in different studies.

1. There are many different cut-points used to define pregnancies as high or low risk for Down's syndrome. This means that direct comparison is more difficult than if all studies used the same cut-point to dichotomise their populations.

2. There are many different risk equations and software applications in use for combination of multiple markers, which were often not described in the papers. This means that risks may be calculated by different formulae, and they may not be directly comparable for this reason. It is possible that this is responsible for confounding results.

3. Different laboratories and clinics run different assays and use different machines and methods. This may influence raw results and subsequent risk calculations. Many laboratories have a quality assessment/audit trail, however, this may not necessarily be standard across the board, for example, how many assays are run, how often medians are calculated and adjusted for a given population and how quickly samples are tested from initially being taken.

4. Few papers make direct comparisons between tests, making it difficult to detect if there is a real difference between tests (i.e. how different tests perform in the same population). There are differences in populations, with assay medians being affected, for example, by race. It is not certain whether it is appropriate to make comparisons between populations which are inherently different.

5. We were unable to perform many of the subgroup analyses that we had originally intended to, as the data simply were not available. The vast majority of papers looking at pregnancies conceived by IVF, affected by diabetes, multiple gestation or a family history of Down's syndrome involved unaffected pregnancies only

The major methodological concern in the primary studies relates to the loss of pregnancies from the studies through miscarriage that occurs between serological testing and obtaining the reference standard. In studies where the patient sample were women attending for an amniocentesis no delay would occur between the serum test and reference standard, and data on all pregnancies would be available. In more standard clinical populations invasive testing is only offered to high-risk pregnancies - in these studies to women with high-risk serum test results. The remainder are assessed at birth for phenotypic features of Down's syndrome, but some will be lost during follow-up due to miscarriage, and are suspected to be omitted from study reports. Even though these problems occur, the sensitivity analysis we have undertaken indicate that the ranking of the included tests is not affected by such differential and delayed verification and drop-out.

Applicability of findings to clinical practice and policy

Potentially, where planning screening policy or a clinical screening programme, clinicians and policy makers need to make decisions about a finite number of tests or type of tests that can be offered. These policies are often driven by both the needs of a specific population and by financial resources. Economic analysis was considered to be outside of the scope of this review. Many of the tests examined as part of this review are already commercially available and in use in the clinical setting. The studies were carried out on populations of typical pregnant women and therefore, the results should be considered comparable with most pregnant populations encountered in every day clinical practice.

We were also unable to extract information about harms of testing, information about miscarriage rates and uptake of definitive testing as the data were not available the majority of the time. Whilst it is unlikely that major differences between the tests evaluated here exist in terms of direct harms of testing, as they are all based on a single blood sample, differences in accuracy may lead to differences in the use of definitive testing and its consequent adverse outcomes.

In some countries with a defined screening policy (i.e. the UK), second trimester screening no longer plays a major role. In others however, there may only be a limited range of tests or markers available - often second trimester markers. The results of this review should be interpreted and applied in the context of test availability and local restrictions, populations or policies.

AUTHORS' CONCLUSIONS

Implications for practice

The evidence for tests involving inhibin as a marker suggests a superiority that is not found to be statistically significant, and based on small populations of women. We would not recommend that these tests should be introduced into wider clinical practice without careful consideration of cost.

The review has shown that tests involving two or three markers in combination with maternal age are significantly better than those involving one marker. We would therefore recommend that one marker tests are not used for Down's syndrome screening. The choice of multiple markers will depend on the availability of certain assays in local laboratories. There was no test combination shown to be superior to others therefore, we cannot recommend a specific test combination.

The performance of tests at earlier gestations will be the subject of a separate Cochrane review and the alternative first trimester, cross-trimester, ultrasound and combinations of serum and ultrasound should also be considered when making policy decisions.

Implications for research

Further evaluation of inhibin-based test combinations are required to determine whether their apparent advantages are not chance findings. Further study of the attenuated performance of test combinations in women over 35 is required, as this age group has the highest incidence of Down's syndrome and has the greatest requirement for tests with high detection rates.

Future studies should ensure that adequate sample sizes are recruited, and take opportunities to make comparisons of test performance testing several alternative test combinations on the same serum samples. Such direct comparison removes issues of confounding when making test comparisons, and allows a clear focus on testing the incremental benefit of increasingly complex and expensive testing strategies. The reporting of studies of test accuracy can be improved and more closely adhere to the STARD reporting standards. Three key aspects of this are 1) formally testing the statistical significance of differences in test performance in direct comparisons and estimating incremental changes in detection rates (together with confidence intervals), 2) clearly reporting the number of mothers studied and their results, and 3) reporting the numbers of women who are lost to follow-up. Many authors reported results of extrapolating findings to age-standardised national cohorts to demonstrate the performance of the test, and failed to report the actual numbers studied and evaluated.

For the purposes of meta-analysis and to allow for comparisons to be made between different tests and combinations, we would recommend the publication of consensus standard algorithms for estimating risk, and reporting of test performance at a standard set of thresholds. This would be difficult to achieve and implement, but an attempt at consensus should be made.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Anandakumar 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing based on age
Participants	1208 participants Singapore - single centre 1989-1991 Pregnant women over 35 years of age Singleton pregnancies Karyotyping performed at same time as serum sampling 12-22 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 7 affected cases Reference standard - Amniocentesis
Index and comparator tests	Maternal age Second trimester serum AFP - Amerlite AFP assay Second trimester serum ß hCG - Amerlite HCG-60 assay
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate

Anandakumar 1999 (*Continued*)

Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Unclear	No information given
Withdrawals explained? All tests	Yes	No withdrawals

Audibert 2001a

Clinical features and settings	Request for Down's syndrome screening in pregnancy Routine screening
Participants	3790 participants France - single centre May 1994-December 1997 Pregnant women Singleton pregnancies CRL between 38 and 84 mm Under 38 years of age
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 10 affected cases Reference standards; Amniocentesis CVS Postnatal karyotype Miscarriage with cytogenetic testing Neonatal examination
Index and comparator tests	Maternal age Second trimester serum hCG Second trimester serum AFP (Nuchal Translucency - see 1st trimester US review) Amerlite, Orthoclinical diagnostics machine Prenata software
Follow-up	Delivery and postnatal paediatric examination 35 lost to follow-up and excluded from analysis

Audibert 2001a (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Typical pregnant population with exception that women over 38 years of age excluded
Acceptable reference standard? All tests	Yes	Amniocentesis CVS Postnatal karyotype Miscarriage with cytogenetic testing Neonatal examination
Partial verification avoided? All tests	Yes	All pregnancies verified by acceptable reference standard
Differential verification avoided? All tests	No	Different reference standards used within same population
Incorporation avoided? All tests	Yes	Index and reference tests separate
Reference standard results blinded? All tests	Yes	Results of index tests known prior to reference standard being performed
Index test results blinded? All tests	Yes	Results of index test unknown to operator providing reference standard
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Bahado-Singh 1999a

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	926 participants USA November 1995 - March 1999

Bahado-Singh 1999a (*Continued*)

	Pregnant women Singleton pregnancies Serum screening performed 15-24 weeks gestation Euploid/Down's karyotype only
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 21 affected cases Reference standard - Amniocentesis
Index and comparator tests	Maternal age 2nd trimester urinary ß core fragment 2nd trimester serum AFP 2nd trimester serum uE3 2nd trimester serum ß hCG Spot specimens of urine - 2 step sandwich assay B120 monoclonal antibody Serum not described
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Typical pregnant population
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Performed after index test
Index test results blinded? All tests	Unclear	No information given

Bahado-Singh 1999a (*Continued*)

Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Bahado-Singh 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	2371 participants USA January 1992 - November 1997 Pregnant women Singleton pregnancies 14-24 weeks gestation Euploid/Down's karyotype only
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 46 affected cases Reference standard - amniocentesis
Index and comparator tests	Maternal age 2nd trimester serum AFP 2nd trimester serum uE3 2nd trimester serum ß hCG Not described
Follow-up	100% karyotype
Notes	Serum data analysed only

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Typical pregnant population
Acceptable reference standard? All tests	Yes	Amniocentesis

Bahado-Singh 2000 (*Continued*)

Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Reference standard performed after index test performed
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Bartels 1990

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	325 participants Germany and USA Dates not specified Known normal outcome or known aneuploidy 14-24/40 gestation
Study design	Retrospective multi-centre case-control study
Target condition and reference standard(s)	Down's syndrome 43 affected cases Reference standard not specified, but known karyotype
Index and comparator tests	2nd trimester hCG 2nd trimester SP-1 Tandem E hCG immunoenzymetric assay Enzygnost SP1 assay
Follow-up	100% methods not specified

Bartels 1990 (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Unclear	Implied karyotype - known outcome
Partial verification avoided? All tests	Yes	All outcomes known
Differential verification avoided? All tests	Unclear	No information given regarding method of karyotype
Incorporation avoided? All tests	Yes	Reference and index test separate
Reference standard results blinded? All tests	Yes	Known prior to index test
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Bartels 1994a

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk women referred for invasive testing
Participants	370 participants Germany 14-21 weeks gestation
Study design	Prospective consecutive series study

Bartels 1994a (*Continued*)

Target condition and reference standard(s)	Down's syndrome 50 affected cases Reference standard - Amniocentesis
Index and comparator tests	Second trimester hCG, SP-1, uE3 and AFP with or without age Amerlex M 2nd trimester kit for AFP, hCG and uE3 Enzygnost SP-1 for SP-1
Follow-up	100% Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk women referred for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Bartels 1994b

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk women referred for invasive testing
Participants	655 participants Germany 14-21 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 10 affected cases Reference standard - Amniocentesis
Index and comparator tests	Second trimester hCG, SP-1, uE3 and AFP with or without age Amerlex M 2nd trimester kit for AFP, hCG and uE3 Enzygnost SP-1 for SP-1
Follow-up	100% Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk women referred for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information provided

Bartels 1994b (*Continued*)

Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Beekhuis 1993

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	2282 participants Netherlands multi-centre October 1st 1990-December 1st 1991 Pregnant women Singleton pregnancies 15-20 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 6 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	Maternal age Second trimester serum hCG Second trimester serum AFP EIA Alpha software
Follow-up	100% karyotype
Notes	Dutch language paper

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received reference standard

Beekhuis 1993 (*Continued*)

Differential verification avoided? All tests	No	Reference standard differs according to index test result
Incorporation avoided? All tests	Yes	Index and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Benattar 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	1649 participants France January to December 1995 Pregnant women Singleton pregnancies Less than 13 weeks gestation at enrolment 15-18 weeks at time of second trimester serum screening
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 5 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	Maternal age Second trimester serum AFP Second trimester serum free ß hCG Second trimester serum hCG No test characteristics specified

Benattar 1999 (*Continued*)

Follow-up	Birth
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differs depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	12 lost to follow-up

Brajenovic 1998

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	3188 participants Croatia January 1996-December 1996 Pregnant women Same ethnic group (not specified) 14-22 weeks

Brajenovic 1998 (*Continued*)

Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 9 affected cases Reference standard; Amniocentesis Maternity record, cytogenetics records and patient questionnaires
Index and comparator tests	Second trimester maternal serum AFP Second trimester maternal serum free ß hCG EMIA coated tubes ELISA assay - CIS Bio international
Follow-up	3 months after delivery
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Maternity record, cytogenetics records and patient questionnaires
Partial verification avoided? All tests	Yes	All pregnancies verified by reference standard
Differential verification avoided? All tests	No	High risk received amniocentesis, low risk received postnatal verification as stated above
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to references standard
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None

Brajenovic 1998 (*Continued*)

Withdrawals explained? All tests	Yes	None

Brizzi 1989a

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	1472 participants Italy Dates not specified Pregnant women Biparietal diameter 32-48 mm
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 27 affected cases Amniocentesis
Index and comparator tests	Second trimester Maternal serum AFP No technical information provided
Follow-up	100% Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given

Brizzi 1989a (*Continued*)

Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Chao 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	10098 participants Taiwan 1st July 1994- 30th April 1996 Pregnant women 15-23 weeks gestation Singleton pregnancies
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 15 affected cases Reference standards; Amniocentesis Postnatal examination Telephone follow-up of high-risk cases
Index and comparator tests	Maternal age Second trimester maternal serum AFP Second trimester maternal serum free ß hCG Beta hCG - solid phase 2 site immunoradiometric assay AFP - enzyme immunoassay kit
Follow-up	85% Known outcome
Notes	85% follow up only

Table of Methodological Quality

Item	**Authors' judgement**	**Description**

Chao 1999 (*Continued*)

Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination Telephone follow-up of high-risk cases
Partial verification avoided? All tests	No	Outcome known in 85% only, but all high-risk women offered invasive testing
Differential verification avoided? All tests	No	Reference standard differed depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	No	15% loss to follow-up not explained

Christiansen 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	261 participants Denmark Dates not specified Pregnant women Known outcome 5-20 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 105 affected cases Karyotyping

Christiansen 1999 (*Continued*)

Index and comparator tests	1st trimester serum ProMBP 2nd trimester serum ProMBP 2 site immunoradiometric assay samples reduced and alkylated and added to microtitre wells coated with monoclonal antibody J13 6B6
Follow-up	100% Birth/karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Typical screening population
Acceptable reference standard? All tests	Yes	Karyotyping
Partial verification avoided? All tests	Yes	All women received karyotyping
Differential verification avoided? All tests	No	All women received karyotyping
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrsopective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Christiansen 2004

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	334 participants Denmark Dates not specified Pregnant women Singletons
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 107 affected cases Reference standard; Amniocentesis CVS Postnatal karyotype
Index and comparator tests	Maternal age Second trimester maternal serum ß hCG Second trimester maternal serum AFP Second trimester maternal serum Pro MBP Pro-MBP - 2 site immunoradiometric assay (IRMA) Free ß hCG and AFP - AutoDELFIA analytical system
Follow-up	Retrospective - Known outcome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis CVS Postnatal karyotype
Partial verification avoided? All tests	Yes	All outcomes known
Differential verification avoided? All tests	No	Different reference standards used within population
Incorporation avoided? All tests	Yes	Index test and reference standards separate

Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Outcome unknown to assessor
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Cioffi 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	717 participants Italy Dates not specified Pregnant women 15-21 weeks gestation No family history of NTD/DM/DS
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 17 affected cases Reference standard; Amniocentesis Postnatal follow-up
Index and comparator tests	Maternal age Second trimester maternal serum uE3 Second trimester maternal serum AFP Second trimester maternal serum ß hCG Isotopic methods uE3 - Bio rad clin division AFP and ß hCG - Immunosystems company
Follow-up	Postnatal period
Notes	

Table of Methodological Quality

Cioffi 2000 (*Continued*)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal follow-up
Partial verification avoided? All tests	Yes	All outcomes verified
Differential verification avoided? All tests	No	High risk only received amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Crossley 1994

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	30084 participants UK 1991-1992 Singleton pregnancies 15-20 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 37 affected cases Reference standards;

Crossley 1994 (*Continued*)

	Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing Postnatal examination
Index and comparator tests	Maternal age 2nd Trimester serum AFP 2nd Trimester serum total hCG Serone MAIA clone
Follow-up	100% birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing Postnatal examination
Partial verification avoided? All tests	Yes	All verified by reference standard
Differential verification avoided? All tests	No	Different women received different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	No	No explanation given
Withdrawals explained? All tests	Yes	None

David 1996

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	9500 participants Israel June 1991-October 1993 Pregnant women SIngleton pregnancies
Study design	Case-control study - controls collected prospectively and cases collected retrospectively
Target condition and reference standard(s)	Down's syndrome 47 affected cases Amniocentesis Postnatal examination
Index and comparator tests	Maternal age Second trimester maternal serum uE3 Second trimester maternal serum AFP Second trimester maternal serum hCG DELFIA Wallac - AFP and hCG Amerlex radioimmunoassay - uE3
Follow-up	Not stated
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	No	Reference standard differed
Differential verification avoided? All tests	No	Different women received different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given

David 1996 (*Continued*)

Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Debieve 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	218 participants Belgium Dates not specified Pregnant women 15-20 weeks gestation Singletons
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 18 affected pregnancies Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	Maternal age Second trimester maternal serum hCG Second trimester maternal serum uE3 Second trimester maternal serum AFP Second trimester maternal serum Inhibin A Amerlex M 2T RIA kits for hCG, uE3 and AFP 2 monoclonal antibody solid-phase sandwich microtitre plate ELISA Serotec Oxford for Inhibin A
Follow-up	Known outcome
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description

Debieve 2000 (*Continued*)

Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All outcomes verified
Differential verification avoided? All tests	No	Only high-risk women had amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Extermann 1998

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	2539 participants Switzerland June 1992 - June 1993 Pregnant women Known outcome 15-18 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 23 affected cases Reference standard - amniocentesis and implied postnatal verification but not specified

Extermann 1998 (*Continued*)

Index and comparator tests	Maternal age Second trimester maternal serum hCG Second trimester maternal serum free ß hCG Second trimester maternal serum AFP Second trimester maternal serum uE3 AFP - IMX (Abbott)/ES 600 Total hCG - IMX/Status (Baxter) uE3 and free ß hCG - RIA using Kodak Amerlex M Estriol kit
Follow-up	Birth
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis and implied postnatal verification
Partial verification avoided? All tests	Yes	All outcomes ascertained
Differential verification avoided? All tests	No	Reference standard offered depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Forest 1995

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	511 participants Canada June 1989 - October 1993 Singleton pregnancies 9-18 weeks gestation
Study design	Prospective consecutive series
Target condition and reference standard(s)	Down's syndrome 11 affected cases Reference standard; Amniocentesis Review of maternal and neonatal records
Index and comparator tests	AFP uE3 Total hCG Free alpha hCG Free ß hCG AFP/hCG - Enzymum test enzyme immunoassay (Boehringer Mannheim, Canada) uE3 - Radioimmunometric assay (DSL Canada) Free alpha and ß hCG - Radioimmunometric assay (Bioclone Austria pty Ltd)
Follow-up	100% Birth/Karyotype
Notes	3 different models used for risk calculation (Wald, Spencer and Ryall)

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Maternity and neonatal records
Partial verification avoided? All tests	Yes	All outcomes verified
Differential verification avoided? All tests	No	Reference standard differs depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate

Forest 1995 (*Continued*)

Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Greenberg 1991

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	3282 participants USA 1985 onwards Pregnant women Singleton pregnancy
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 51 affected cases Reference standards; Amniocentesis Postnatal karyotype Postnatal examination
Index and comparator tests	Second trimester serum AFP alone Clinical assays, Cambridge, Massachusetts
Follow-up	100% - known outcome
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting -screening programme

Greenberg 1991 (*Continued*)

Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Postnatal examination
Partial verification avoided? All tests	Yes	All women received verification by reference standard
Differential verification avoided? All tests	No	Different reference standards used depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Known outcome - retrospective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Haddow 1994

Clinical features and settings	Request for Down's syndrome screening in pregnancy HIgh-risk referral for invasive testing
Participants	5336 participants USA December 1990 - October 1992 Pregnant women 35 years of age and over
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 54 affected cases Reference standard - Amniocentesis
Index and comparator tests	2nd trimester serum AFP 2nd trimester serum uE3 2nd trimester serum hCG Maternal age at delivery

Haddow 1994 (*Continued*)

	AFP - In house assay uE3 - Amerlex M radioimmunoassay kit specific for uE3 hCG - Amerlex M extended range hCG radioimmunoassay kit
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Index test result not known to operator
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Haddow 1998

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	308 participants USA

Haddow 1998 (*Continued*)

	December 1990 - October 1992 Pregnant women 35 years of age and over
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down' syndrome 52 affected cases Reference standard - amniocentesis
Index and comparator tests	2nd trimester serum Inhibin A Inhibin A measured in duplicate using a solid phase sandwich enzyme linked immunosorbent assay (ELISA)
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Index test result unknown to operator
Index test results blinded? All tests	Yes	Carried out prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None

Withdrawals explained? All tests	Yes	None

Heyl 1990

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	101 participants USA January 1986 - January 1990 Greater than 35 years Singleton pregnancies
Study design	Retrospective single-centre case-control study
Target condition and reference standard(s)	Down's syndrome 16 affected cases Reference standard - Amniocentesis
Index and comparator tests	2nd trimester AFP 2nd trimester hCG 2nd trimester uE3 Maternal Age 1 in 365 risk AFP - Abbott enzyme immunoassay hCG - IMx system total ß hCG assay uE3 - Amerlex M unconjugated oestriol
Follow-up	100%
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis

Heyl 1990 (*Continued*)

Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Index test result not known at time of reference standard being carried out
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Hsu 1997a

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	8265 participants Taiwan 1992-1996 Pregnant women Singletons 14-23 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 47 affected cases Reference standards; Amniocentesis Neonatal examination
Index and comparator tests	Maternal age Second trimester serum free ß hCG Second trimester serum AFP Abbott EIA-AFP Free ß hCG ELSA kit Cuckle 1987 formula
Follow-up	Birth/Karyotype
Notes	

Hsu 1997a (*Continued*)

Table of Methodological Quality		
Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Neonatal examination
Partial verification avoided? All tests	Yes	All women had reference standard performed
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Reference standard performed prior to index test
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Huderer-Duric 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy HIgh-risk referral for invasive testing
Participants	2833 participants Croatia 1996-1998 Pregnant women Singletons 15-22 weeks gestation
Study design	Prospective consecutive series study

Huderer-Duric 2000 (*Continued*)

Target condition and reference standard(s)	Down's syndrome 12 affected cases Amniocentesis
Index and comparator tests	Maternal age Second trimester maternal serum uE3 Second trimester maternal serum hCG Second trimester maternal serum AFP Amerlax M Prenata software
Follow-up	Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Operator not aware of index test result at time of amniocentesis
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Jou 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	17742 participants Taiwan June 1994-July 1998 Pregnant women 14-22 weeks gestation Singletons
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 16 affected cases Reference standard; Amniocentesis Postnatal examination Postnatal karyotype
Index and comparator tests	Maternal age Second trimester maternal serum hCG Second trimester maternal serum AFP AFP - microparticle enzyme immunoassay kit hCG - CMEIA AFP kit and MEIA ß hCG kit RAM programme - body weight corrected MoM and maternal age
Follow-up	Birth/Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination Postnatal karyotype
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Refence standard differs depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate

Jou 2000 (*Continued*)

Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Kadir 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	4427 participants England 1/4/93 - 31/3/95
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 13 affected cases Reference standards; Amniocentesis Postnatal examination
Index and comparator tests	2nd trimester AFP 2nd trimester free ß hCG Maternal age 1 in 250 risk at EDD AFP - immunoradiometric assay (omnia alpha FP) Free ß hCG - specific solid phase 2 site immunoradiometric assay (ELISA fBhCG)
Follow-up	100% karyotype or postnatal examination
Notes	9.4% of study population older than 37 years

Table of Methodological Quality

Item	**Authors' judgement**	**Description**

Kadir 1999 (*Continued*)

Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women had reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Index test performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Kishida 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme, but 63% of women over 35 yrs of age
Participants	1055 participants Japan May 1995-Feb 1998 Pregnant women Singleton pregnancies 14-20 weeks No major pregnancy complications
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 10 affected cases Reference standards; Amniocentesis

Kishida 2000 (*Continued*)

	Clinical neonatal examination	
Index and comparator tests	Maternal age Second trimester maternal serum AFP Second trimester maternal serum hCG Second trimester maternal serum uE3 AFP - Abbott laboratories hCG - Wallac uE3 - Diagnostic products, LA	
Follow-up	100% birth/karyotype	
Notes	63% of women over 35 yrs of age	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Neonatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Index test results unknown to operator
Index test results blinded? All tests	Yes	Index test performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Knight 1998

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	5117 participants USA December 1990 - October 1992 Pregnant women 35 years of age and over
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 52 affected pregnancies reference standard - Amniocentesis
Index and comparator tests	Second trimester serum hCG Second trimester serum free ß hCG Second trimester serum AFP Second trimester serum uE3 Maternal age Free ß hCG and AFP - Wallac DELFIA hAFP/free ß hCG dual assay hCG - hCG MAIA clone uE3 - Amerlex M radioimmunoassay kit specific for uE3
Follow-up	100% Karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Yes	Results of index test unknown to operator

Knight 1998 (*Continued*)

Index test results blinded? All tests	Yes	Index test performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Lam 2002

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	16237 participants Taiwan June 1994-July 1998 Pregnant women 15-20 weeks gestation for serum analysis
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 35 affected cases Reference standards; Amniocentesis CVS Neonatal examination
Index and comparator tests	Maternal age Second trimester maternal serum AFP Second trimester maternal serum hCG Test characteristics not described in paper
Follow-up	Birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme

Lam 2002 (*Continued*)

Acceptable reference standard? All tests	Yes	Amniocentesis CVS Neonatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differs depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	No	Index test result known to operator
Index test results blinded? All tests	Yes	Index test performed prior to reference standard
Relevant clinical information? All tests	Unclear	No clear information given
Uninterpretable results reported? All tests	Yes	Miscarriages etc excluded from analysis
Withdrawals explained? All tests	Yes	None

Lemay 1995

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	18600 participants France October 1989 to December 1993 Pregnant women 15-18 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 32 affected cases Refernce standards; Amniocentesis Postnatal karyotype Postnatal examination

Lemay 1995 (*Continued*)

Index and comparator tests	Second trimester serum total hCG Second trimester serum total AFP Maternal age Arcus 1230 2 site immunofluorimetric assay (DELFIA)
Follow-up	100% birth/karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differs depending on index test result
Incorporation avoided? All tests	Yes	Index test and reference standard separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Results not available to operator
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Malone 2005

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	35236 participants USA - 15 centres October 1999 - December 2002 Pregnant women Maternal age >16 years Singleton live fetus Fetal CRL 36-79 mm (10+3 - 13+6/40 at recruitment)
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 87 affected cases Reference standards; Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing Neonatal examination
Index and comparator tests	Maternal age Second trimester maternal serum AFP Second trimester maternal serum total hCG Second trimester maternal serum uE3 Second trimester maternal serum Inhibin A Test characteristics not specified
Follow-up	Birth/karyotype
Notes	Cystic hygroma analysed separately

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing Neonatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differs depending on index test result

Malone 2005 (*Continued*)

Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Mancini 1991

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	731 participants Italy 1989-1990 Pregnant women 15-18 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 9 affected cases Reference standard - amniocentesis
Index and comparator tests	2nd trimester serum uE3 2nd trimester serum hCG 2nd trimester serum AFP Maternal age AFP - DELFIA hAFP kit hCG - DELFIA hCG uE3 - Unconjugated RIA
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Mancini 1991 (*Continued*)

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	No	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index test and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Milunsky 1993

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	511 participants USA Dates not specified Normal singleton pregnancy versus known Down's pregnancy
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 31 affected cases Reference standards; Amniocentesis Postnatal examination

Index and comparator tests	Intact hCG Free ß hCG MSAFP Maternal age Cases that had not undergone more than 2 freeze thaw cycles Free ß by commercial immunoradiometric assay (CIS UK Ltd) Intact hCG by immunoradiometric solid phase assay (Serone MAIA clone)
Follow-up	100% Birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Yes	Performed without knowledge of reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information provided
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Muller 1996a

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	51048 participants France 1989-1993 15-17/40 gestation Singleton pregnancies
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 135 affected cases Reference standard; Amniocentesis Postnatal Karyotype Postnatal examination
Index and comparator tests	2nd trimester maternal serum hCG Greater than or equal to 1% risk of Down's hCG high values, SFRI, Bordeaux, France
Follow-up	100% by karyotype or postnatal examination
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting- screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal Karyotype Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given

Muller 1996a (*Continued*)

Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Palomaki 2004

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	283 participants USA 14 centres 1990-1992 Pregnant women 14-21 weeks gestation SIngleton pregnancies
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 45 affected cases Reference standard - amniocentesis
Index and comparator tests	2nd trimester serum Invasive trophoblast antigen Maternal age Automated immuno chemiluminometric assay
Follow-up	100% birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis

Palomaki 2004 (*Continued*)

Partial verification avoided? All tests	Yes	All women had amniocentesis
Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Yes	Outcome unknown to operator
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Palomaki 2006

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	540 participants USA single centre Dec 1 1999-October 31 2003 Pregnant women Known Down's or normal pregnancy
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 32 affected cases Reference standard - Outcome obtained from Ontario Multiple Marker screening database
Index and comparator tests	Second trimester maternal serum PAPP-A Alpha logical medical systems software Repeated measures method Perkin Elmer assay
Follow-up	Known outcome

Palomaki 2006 (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Genetic database Karyotyping
Partial verification avoided? All tests	Yes	All women had known outcome
Differential verification avoided? All tests	No	Reference standard differed
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Pandian 2004

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme AND High-risk referral for invasive testing
Participants	100 participants USA Dates not specified Pregnant women Singleton pregnancies
Study design	Retrospective case-control study

Target condition and reference standard(s)	Down's syndrome 16 affected cases Reference standard ; Amniocentesis Known pregnancy outcome
Index and comparator tests	Second trimester maternal serum ITA Second trimester maternal serum AFP Second trimester maternal serum uE3 Second trimester maternal serum hCG Second trimester maternal serum Inhibin A ITA - Diagnostic System Laboratories AFP/uE3/hCG/Inhibin A - Immulite 2000
Follow-up	Birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme AND High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis 'Known pregnancy outcome'
Partial verification avoided? All tests	Yes	All had reference standard
Differential verification avoided? All tests	No	Reference standard differed
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Performed prior to index test analysis
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None

Pandian 2004 (*Continued*)

Withdrawals explained? All tests	Yes	None

Perona 1997

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	20856 participants Italy October 1991-December 1995 Pregnant women Singleton pregnancies 30-35 years of age 15-18 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 41 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	2nd trimester serum uE3 2nd trimester serum hCG 2nd trimester serum AFP Maternal age ALPHA software package AFP - DELFIA hAFP kit hCG - DELFIA hCG uE3 - Unconjugated RIA
Follow-up	100% birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination

Perona 1997 (*Continued*)

Partial verification avoided? All tests	Yes	All women had reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Piggott 1994

Clinical features and settings	Request for Down's syndrome screening in pregnancy CLinical setting - screening programme
Participants	6990 participants UK January 1991-December 1992 Pregnant women Singleton pregnancies 15-22 weeks gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 11 affected cases Reference standard; Amniocentesis Neonatal examination and birth registers
Index and comparator tests	Maternal age Second trimester maternal serum AFP Second trimester maternal serum uE3 Second trimester maternal serum hCG

Piggott 1994 (*Continued*)

	AFP and hCG - Delfia kits uE3 - Amerlex M Alpha software
Follow-up	Birth/karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Neonatal examination and birth registers
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to references standard
Relevant clinical information? All tests	Unclear	No information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Qin 1997

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme

Qin 1997 (*Continued*)

Participants	352 participants Denmark - single centre Dates not specified Pregnant women Known Down's or normal pregnancy 5-9 weeks (for USS) or 14-20 weeks for serum
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 116 affected pregnancies Reference standard; Amniocentesis CVS Postnatal karyotype Postnatal examination
Index and comparator tests	Second trimester maternal serum SP1 Non-competitive time resolved immuno fluorometric assay using rabbit antibody against SP1 Multicalc software package
Follow-up	Birth/Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis CVS Postnatal karyotype Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differs
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study

Qin 1997 (*Continued*)

Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Roberts 2000

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	26080 participants England February 1992 - January 1997
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 41 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	2nd trimester AFP 2nd trimester hCG Maternal age 1 in 250 risk Amerlex-M 2T kit, ortho clinical diagnostics
Follow-up	100% karyotype/postnatal examination
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination

Roberts 2000 (*Continued*)

Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Rose 1994

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	3896 participants USA 1974-1990 Pregnant women Greater than 35 years of age
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 33 affected cases Reference standard; Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing
Index and comparator tests	Maternal age Second trimester maternal serum AFP Test characteristics not described

Follow-up	Birth/Karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differed depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Rosen 2002

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	1006 participants Belgium January 1991-September 1992 Pregnant women 14-24 weeks by USS

Rosen 2002 (*Continued*)

Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 13 affected cases Reference standard - Amniocentesis
Index and comparator tests	Maternal age Second trimester maternal serum uE3 Second trimester maternal serum hCG Second trimester maternal serum AFP Dried blood samples on blotting paper card Non radioactive immunologic step followed by colorimetric quantification of a horseradish pre oxidase
Follow-up	Birth/Karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Performed immediately after venepuncture for index test
Index test results blinded? All tests	Yes	Reference standard results not known
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None

Withdrawals explained? All tests	Yes	None

Rozenberg 2002

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	8297 participants France (multi-centre) March 1994 - December 1997 18-37 years of age Singleton pregnancy No family history of Down's syndrome 12-14 weeks gestation at time of scan and 14+1 to 17 weeks at time of serum sample
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 20 affected cases Reference standards; Amniocentesis Postnatal examination
Index and comparator tests	NT Second trimester serum free ß hCG Second trimester serum AFP Secdond trimester serum Maternal age NT - FMF methods SERUM - ß hCG ELISA immunoradiometric assay AFP ELISA immunoradiometric assay
Follow-up	100% Birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard

Rozenberg 2002 (*Continued*)

Differential verification avoided? All tests	No	Different references standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Sancken 2003

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	221 participants Germany Dates not specified Pregnant women 15-22 weeks gestation Known pregnancy outcome
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 33 affected cases Reference standard; Amniocentesis Postnatal karyotype Postnatal examination
Index and comparator tests	Maternal Age Total hCG Free ß hCG AFP uE3 Second trimester serum samples AFP and uE3 - Radioimmunoassay

Sancken 2003 (*Continued*)

	Free ß hCG - Immunoradiometric assay
Follow-up	100% birth/karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Different reference standard depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	One outlier excluded from analysis

Su 2002

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	356 participants Taiwan January 1995 - November 1998

	Singleton pregnancies 14-21 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 36 affected cases Reference standard; Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing
Index and comparator tests	Placental growth factor 95th percentile Sandwich enzyme immunoassay technique (R and D systems, Minneapolis USA)
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal karyotype Miscarriage with cytogenetic testing
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Refence standard differs
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given

Su 2002 (*Continued*)

Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Suzumori 1997

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	1078 participants 15-18 weeks gestation Japan April 1994 - March 1996 Singleton pregnancy
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 14 affected cases Reference standard - amniocentesis
Index and comparator tests	Second trimester serum AFP Second trimester serum hCG Second trimester serum uE3 Maternal age AFP - Abbott Ltd USA hCG - Wallac Finland uE3 - Diagnostic products corps, USA
Follow-up	100% Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women had amniocentesis

Suzumori 1997 (*Continued*)

Differential verification avoided? All tests	Yes	All women had amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Yes	Outcome unknown to operator
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Talbot 2003

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	328 participants UK Dates not specified Singleton pregnancies 2nd trimester
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 50 affected cases Reference standards; Amniocentesis Postnatal examination
Index and comparator tests	Second trimester serum hCG glycoform Second trimester serum free ß hCG Second trimester serum AFP Maternal age hCG Glycoforms - Lectin immunoassay free ß hCG, AFP, total hCG - Kryptor analyser (TRACE)
Follow-up	100% Birth/Karyotype

Talbot 2003 (*Continued*)

Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting- screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Poatnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differed according to index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Index test results not known to operator
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Van Lith 1992

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	90 participants The Netherlands Dates not specified Pregnant women 14-18 weeks gestation
Study design	Retrospective case-control study

Van Lith 1992 (*Continued*)

Target condition and reference standard(s)	Down's syndrome 10 affected cases Reference standard - Amniocentesis
Index and comparator tests	2nd trimester serum Inhibin A 2 site enzyme immunoassay specific for alpha peptide of human Inhibin AEASIA apparatus
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Verloes 1995

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	10454 participants Belgium January 1991-September 1992 Pregnant women 14-24 weeks by USS
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 15 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	Maternal age Second trimester maternal serum uE3 Second trimester maternal serum hCG Second trimester maternal serum AFP Dried blood samples on blotting paper card Non radioactive immunologic step followed by colorimetric quantification of a horseradish pre oxidase
Follow-up	Birth/Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Refence standard differed
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given

Verloes 1995 (*Continued*)

Index test results blinded? All tests	Yes	Analysed without knowledge of karyotype
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Wald 2003a

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	1092 participants UK and Austria (multi-centre trial) September 1996 to April 2000 Pregnant women booking at 8-14 weeks gestation by LMP and confirmed by USS Viable pregnancy
Study design	Prospective nested case-control study
Target condition and reference standard(s)	Down's syndrome 82 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	NT at 9-13 weeks Serum AFP, hCG, uE3, PAPP-A, free ß hCG, Inhibin A - 1st and 2nd trimester Urinary ß core fragment, total hCG, ITA and free ß hCG - 1st and 2nd trimester NT - midsaggital section, optimal magnification of thickness of translucent space between inner skin surface and fascia covering cervical spine (white black interface (outer) - black white interface (inner)) 41 models of ultrasound machine 20 minutes allotted scanning time SERUM - Each Down's pregnancy matched with 5 controls AFP, free ß hCG, total hCG, uE3 and PAPP-A measured with time resolved fluoroimmunoassay (AutoDELFIA) Inhibin A - Sandwich enzyme linked immunosorbent assay (Oxford bio innovation) URINE - ITA and ß core fragment (Quest diagnostics USA) Total hCG and free ß hCG as per serum
Follow-up	96% Birth/Karyotype full outcome documentation obtained

Wald 2003a (*Continued*)

Notes	Performance of screening assessed at 17 weeks gestation

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differed depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	No	Known to operator prior to performing reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Ward 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy Clinical setting - screening programme
Participants	13613 participants UK - single centre 1992-1997 Singleton pregnancies 15-18 weeks gestation
Study design	Prospective consecutive series study

Target condition and reference standard(s)	Down's syndrome 16 affected cases Reference standard; Amniocentesis Postnatal examination
Index and comparator tests	2nd trimester serum AFP 2nd trimester serum uE3 2nd trimester serum hCG Maternal age AFP - Amerlite 2T hCG and uE3 - Amerlex M2T
Follow-up	100% birth/karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	Clinical setting - screening programme
Acceptable reference standard? All tests	Yes	Amniocentesis Postnatal examination
Partial verification avoided? All tests	Yes	All women received reference standard
Differential verification avoided? All tests	No	Reference standard differs depending on index test result
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Yes	Performed prior to reference standard
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None

Withdrawals explained? All tests	Yes	None

Watanabe 2002

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	25 participants Japan Dates not specified Singleton pregnancies 15-17 weeks gestation
Study design	Prospective case-control study
Target condition and reference standard(s)	Down's syndrome 5 affected cases Refernce standard - Amniocentesis
Index and comparator tests	Second trimester serum PAPP-A Second trimester serumInhibin A Maternal age PAPP-A - Amerlex M PAPP-A IRMA kit Inhibin A - serotec dimeric Inhibin A immunoassay kit
Follow-up	100% Birth/Karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	No	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate

Watanabe 2002 (*Continued*)

Reference standard results blinded? All tests	Yes	Index test result unknown to operator
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Wenstrom 1997

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	349 participants USA 1992-1996 Pregnant women 14-20 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 33 affected pregnancies Reference standard - Amniocentesis
Index and comparator tests	Second trimester serum Inhibin A Second trimester serum AFP Second trimester serum uE3 Second trimester serum hCG Maternal age Inhibin A - ELISA (Serotec, Oxford) Other serum tests see Wenstrom 1997a
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**

Wenstrom 1997 (*Continued*)

Representative spectrum? All tests	Yes	HIgh-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Wenstrom 1997a

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	344 participants USA 1992-1996 Pregnant women 14-20 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 31 affected cases Reference standard - amniocentesis

Wenstrom 1997a (*Continued*)

Index and comparator tests	Second trimester serum AFP Second trimester serum uE3 Second trimester serum hCG Second trimester serum free ß hCG Second trimester serum Maternal age AFP - Sarofi Pasteur Intact hCG - Nichols institute uE3 - Diagnostic systems laboratory Free ß hCG - CIS - US Bedford, solid phase 2 site sandwich immunoradiometric assay
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	**Authors' judgement**	**Description**
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Wenstrom 1997b

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	328 participants USA 1993-1995 Pregnant women 14-20 weeks gestation
Study design	Retrospective case-control study
Target condition and reference standard(s)	Down's syndrome 22 affected cases Reference standard - amniocentesis
Index and comparator tests	Maternal age Second trimester maternal serum CA125 Second trimester maternal serum AFP Second trimester maternal serum hCG CA125 - ELISA (Centocor) AFP - Sanofi pasteur hCG - Nichols institute
Follow-up	Birth/Karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description
Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Yes	Retrospective study

Wenstrom 1997b (*Continued*)

Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

Wenstrom 1999

Clinical features and settings	Request for Down's syndrome screening in pregnancy High-risk referral for invasive testing
Participants	1256 participants USA August 1996 - August 1998 Pregnant women 14-20/40 gestation
Study design	Prospective consecutive series study
Target condition and reference standard(s)	Down's syndrome 13 affected cases Reference standard - amniocentesis
Index and comparator tests	Second trimester serum AFP Second trimester serum uE3 Second trimester serum total hCG Second trimester serum free ß hCG Second trimester serum Inhibin A Maternal age uE3 - Diagnostic systems laboratory, Texas Inhibin A - Serotec, Oxford AFP and total hCG - Chemoluminescent procedure on Chiron ACS automatic analyser Free ß hCG - Solid phase 2 site immunoradiometric assay
Follow-up	100% karyotype
Notes	

Table of Methodological Quality

Item	Authors' judgement	Description

Wenstrom 1999 (*Continued*)

Representative spectrum? All tests	Yes	High-risk referral for invasive testing
Acceptable reference standard? All tests	Yes	Amniocentesis
Partial verification avoided? All tests	Yes	All women received amniocentesis
Differential verification avoided? All tests	Yes	All women received amniocentesis
Incorporation avoided? All tests	Yes	Index and reference standards separate
Reference standard results blinded? All tests	Unclear	No information given
Index test results blinded? All tests	Unclear	No information given
Relevant clinical information? All tests	Yes	Appropriate clinical information given
Uninterpretable results reported? All tests	Yes	None
Withdrawals explained? All tests	Yes	None

AFP: alpha-fetoprotein
CVS: chorionic villus sampling
hCG: human chorionic gonadotrophin
MoM: multiples of the median
NTD: neural tube defect
PAPP-A: pregnancy-associated plasma protein A
ProMBP: Proform of Eosinophil Major Basic Protein
uE3: unconjugated oestriol

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Abbas 1995	Unable to extract useful data.
Abdul-Hamid 2004	No Down's syndrome pregnancies.
Abraha 1999	Unable to extract useful data.
Adekunle 1999	Unable to extract useful information.
Aitken 1993	Unable to extract useful data.
Aitken 1996a	Fewer than 80% of pregnancies had gestational age confirmed by USS
Aitken 1996b	Fewer than 80% of pregnancies had gestational age confirmed by USS
Akbas 2001	Less than 5 Down's syndrome pregnancies.
Antona 1998	Likely fewer than 80% of pregnancies dated by USS.
Antsaklis 1999	Women screened at greater than 24 weeks gestation.
Ashwood 1987	Unable to extract useful data.
Asrani 2005	Review article.
Audibert 2001b	Unable to ascertain whether part of screening population in Rozenberg et al. No response from authors therefore excluded to reduce risk of data replication
Axt-Fleidner 2006	Unable to extract useful data.
Azuma 2002	Unable to extract useful data.
Baghagho 2004	Unable to obtain paper.
Bahado-Singh 1995	USS markers greater than 14 weeks gestation.
Bahado-Singh 1996	USS markers greater than 14 weeks gestation.
Bahado-Singh 1999b	USS markers greater than 14 weeks gestation.
Bahado-Singh 2002	USS markers greater than 14 weeks gestation.
Bahado-Singh 2003	Review article.
Bar-Hava 2001	No Down's pregnancies in study population.

(*Continued*)

Barkai 1996	No Down's pregnancies in study population.
Barnabei 1995	No Down's pregnancies in study population.
Bartels 1988	Unable to extract useful data.
Bartels 1993	No Down's pregnancies in study population.
Barth 1991	Second trimester ultrasound study.
Baviera 2004	Unclear method of confirmation of gestational age.
Bazzett 1998	Male versus female fetuses.
Bellver 2005	No Down's syndrome pregnancies in study.
Benn 1995	Less than 80% follow-up.
Benn 1996	Less than 80% follow-up.
Benn 1997	No Down's pregnancies in study population.
Benn 1998	Less than 80% follow-up.
Benn 2001	Statistical modelling (computer simulation).
Benn 2002	Modelled data.
Benn 2003a	Less than 80% of pregnancies dated by USS.
Benn 2003b	Editorial.
Benn 2005a	No Down's pregnancies included.
Benn 2005b	Mathematical model.
Berry 1995	Less than 80% of pregnancies USS dated.
Berry 1997	Less than 80% of pregnancies USS dated.
Bersinger 1994	Gestational age not USS estimated.
Bersinger 2000	Unable to extract useful data.
Bersinger 2001	No Down's syndrome pregnancies in study population.
Bersinger 2003	Unable to extract useful data.

(*Continued*)

Bersinger 2004	No Down's syndrome pregnancies in study population.
Bersinger 2005	No Down's syndrome pregnancies in study population.
Biggio 2004	Cost-effectiveness analysis.
Bindra 2002	Review article.
Blundell 1999	Unable to extract useful data.
Boots 1989	Population risk factor calculations.
Borruto 2002	Unable to extract useful data.
Boue 1990	Review article.
Bradley 1994	Screen negative population gestations not confirmed by ultrasound
Braithwaite 1996	Review article.
Brambati 1995	USS screening inclusive of women greater than 14 weeks gestation
Brambati 1996	Review article.
Brizot 1995a	Unable to extract useful data.
Brizot 1995b	Unable to extract useful data.
Brizzi 1989b	Second trimester ultrasound.
Brock 1990	Unable to extract useful data.
Campogrande 2001	Unable to extract useful data.
Canick 1988	Unable to extract useful data.
Canick 1995b	Unable to extract useful data.
Canini 2002	No Down's syndrome pregnancies in study population.
Cans 1998	Second trimester ultrasound.
Carreras 1991	Second trimester ultrasound.
Chen 1999	Review article.
Chen 2002	No Down's syndrome pregnancies in study population.
Chen 2004	Less than 5 Down's cases in study population.

(*Continued*)

Chen 2005	Unable to extract useful data.
Cheng 1993	Likely that fewer than 80% of gestational age confirmed by USS
Cheng 1999	Case series. No Down's syndrome pregnancies in study population
Cheng 2004a	No Down's syndrome pregnancies in study population.
Cheng 2004b	No Down's syndrome pregnancies in study population.
Chitayat 2002	Less than 5 Down's cases in study population.
Christiansen 2002	Unable to extract useful data.
Christiansen 2007	Unable to extract useful data.
Chung 2000	Less than 5 Down's syndrome pregnancies in study population.
CNGOF 1996	Unable to obtain translation.
Cole 1996	Review article.
Comas 2001	USS at greater than 14 weeks.
Comas 2002a	USS at greater than 14 weeks.
Comas 2002b	USS at greater than 14 weeks.
Comstock 2006	Unable to extract useful data.
Conde 1998	Review article.
Crossley 1991	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1993	Less than 80% of pregnancies had gestational age confirmation by ultrasound
Crossley 1996	No Down's syndrome pregnancies in study population.
Crossley 2002a	Adjustment factors for smokers.
Cuckle 1984	Gestational age not confirmed by USS.
Cuckle 1987a	Gestational age not confirmed by USS.
Cuckle 1987b	No gestational age limits given.
Cuckle 1990	Paper presenting adjustment factors.

(*Continued*)

Cuckle 1996	Data modelled on 4 meta-analysed studies.
Cuckle 1999a	Unable to extract useful data.
Cuckle 1999b	Review article.
Cullen 1990	Abnormal scans only in study population.
Cusick 2004	Less than 5 Down's syndrome pregnancies in study population.
D'Ottavio 1997	Second trimester USS.
Dancoine 2001	No Down's syndrome pregnancies in study population.
De Biasio 2000	Unable to extract useful information.
De Biasio, 1999	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Biasio, 2001	Unable to ascertain whether overlapping populations between several papers - attempted to contact author with no response
De Graaf 1991	Unable to extract useful data.
De Graaf 1999	Modelled data.
DeVore 2001	Second trimester ultrasound.
Dickerson 1994	Comment.
Dimaio 1987	Gestational age by USS only in screen positive population.
Doran 1986	Ultrasound confirmation of gestational age performed in screen positive women only
Drugan 1996a	Second trimester ultrasound.
Drugan 1996b	Unable to extract useful data.
Drysdale 2002	Fewer than 5 Down's syndrome pregnancies in population.
Ebell 1999	Review article.
Economides 1998	Unable to extract useful data.
Erickson 2004	No Down's syndrome pregnancies in population.
Evans 1996	No Down's syndrome pregnancies in population.

(*Continued*)

Falcon 2005	Unable to extract useful data.
Falcon 2006	Unable to extract useful data.
Ford 1998	Audit.
Frishman 1997	No Down's syndrome pregnancies in population.
Fukada 2000	Unable to extract useful data.
Ghidini 1998	Comparison of male versus female fetuses.
Goldie 1995	Fewer than 80% of study population ahd gestational age confirmed by USS
Gonçalves 2004	Greater than 14 weeks USS screening.
Goodburn 1994	Likely that fewer than 80% of pregnancies had gestational age estimated by USS
Grozdea 2002	Unable to extract useful data.
Gyselaers 2004a	Less than 80% follow-up.
Gyselaers 2004b	Less than 80% follow-up.
Gyselaers 2006a	Unaffected pregnancies only.
Gyselaers 2006b	Unable to extract useful data.
Hackshaw 1995	No Down's syndrome pregnancies in population.
Hackshaw 2001	No Down's syndrome pregnancies in population
Haddow 1992	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Hafner 1995	Less than 5 Down's pregnancies in study population.
Hallahan 1998	Gestational age greater than 24 weeks.
Harrison 2006	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Harry 2006	Editorial.
Hayashi 1995	Unable to extract useful data.
Hayashi 1996	Less than 5 Down's pregnancies in study population.
Heikkila 1997	Fewer than 80% of pregnancies had gestational age confirmed by USS

(*Continued*)

Heinonen 1996	No Down's syndrome pregnancies in population.
Herman 2000	No Down's syndrome pregnancies in study population.
Herman 2003	Correlation between markers, not evaluation of screening tests
Herrou 1992	Unable to extract useful data.
Hershey 1985	Gestation unclear.
Hershey 1986	Gestation based on LMP.
Hewitt 1993	Unable to extract useful data.
Hogdall 1992	Unclear method of determination of gestational age. Unable to extract useful data
Hong Kong Practitioner	CME.
Howe 2000	Second trimester ultrasound scans.
Hsiao 1991	Unable to obtain translation.
Hsieh 1999	No Down's syndrome pregnancies in study population.
Hsu 1997b	Adjustment factors.
Hsu 1998a	No Down's syndrome pregnancies in study population.
Hsu 1999b	No Down's pregnancies.
Huang 2003	No Down's syndrome pregnancies in study population.
Huggon 2004	Study of cardiac function in pregnancies with normal and abnormal NT results
Hui 2003	No Down's syndrome pregnancies in population.
Hui 2005	No Down's syndrome pregnancies in population.
Hultén 2004	Editorial/commentary.
Hung 2003	Modelling.
Hurley 1993	Unable to extract useful data.
Huttly 2004	No Down's syndrome pregnancies in population.
Hwa 2004	Less than 5 Down's pregnancies in population.

(*Continued*)

Iles 1996	Review.
Ind 1994	Unable to extract useful data.
Jean-Pierre 2005	Review article.
Johnson 1991	Gestatiojnal age estimated by USS in fewer than 80% of cases
Johnson 1993	Normal pregnancies only.
Jorgensen 1999	Gestation greater than 14 weeks for USS.
Josefsson 1998	No Down's syndrome pregnancies in study population.
Jou 2001	Less than 5 Down's syndrome pregnancies in study population.
Jun-Tao 2003	Unable to obtain translation.
Kagan 2006	Screen positive pregnancies only.
Kautzmann 1995	Fewer than 80% pregnancies had gestational age estimated by USS
Keith 1992	Summary article.
Kelekci 2004	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995a	Less than 5 Down's syndrome pregnancies in population.
Kellner 1995b	Less than 80% follow-up. Unable to ascertain proportion of population with gestational age confirmed by USS
Kellner 1997	Assumption of normal karyotype without reference standard in significant proportion of control pregnancies
Knight 1990	Review article.
Knight 2001	Validation of a specific assay.
Knight 2005	Less than 80% of pregnancies had gestational age confirmed by ultrasound scan
Koos 2006	Review article.
Kornman 1996	Less than 5 Down's syndrome pregnancies in population.
Kornman 1997	Unable to extract useful information.
Kramer 1998	No Down's syndrome pregnancies in study population.

(*Continued*)

Krantz 1996	Modelled data.
Krantz 2005	Adjustment factor.
Kulch 1993	No Down's cases in population.
Lai 1998	Modelled population..
Lai 2003	No Down's syndrome pregnancies in study population.
Laigaard 2006a	Unable to extract useful data.
Laigaard 2006b	Simulation.
Lam 1997	Unable to extract useful data.
Lam 1998	Fewer than 80% pregnancies had gestational age estimated by USS
Lam 1999a	No Down's syndrome pregnancies in population
Lam 1999b	Unable to extract useful data
Lam 2000	Study of women's decisions about screening.
Lam 2001	Male versus female fetuses.
Lambert-Messerlian 1996	Fewer than 80% of pregnancies USS dated.
Lambert-Messerlian 1998	Unable to extract useful data.
Lehavi 2005	Down's syndrome pregnancies only.
Leung 2006	Unable to separate twins from singletons therefore unable to extract useful data
Leymarie 1993	Appears to be a review article (French).
Li 1998	Unable to obtain translation.
Li 1999	Unable to obtain translation.
Liao 1997	Unable to obtain translation.
Liao 2001	Unable to extract useful data.
Lim 2002	Second trimester ultrasound.
Lippman 1987	Editorial.

(*Continued*)

Lustig 1988	Gestational age by LMP only.
MacDonald 1991	Fewer than 80% of gestational ages estimated by USS.
Macintosh 1994	Unable to extract useful data.
Macintosh 1997	Unable to extract useful data.
Macri 1994	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Macri 1996	Likely fewer than 80% evaluated for gestational age by ultrasound examination
Malone 1998	Review article.
Malone 2003	Review article.
Mangione 2001	Abnormal screening results only.
Maymon 2001a	No Down's syndrome pregnancies in study population.
Maymon 2001b	No normal test results included therefore unable to extract meaningful data
Maymon 2002	No Down's syndrome pregnancies in study population.
Maymon 2004	No Down's syndrome pregnancies in study population.
Maymon 2005	Modelled data.
McDuffie 1996	USS dating on screen positive women only.
Meier 2002	Observed vs expected cases of Down's syndrome in a population
Merkatz 1984	Gestational age not confirmed by ultrasound scan.
Merz 2005	Editorial.
Metzenbauer 2001	Normal pregnancies only.
Metzenbauer 2002	Unable to extract useful data.
Mikic 1999	No Down's syndrome pregnancies in study population.
Miller 1991	Unable to extract useful data.
Milunsky 1989	Fewer than 80% gestational age estimated by USS.
Milunsky 1996	Fewer than 80% gestational age estimated by USS.

(*Continued*)

Minobe 2002	Gestational age greater than specified limits.
Miyamura 1999	Unable to extract useful data.
Moghadam 1998	Unable to extract useful data.
Monni 2000	Less than 5 Down's syndrome pregnancies.
Monni 2002	Review article.
Mooney 1994	Greater than 24 weeks gestation.
Muller 1994	No Down's syndrome pregnancies in study population.
Muller 1996b	Unable to extract useful data.
Muller 1999	Unable to extract useful data.
Muller 2002a	Getstional age greater than 24 weeks.
Muller 2002b	Unable to extract meaningful data - unable to separate double and triple test data
Muller 2003	No Down's syndrome pregnancies in study population.
Murta 2002	Unable to extract useful data.
Musone 2000	Unable to extract useful data.
Musto 1986	Fewer than 80% USS dated.
Myrick 1990	Unable to extract useful data.
Neveux 1996a	No Down's syndrome pregnancies in population.
Neveux 1996b	Unable to extract useful data.
Ng 2004	Unable to extract useful data.
Nicolaides 1992	Study of outcomes of abnormal NT results.
Nicolaides 2000	Review article.
Nicolaides 2004	Review article.
Nicolaides 2005a	Unable to obtain translation - appears to be a review article
Nicolaides 2005b	Unable to obtain translation - appears to be a review article

(*Continued*)

Nicolaides 2005c	Unable to obtain translation - appears to be a review article
Nicolaides 2005d	Unable to obtain translation - appears to be a review article
Nicolaides 2005e	Unable to obtain translation - appears to be a review article
Nicolaides 2005f	Review article.
Niemimaa 2001	No Down's pregnancies in study population.
Niemimaa 2002	No Down's syndrome pregnancies in population.
Niemimaa 2003	No Down's syndrome pregnancies in population.
Noble 1997	Unable to extract useful data.
Norgaard 1990	Less than 80% of gestational ages confirmed by USS.
Norton 1992	Unable to extract useful data.
O'Brien 1997a	No Down's syndrome pregnancies in population.
O'Brien 1997b	No Down's syndrome pregnancies in population.
Odibo 2004	Gestational age of greater than 14 weeks in USS population.
Ognibene 1999	Unable to extract useful data.
Olajide 1989	Unable to extract useful data.
Onda 1996	Unable to extract useful data
Onda 1998	Unable to extract useful data.
Onda 2000	Less than 80% follow-up.
Orlandi 2002	No Down's syndrome pregnancies in study population
Palka 1998	Twin data used in calculation of the median.
Palomaki 1989	Fewer than 80% USS dated.
Palomaki 1993	No Down's syndrome pregnancies in population.
Palomaki 1994	No Down's syndrome pregnancies in population.
Palomaki 1996	Meta-analysis.

(*Continued*)

Palomaki 2005	Unable to extract meaningful data.
Panburana 2001	Less than 5 Down's syndrome pregnancies in population.
Pandya 1994	Study of outcomes of abnormal NT results.
Pandya 1995	Review article.
Paul 2001	Unable to extract useful data.
Peralta 2005	Unable to extract useful data.
Perenc 1998	No Down's syndrome pregnancies in study population.
Perheentupa 2002	No Down's syndrome pregnancies in population.
Perona 1998	Smokers versus non smokers.
Petervari 2000	Unable to extract useful data.
Petrocik 1989	Likely fewer than 80% USS dated.
Phillips 1992	Gestational age confirmed by USS in less than 80% of population
Phillips 1993	Gestational age confirmed by USS in less than 80% of population
Pinette 2003	Women screened prior to recruitment.
Platt 2004	Unable to extract useful data.
Podobnik 1995	Abnormal results only.
Prefumo 2002	Comparison of prevalence and predicition.
Prefumo 2004	Comparison of a marker in women of different ethnic origins.
Price 1998	Unable to extract useful data.
Páez 2004	Unable to obtain translation.
Raty 2000	No Down's syndrome pregnancies in population.
Rembouskos 2004	Unable to extract useful data.
Ren 1992	Review article.
Renier 1998	Method of ascertainment of gestational age unclear. Twin gestations included in general population

(*Continued*)

Resta 1990	Second trimester USS.
Reynders 1997	Fewer than 5 Down's cases.
Reynolds 1989	Explanation of mathematical techniques.
Reynolds 1999	Unable to extract useful data.
Ribbert 1996	No Down's syndrome pregnancies in study population.
Rice 2005	Down's syndrome pregnancies excluded from study.
Rich 1991	Unable to extract useful data.
Roberts 1995	No Down's syndrome pregnancies in study population.
Robertson 1991	Editorial.
Rode 2003	No Down's pregnancies.
Ronge 2006	Editorial - summary of FASTER results.
Rose 1995	Review article.
Ross 1997	Review article.
Rotmensch 1996	Unable to extract useful data.
Rotmensch 1999	No Down's syndrome pregnancies in study population.
Rozenberg 2006	USS greater than 14 weeks gestation.
Rudnicka 2002	No Down's syndrome pregnancies in population.
Ryall 1992	Unable to determine method of confirmation of gestational age
Ryall 2001	High-risk results only included (i.e. no screen negative group for comparison)
Räty 2002	No Down's pregnancies in population.
Sabriá 2002	Unable to ascertain hjow numbers calculated and from which populations
Sacchini 2003	Unable to extract useful data.
Saller 1997	Down's syndrome secondary to Robertsonian translocation only. No controls
Salomon 2001	No Down's syndrome pregnancies in population.

(*Continued*)

Salonen 1997	Fewer than 80% had gestational age estimated by USS.
Saltvedt 2005	Gestation greater than 14 weeks for nuchal scanning.
Saridogan 1996	Down's syndrome and Edward's syndrome affected pregnancies only
Savoldelli 1993	Unable to extract useful data.
Schiott 2006	Unable to extract useful data.
Schuchter 1998	No Down's pregnancies in study population.
Scott 1995	Less than 5 Down's syndrome pregnancies in study population.
Seeds 1990	Review article.
Seki 1995	No Down's syndrome pregnancies in study population.
Shenhav 2003	No Down's syndrome pregnancies.
Shintaku 1989	Unable to extract useful data.
Shulman 2003	No Down's syndrome pregnancies in population.
Simon-Bouy 1999	Review article.
Simpson 1986	Gestational age confirmed by USS in less than 80% of population
Smith 1990	Analysis of screen positive results.
Smith 1996	Review/meta-analysis.
Smith 1999	Unable to extract useful data.
Smith-Bindman 2001	Meta-analysis of second trimester ultrasound markers.
Smith-Bindman 2003	Population study, not examining DTA.
Snijders 1995	Study of prevalence, not screening.
Snijders 1999	Study of prevalence, not screening.
Soergel 2006	Less than 80% follow-up.
Sokol 1998	Observation of Down's prevalence stratified by age.
Sonek 2003	Editorial.

(*Continued*)

Spencer 1985	Fewer than 80% USS dated.
Spencer 1991a	Likely fewer than 80% USS dated.
Spencer 1991b	Unable to extract useful data.
Spencer 1992	Unable to extract useful data.
Spencer 1993a	Fewer than 80% USS dated.
Spencer 1993b	No Down's pregnancies in study population.
Spencer 1993c	Unable to extract useful data.
Spencer 1993d	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1993e	Unable to extract useful data.
Spencer 1995	No Down's pregnancies in population.
Spencer 1996a	Fewer than 80% of pregnancies had gestational age confirmed by USS
Spencer 1997	Statistical modelling, aneuploid pregnancies only in study population
Spencer 1998a	No Down's pregnancies in population.
Spencer 1998b	Unable to extract useful data.
Spencer 1999a	Review.
Spencer 1999b	Statistical methods paper.
Spencer 2000a	Examination of median shifts rather than an evaluation of screening
Spencer 2000b	No Down's syndrome pregnancies in population.
Spencer 2000c	No Down's syndrome pregnancies in population.
Spencer 2000d	No Down's cases.
Spencer 2000e	Male versus female fetuses.
Spencer 2000f	No Down's cases in population.
Spencer 2000g	No Down's pregnancies in population.

(*Continued*)

Spencer 2000h	No Down's pregnancies in population.
Spencer 2000i	Comparsison of fetal sex.
Spencer 2001	No Down's syndrome pregnancies in population.
Spencer 2001a	Unable to extract useful data.
Spencer 2001b	Unable to extract useful data.
Spencer 2001c	Unable to extract useful data.
Spencer 2001d	No Down's syndrome pregnancies in population.
Spencer 2002a	No Down's pregnancies.
Spencer 2002b	Risk validation study.
Spencer 2002c	No Down's syndrome pregnancies in population.
Spencer 2002d	Demonstration of median changes with time, rather than evaluation of screening
Spencer 2003a	No Down's pregnancies in population.
Spencer 2003b	No Down's pregnancies in population.
Spencer 2003c	Calculation of weight correction factor.
Spencer 2003d	Fewer than 5 Down's syndrome pregnancies.
Spencer 2004	Calculation of smoking correction factor.
Spencer 2005a	No Down's pregnancies.
Spencer 2005b	No Down's pregnancies.
Spencer 2005c	Comparison of two different assays - not actual screening evaluation
Spong 1999	Comparison of male and female fetuses.
Stevens 1998	Literature review.
Stoll 1992	Review article.
Su 2002a	Unable to extract useful data.
Suchet 1995	Review article.

(*Continued*)

Suchy 1990	Unable to ascertain method of confirmation of gestational age
Summers 2003a	Only 55% gestational ages estimated by USS.
Summers 2003b	No Down's syndrome pregnancies in study population.
Suntharasaj 2005	Examination of inter-observer variation in NT scanning.
Sutton 2004	Unable to extract useful data.
Suzuki 1998	Unable to extract useful data.
Tabor 1987	Geststional age not confirmed by USS.
Tanski 1999	Information on screen positive pregnancies only.
Thilaganathan 1998	No Down's syndrome pregnancies in study population.
Thilaganathan 1999	Editorial.
Tislaric 2002	No Down's syndrome pregnancies in population.
Torok 1997	Unable to extract useful data.
Tsai 2001	Less than 5 Down's syndrome pregnancies in study population.
Valerio 1996	Fewer than 80% pregnancies had gestational age estimated by USS
Van Blerk 1992	Unable to extract useful data.
Van Heesch, 2006	No Down's syndrome pregnancies in study population. Software comparison study
Van Lith 1991	Unable to extract useful data.
Van Lith 1993	Unable to extract useful data.
Van Lith 1994	Unable to extract useful data.
Veress 1986	Unable to extract useful data.
Veress 1988	Unable to extract useful data.
Vintzileos 2003	Second trimester USS.
Wald 1988a	Less than 80% had gestational age confirmed by ultrasound.
Wald 1988b	Gestational age not confirmed by USS.

(*Continued*)

Wald 1991	No Down's pregnancies in study.
Wald 1992a	Less than 80% had gestational age confirmed by ultrasound.
Wald 1992b	No Down's pregnancies in study.
Wald 1992c	No Down's pregnancies in study.
Wald 1993	No USS dating.
Wald 1994a	No Down's syndrome pregnancies in population.
Wald 1994b	Review article.
Wald 1996a	No Down's pregnancies.
Wald 1996b	Dated by LMP.
Wald 1996d	No Down's syndrome pregnancies in population.
Wald 1996e	Gestational age greater than 24 weeks.
Wald 1997	Data modelled on 3 separate populations of women.
Wald 1998	Unable to extract useful data.
Wald 1999a	Unable to extract useful data.
Wald 1999b	Gestational age not confirmed by USS.
Wald 1999c	No Down's syndrome pregnancies.
Wald 1999d	Modelled on several studies, some of which have no USS dating
Wald 2003b	No cases.
Wald 2003c	Less than 80% had gestational age confirmed by USS.
Wald 2006	Modelled on SURRUS data.
Wallace 1994	Unable to extract useful data.
Wallace 1997	No Down's syndrome pregnancies in study population.
Ward 2005	Review article.
Watt 1996a	No Donw's syndrome pregnancies in study population.

(*Continued*)

Watt 1996b	No Down's syndrome pregnancies in study population.
Weinans 2001	Unable to extract useful data.
Weinans 2004	Study of women's views on screening.
Welborn 1994	Abnormal results only (cystic hygroma).
Wenstrom 1993	Less than 80% of pregnancies had gestational age confirmed by USS
Wenstrom 1995a	Adjustment factors.
Wenstrom 1995b	Less than 80% of pregnancies had gestational age confirmed by USS
Whitlow 1998a	Unable to extract useful data.
Whitlow 1998b	Unable to extract useful data.
Whitlow 1999	Unable to extract useful data.
Williamson 1994	Likely fewer than 80% USS dated.
Wilson 2000	Review.
Wojdemann 2001	No Down's syndrome pregnancies in study population.
Wong 2003	Less than 5 Down's syndrome pregnancies in population.
Wright 2006	Mathematical model.
Yagel 1998	Second trimester USS.
Yamamoto 2001a	Unable to extract useful data.
Yamamoto 2001b	Method of determination of gestational age unclear.
Yamamoto 2001c	Unable to extact useful data.
Yaron 2001	Male versus female fetuses.
Ye 1995	Unable to obtain translation.
Yoshida 2000	Fewer than 80% pregnancies had gestational age estimated by USS
Zeitune 1991	Only aneuploid pregnancies included in study.
Zelop 2005	No Down's cases in population.

(*Continued*)

Zhao 1998	Unable to obtain translation.
Zoppi 2003	Inappropriate study design.

LMP: last menstural period
USS: ultrasound screening

DATA

Presented below are all the data for all of the tests entered into the review.

Tests. Data tables by test

Test	No. of studies	No. of participants
1 Inhibin A at mixed cutpoints	4	1590
2 Inhibin A at 5% FPR	2	1192
3 Inhibin A at 2.4MoM	1	90
4 Inhibin A at 2 MoM	2	398
5 SP1 at mixed cutpoints	3	777
6 SP1 at 2.5MoM	1	325
7 SP1 at 5% FPR	2	452
8 AFP at mixed cutpoints	5	14201
9 AFP at 0.8MoM	1	3272
10 AFP at 5% FPR	3	9457
11 AFP at SD (specified in paper)	1	1472
12 Total hCG at 5% FPR	2	1192
13 Total hCG at 2.5MoM	1	246
14 Total hCG at mixed cutpoints	3	1438
15 Free ßhCG at 5% FPR	2	9357
16 uE3 at 5% FPR	2	1192
17 Troponin at 5% FPR	1	283
18 Free ßhCG to AFP ratio at 5% FPR	1	8265
19 PAPP-A at 5% FPR	1	1092
20 PGF at 95th percentile	1	356
21 CA125 at 1.5MoM	1	328
22 Age and Total hCG at 5% FPR/95th percentile	3	57257
23 Age and Total hCG at mixed cutpoints	4	57768
24 Age and Total hCG at 1:384 risk	1	511
25 Age and AFP at 1:270 risk	1	3896
26 Age and AFP at 5% FPR	2	9357
27 Age and AFP at mixed cutpoints	4	13764
28 Age and Free ßhCG at mixed cutpoints	4	14985
29 Age and Free ßhCG at 1:384 risk	1	511
30 Age and uE3 at mixed cutpoints	2	1603
31 Age and uE3 at 1:384 risk	1	511
32 Age and Free ßhCG to AFP at 5% FPR	1	8265
33 Age and inhibin at 5% FPR	2	1117

34 Age and PAPP-A at 5% FPR	2	1117
35 Age and ProMBP at 1:250 risk	1	256
36 Age and Free αhCG at 1:384 risk	1	511
37 Age, Total hCG and Free ßhCG at 1:384 risk	1	511
38 Age, Total hCG and uE3 at 5% FPR	1	370
39 Age, Total hCG and uE3 at 1:384 risk	1	511
40 Age, Total hCG and uE3 at mixed cutpoints	2	881
41 Age, Total hCG and AFP at 5% FPR	4	22816
42 Age, Total hCG and AFP at 1:250 risk	6	43519
43 Age, Total hCG and AFP at mixed cutpoints	15	133783
44 Age, Total hCG and SP1at 5% FPR	1	370
45 Age, Total hCG and Free αhCG at 1:384 risk	1	511
46 Age, Free ßhCG and uE3 at 1:384 risk	1	511
47 Age, Free ßhCG and AFP at 1:250 risk	3	15912
48 Age, Free ßhCG and AFP at 5% FPR	5	23979
49 Age, Free ßhCG and AFP at mixed cutpoints	12	45597
50 Age, Free ßhCG and Free αhCG at 1:384 risk	1	511
51 Age, AFP and uE3 at 1:384 risk	1	511
52 Age, AFP and uE3 at 5% FPR	1	370
53 Age, AFP and uE3 at mixed cutpoints	2	881
54 Age, uE3 and Free αhCG at 1:384 risk	1	511
55 Age, uE3 and SP1 at 5% FPR	1	370
56 Age, AFP and SP1 at 5% FPR	1	370
57 Age, AFP and Hyperglycosylated hCG at 5% FPR	1	328
58 Age, AFP and Free αhCG 1:384 risk	1	511
59 Age, Total hCG, Free ßhCG and AFP at 1:266 risk	1	344
60 Age, Total hCG, AFP and uE3 at 5% FPR	7	15453
61 Age, Total hCG, AFP and uE3 at 1:250 risk	5	30910

62 Age, Total hCG, AFP and uE3 at mixed cutpoints	24	89047
63 Age, Total hCG, uE3 and SP1 at 5% FPR	1	370
64 Age, Total hCG, AFP and Inhibin A at 1:190 risk	2	564
65 Age, Total hCG, AFP and Inhibin A at 1:250 risk	1	218
66 Age, Total hCG, AFP and SP1 at 5% FPR	1	370
67 Age, Total hCG, AFP and CA125 at 1:190 risk	1	328
68 Age, Free ßhCG, AFP and uE3 at 5% FPR	3	6430
69 Age, Free ßhCG, AFP and uE3 at 1:250 risk	2	1809
70 Age, Free ßhCG, AFP and uE3 at mixed cutpoints	7	10541
71 Age, Free ßhCG, AFP and Inhibin A at 1:190 risk	1	1256
72 Age, Free ßhCG, AFP and ProMBP at 5% FPR	1	334
73 Age, Free ßhCG, AFP and ProMBP at 1:250 risk	1	334
74 Age, AFP, uE3 and Free αhCG at 1:384 risk	1	511
75 Age, AFP, uE3 and Inhibin A at 1:233 risk	1	346
76 Age, AFP, uE3 and SP1 at 5% FPR	1	370
77 Age, Total hCG, Free ßhCG, AFP and uE3 at 1:384 risk	1	511
78 Age, Total hCG, AFP, uE3 and Inhibin A at 5% FPR	1	1092
79 Age, Total hCG, AFP, uE3 and Inhibin A at 1:150 risk	3	2014
80 Age, Total hCG, AFP, uE3 and Inhibin A at 1:250 risk	2	758
81 Age, Total hCG, AFP, uE3 and Inhibin A at mixed cutpoints	5	38342
82 Age, Total hCG, AFP, uE3 and Free αhCG at 1:384 risk	1	511
83 Age, Total hCG, AFP, uE3 and SP1 at 5% FPR	1	370
84 Age, Free ßhCG, AFP, uE3 and Inhibin A at 5% FPR	1	1092
85 Age, Free ßhCG, AFP, uE3 and Inhibin A at 1:250 risk	1	1092
86 Age, Free ßhCG, AFP, uE3 and Inhibin A at mixed cutpoints	2	2348

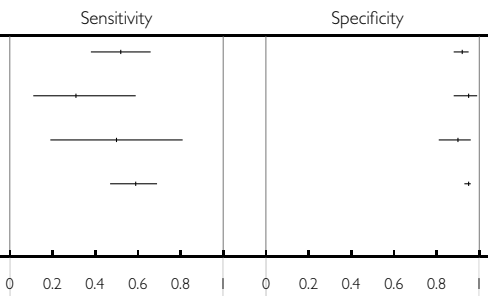
87 Age, Total hCG, AFP, uE3, Inhibin A and PAPP-A at 5% FPR	1	1092
88 Age, Total hCG, Free ßhCG, AFP, uE3 and Free αhCG at 1:384 risk	1	511
89 Age, Free ßhCG, AFP, uE3, Inhibin A and PAPP-A at 5%FPR	1	1092

Test 1. Inhibin A at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 1 Inhibin A at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Haddow 1998	27	21	25	235	0.52 [0.38, 0.66]	0.92 [0.88, 0.95]
Pandian 2004	5	4	11	80	0.31 [0.11, 0.59]	0.95 [0.88, 0.99]
Van Lith 1992	5	8	5	72	0.50 [0.19, 0.81]	0.90 [0.81, 0.96]
Wald 2003a	48	51	34	959	0.59 [0.47, 0.69]	0.95 [0.93, 0.96]

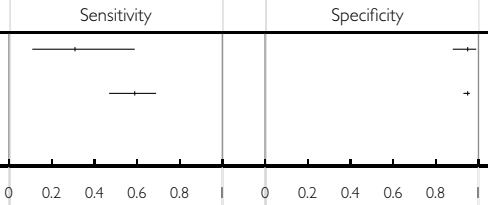


Test 2. Inhibin A at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 2 Inhibin A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Pandian 2004	5	4	11	80	0.31 [0.11, 0.59]	0.95 [0.88, 0.99]
Wald 2003a	48	51	34	959	0.59 [0.47, 0.69]	0.95 [0.93, 0.96]

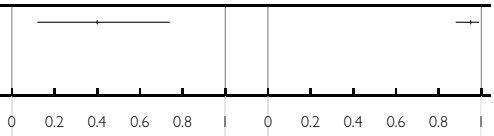


Test 3. Inhibin A at 2.4MoM.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 3 Inhibin A at 2.4MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Van Lith 1992	4	4	6	76	0.40 [0.12, 0.74]	0.95 [0.88, 0.99]

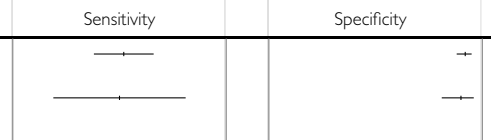


Test 4. Inhibin A at 2 MoM.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 4 Inhibin A at 2 MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Haddow 1998	27	21	25	235	0.52 [0.38, 0.66]	0.92 [0.88, 0.95]
Van Lith 1992	5	8	5	72	0.50 [0.19, 0.81]	0.90 [0.81, 0.96]

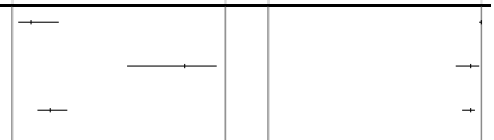


Test 5. SP1 at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 5 SP1 at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1990	4	0	39	282	0.09 [0.03, 0.22]	1.00 [0.99, 1.00]
Pandian 2004	13	4	3	80	0.81 [0.54, 0.96]	0.95 [0.88, 0.99]
Qin 1997	21	12	95	224	0.18 [0.12, 0.26]	0.95 [0.91, 0.97]

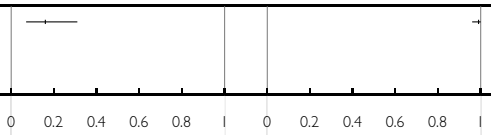


Test 6. SP1 at 2.5MoM.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 6 SP1 at 2.5MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1990	7	4	36	278	0.16 [0.07, 0.31]	0.99 [0.96, 1.00]



Test 7. SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 7 SP1 at 5% FPR

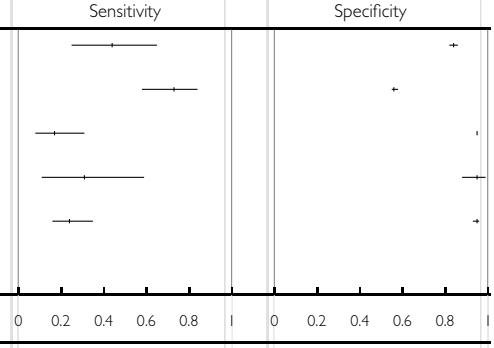
Study	TP	FP	FN	TN	Sensitivity	Specificity
Pandian 2004	13	4	3	80	0.81 [0.54, 0.96]	0.95 [0.88, 0.99]
Qin 1997	21	12	95	224	0.18 [0.12, 0.26]	0.95 [0.91, 0.97]

Test 8. AFP at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 8 AFP at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brizzi 1989a	12	231	15	1214	0.44 [0.25, 0.65]	0.84 [0.82, 0.86]
Greenberg 1991	37	1405	14	1816	0.73 [0.58, 0.84]	0.56 [0.55, 0.58]
Hsu 1997a	8	411	39	7807	0.17 [0.08, 0.31]	0.95 [0.95, 0.95]
Pandian 2004	5	4	11	80	0.31 [0.11, 0.59]	0.95 [0.88, 0.99]
Wald 2003a	20	51	62	959	0.24 [0.16, 0.35]	0.95 [0.93, 0.96]

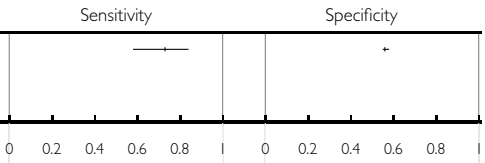


Test 9. AFP at 0.8MoM.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 9 AFP at 0.8MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Greenberg 1991	37	1405	14	1816	0.73 [0.58, 0.84]	0.56 [0.55, 0.58]

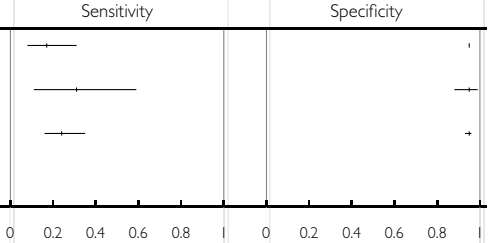


Test 10. AFP at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 10 AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Hsu 1997a	8	411	39	7807	0.17 [0.08, 0.31]	0.95 [0.95, 0.95]
Pandian 2004	5	4	11	80	0.31 [0.11, 0.59]	0.95 [0.88, 0.99]
Wald 2003a	20	51	62	959	0.24 [0.16, 0.35]	0.95 [0.93, 0.96]

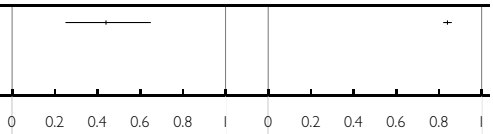


Test 11. AFP at SD (specified in paper).

Review: Second trimester serum tests for Down's Syndrome screening

Test: 11 AFP at SD (specified in paper)

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brizzi 1989a	12	231	15	1214	0.44 [0.25, 0.65]	0.84 [0.82, 0.86]

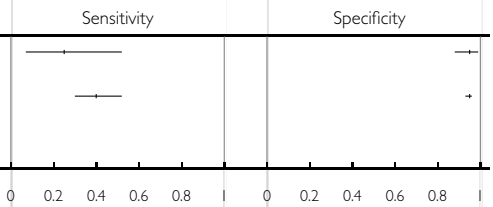


Test 12. Total hCG at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 12 Total hCG at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Pandian 2004	4	4	12	80	0.25 [0.07, 0.52]	0.95 [0.88, 0.99]
Wald 2003a	33	51	49	959	0.40 [0.30, 0.52]	0.95 [0.93, 0.96]

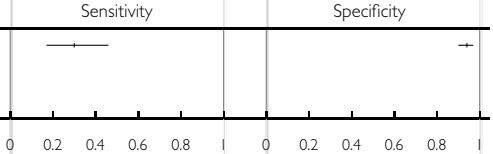


Test 13. Total hCG at 2.5MoM.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 13 Total hCG at 2.5MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1990	13	12	30	191	0.30 [0.17, 0.46]	0.94 [0.90, 0.97]

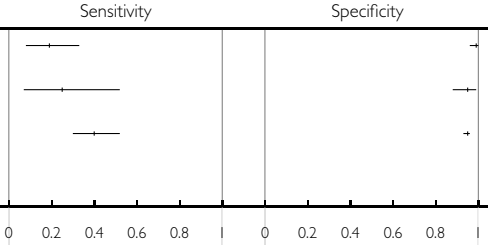


Test 14. Total hCG at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 14 Total hCG at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1990	8	3	35	200	0.19 [0.08, 0.33]	0.99 [0.96, 1.00]
Pandian 2004	4	4	12	80	0.25 [0.07, 0.52]	0.95 [0.88, 0.99]
Wald 2003a	33	51	49	959	0.40 [0.30, 0.52]	0.95 [0.93, 0.96]

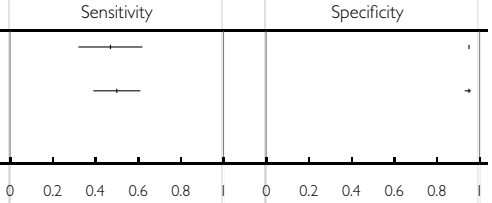


Test 15. Free ßhCG at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 15 Free ??hCG at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Hsu 1997a	22	411	25	7807	0.47 [0.32, 0.62]	0.95 [0.95, 0.95]
Wald 2003a	41	51	41	959	0.50 [0.39, 0.61]	0.95 [0.93, 0.96]

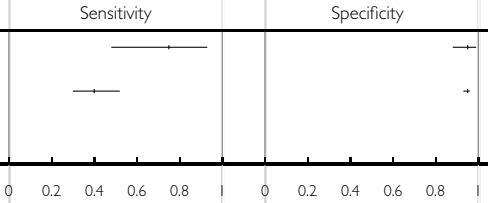


Test 16. uE3 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 16 uE3 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Pandian 2004	12	4	4	80	0.75 [0.48, 0.93]	0.95 [0.88, 0.99]
Wald 2003a	33	51	49	959	0.40 [0.30, 0.52]	0.95 [0.93, 0.96]

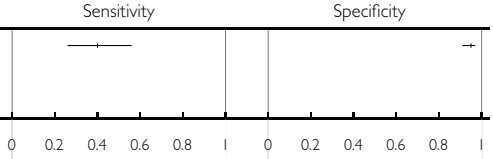


Test 17. Troponin at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 17 Troponin at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Palomaki 2004	18	12	27	226	0.40 [0.26, 0.56]	0.95 [0.91, 0.97]

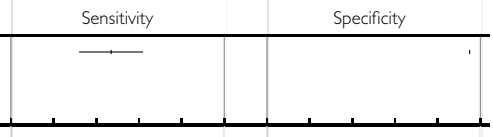


Test 18. Free ßhCG to AFP ratio at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 18 Free ??hCG to AFP ratio at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Hsu 1997a	22	411	25	7807	0.47 [0.32, 0.62]	0.95 [0.95, 0.95]

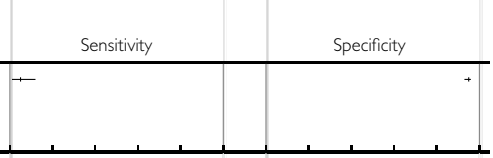


Test 19. PAPP-A at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 19 PAPP-A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	4	51	78	959	0.05 [0.01, 0.12]	0.95 [0.93, 0.96]

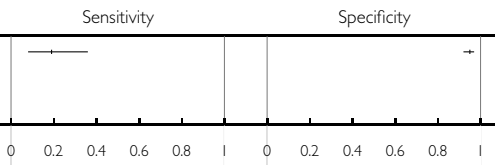


Test 20. PGF at 95th percentile.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 20 PGF at 95th percentile

Study	TP	FP	FN	TN	Sensitivity	Specificity
Su 2002	7	16	29	304	0.19 [0.08, 0.36]	0.95 [0.92, 0.97]

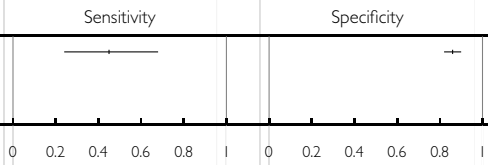


Test 21. CA125 at 1.5MoM.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 21 CA125 at 1.5MoM

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wenstrom 1997b	10	43	12	263	0.45 [0.24, 0.68]	0.86 [0.82, 0.90]

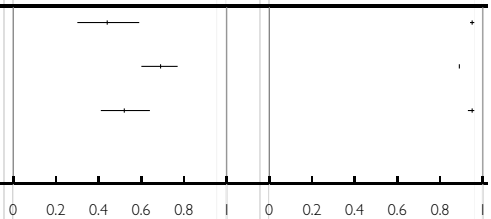


Test 22. Age and Total hCG at 5% FPR/95th percentile.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 22 Age and Total hCG at 5% FPR/95th percentile

Study	TP	FP	FN	TN	Sensitivity	Specificity
Knight 1998	23	253	29	4812	0.44 [0.30, 0.59]	0.95 [0.94, 0.96]
Muller 1996a	93	5562	42	45351	0.69 [0.60, 0.77]	0.89 [0.89, 0.89]
Wald 2003a	43	51	39	959	0.52 [0.41, 0.64]	0.95 [0.93, 0.96]

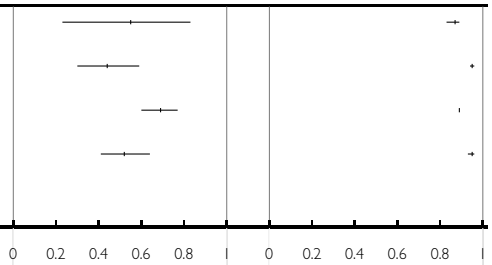


Test 23. Age and Total hCG at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 23 Age and Total hCG at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	6	67	5	433	0.55 [0.23, 0.83]	0.87 [0.83, 0.89]
Knight 1998	23	253	29	4812	0.44 [0.30, 0.59]	0.95 [0.94, 0.96]
Muller 1996a	93	5562	42	45351	0.69 [0.60, 0.77]	0.89 [0.89, 0.89]
Wald 2003a	43	51	39	959	0.52 [0.41, 0.64]	0.95 [0.93, 0.96]

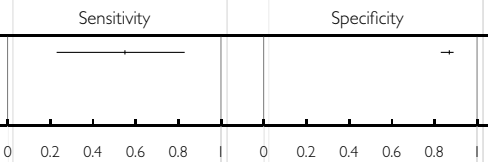


Test 24. Age and Total hCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 24 Age and Total hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	6	67	5	433	0.55 [0.23, 0.83]	0.87 [0.83, 0.89]

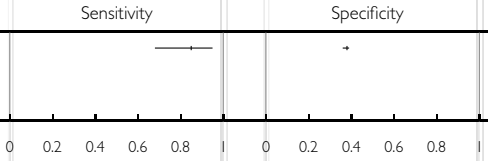


Test 25. Age and AFP at 1:270 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 25 Age and AFP at 1:270 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Rose 1994	28	2398	5	1465	0.85 [0.68, 0.95]	0.38 [0.36, 0.39]

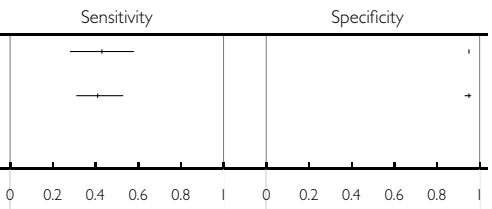


Test 26. Age and AFP at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 26 Age and AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Hsu 1997a	20	411	27	7807	0.43 [0.28, 0.58]	0.95 [0.95, 0.95]
Wald 2003a	34	51	48	959	0.41 [0.31, 0.53]	0.95 [0.93, 0.96]

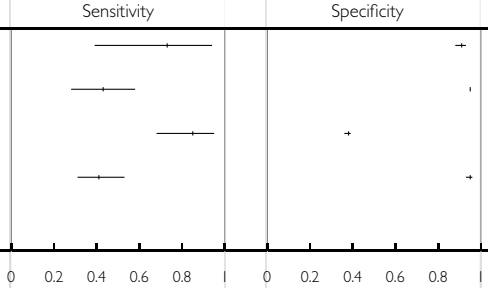


Test 27. Age and AFP at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 27 Age and AFP at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	45	3	455	0.73 [0.39, 0.94]	0.91 [0.88, 0.93]
Hsu 1997a	20	411	27	7807	0.43 [0.28, 0.58]	0.95 [0.95, 0.95]
Rose 1994	28	2398	5	1465	0.85 [0.68, 0.95]	0.38 [0.36, 0.39]
Wald 2003a	34	51	48	959	0.41 [0.31, 0.53]	0.95 [0.93, 0.96]

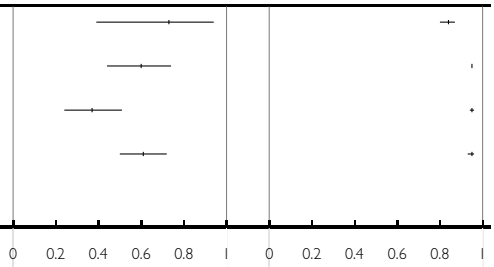


Test 28. Age and Free ßhCG at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 28 Age and Free ??hCG at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	82	3	418	0.73 [0.39, 0.94]	0.84 [0.80, 0.87]
Hsu 1997a	28	411	19	7807	0.60 [0.44, 0.74]	0.95 [0.95, 0.95]
Knight 1998	19	253	33	4812	0.37 [0.24, 0.51]	0.95 [0.94, 0.96]
Wald 2003a	50	51	32	959	0.61 [0.50, 0.72]	0.95 [0.93, 0.96]

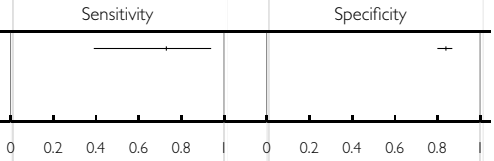


Test 29. Age and Free ßhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 29 Age and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	82	3	418	0.73 [0.39, 0.94]	0.84 [0.80, 0.87]

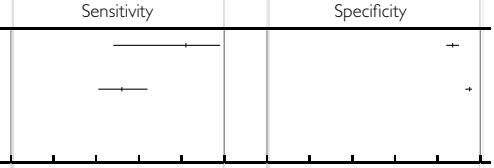


Test 30. Age and uE3 at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 30 Age and uE3 at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	9	64	2	436	0.82 [0.48, 0.98]	0.87 [0.84, 0.90]
Wald 2003a	43	51	39	959	0.52 [0.41, 0.64]	0.95 [0.93, 0.96]

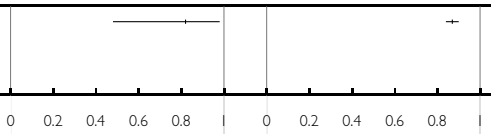


Test 31. Age and uE3 at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 31 Age and uE3 at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	9	64	2	436	0.82 [0.48, 0.98]	0.87 [0.84, 0.90]

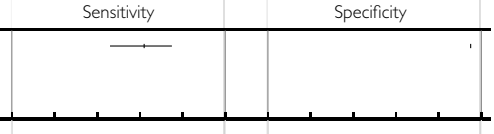


Test 32. Age and Free ßhCG to AFP at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 32 Age and Free ??hCG to AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Hsu 1997a	29	411	18	7807	0.62 [0.46, 0.75]	0.95 [0.95, 0.95]

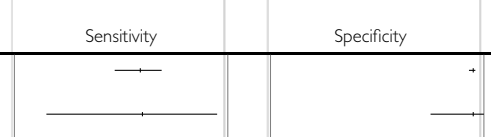


Test 33. Age and inhibin at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 33 Age and inhibin at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	48	51	34	959	0.59 [0.47, 0.69]	0.95 [0.93, 0.96]
Watanabe 2002	3	1	2	19	0.60 [0.15, 0.95]	0.95 [0.75, 1.00]

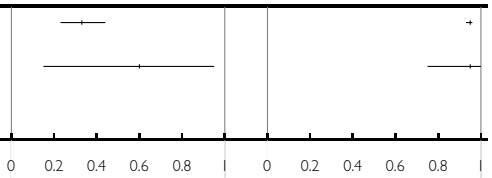


Test 34. Age and PAPP-A at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 34 Age and PAPP-A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	27	51	55	959	0.33 [0.23, 0.44]	0.95 [0.93, 0.96]
Watanabe 2002	3	1	2	19	0.60 [0.15, 0.95]	0.95 [0.75, 1.00]

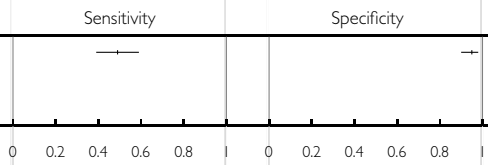


Test 35. Age and ProMBP at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 35 Age and ProMBP at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 1999	51	8	54	143	0.49 [0.39, 0.59]	0.95 [0.90, 0.98]

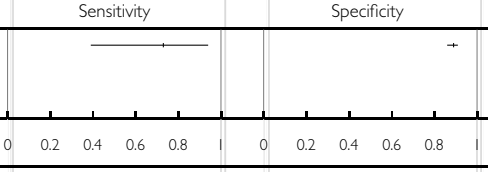


Test 36. Age and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 36 Age and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	56	3	444	0.73 [0.39, 0.94]	0.89 [0.86, 0.91]

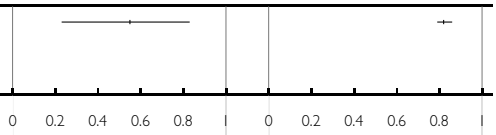


Test 37. Age, Total hCG and Free ßhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 37 Age, Total hCG and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	6	88	5	412	0.55 [0.23, 0.83]	0.82 [0.79, 0.86]		

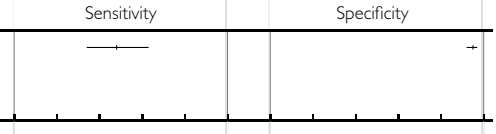


Test 38. Age, Total hCG and uE3 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 38 Age, Total hCG and uE3 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Bartels 1994a	24	16	26	304	0.48 [0.34, 0.63]	0.95 [0.92, 0.97]		

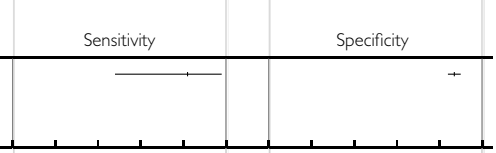


Test 39. Age, Total hCG and uE3 at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 39 Age, Total hCG and uE3 at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	9	66	2	434	0.82 [0.48, 0.98]	0.87 [0.84, 0.90]		

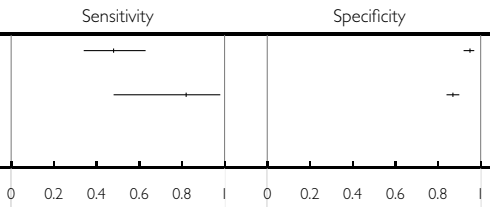


Test 40. Age, Total hCG and uE3 at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 40 Age, Total hCG and uE3 at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	24	16	26	304	0.48 [0.34, 0.63]	0.95 [0.92, 0.97]
Forest 1995	9	66	2	434	0.82 [0.48, 0.98]	0.87 [0.84, 0.90]

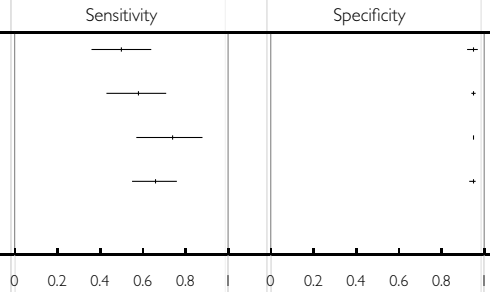


Test 41. Age, Total hCG and AFP at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 41 Age, Total hCG and AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	25	16	25	304	0.50 [0.36, 0.64]	0.95 [0.92, 0.97]
Knight 1998	30	253	22	4812	0.58 [0.43, 0.71]	0.95 [0.94, 0.96]
Lam 2002	26	810	9	15392	0.74 [0.57, 0.88]	0.95 [0.95, 0.95]
Wald 2003a	54	51	28	959	0.66 [0.55, 0.76]	0.95 [0.93, 0.96]

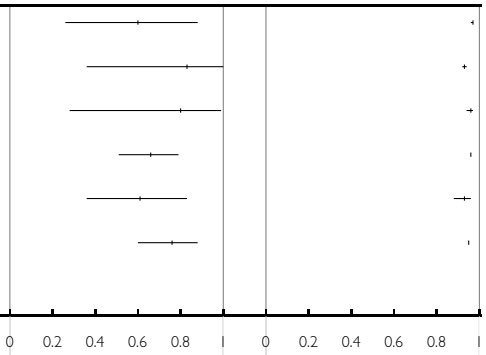


Test 42. Age, Total hCG and AFP at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 42 Age, Total hCG and AFP at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Audibert 2001a	6	124	4	3656	0.60 [0.26, 0.88]	0.97 [0.96, 0.97]
Beekhuis 1993	5	149	1	2127	0.83 [0.36, 1.00]	0.93 [0.92, 0.94]
Benattar 1999	4	73	1	1571	0.80 [0.28, 0.99]	0.96 [0.94, 0.97]
David 1996	31	378	16	9075	0.66 [0.51, 0.79]	0.96 [0.96, 0.96]
Debieve 2000	11	15	7	185	0.61 [0.36, 0.83]	0.93 [0.88, 0.96]
Roberts 2000	31	1323	10	24716	0.76 [0.60, 0.88]	0.95 [0.95, 0.95]

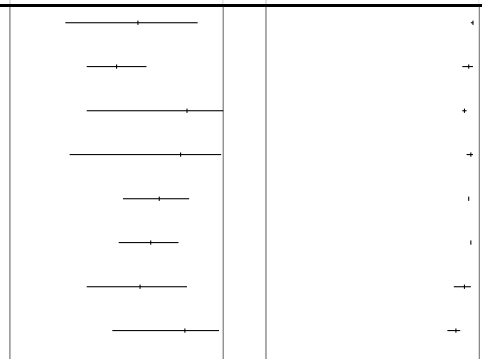


Test 43. Age, Total hCG and AFP at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 43 Age, Total hCG and AFP at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Audibert 2001a	6	124	4	3656	0.60 [0.26, 0.88]	0.97 [0.96, 0.97]
Bartels 1994a	25	16	25	304	0.50 [0.36, 0.64]	0.95 [0.92, 0.97]
Beekhuis 1993	5	149	1	2127	0.83 [0.36, 1.00]	0.93 [0.92, 0.94]
Benattar 1999	4	73	1	1571	0.80 [0.28, 0.99]	0.96 [0.94, 0.97]
Crossley 1994	26	1497	11	28550	0.70 [0.53, 0.84]	0.95 [0.95, 0.95]
David 1996	31	378	16	9075	0.66 [0.51, 0.79]	0.96 [0.96, 0.96]
Debieve 2000	11	15	7	185	0.61 [0.36, 0.83]	0.93 [0.88, 0.96]
Forest 1995	9	57	2	443	0.82 [0.48, 0.98]	0.89 [0.85, 0.91]
Jou 2000	10	1144	6	16582	0.63 [0.35, 0.85]	0.94 [0.93, 0.94]
Knight 1998	30	253	22	4812	0.58 [0.43, 0.71]	0.95 [0.94, 0.96]
Lam 2002	26	810	9	15392	0.74 [0.57, 0.88]	0.95 [0.95, 0.95]
Lemay 1995	21	1013	11	17555	0.66 [0.47, 0.81]	0.95 [0.94, 0.95]



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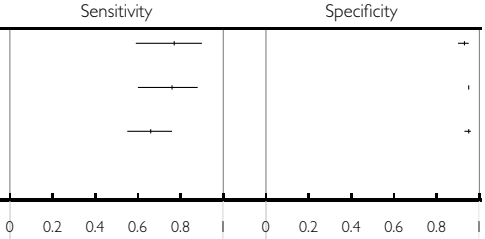
Study	TP	FP	FN	TN	Sensitivity	Specificity
Milunsky 1993	24	36	7	444	0.77 [0.59, 0.90]	0.93 [0.90, 0.95]
Roberts 2000	31	1323	10	24716	0.76 [0.60, 0.88]	0.95 [0.95, 0.95]
Wald 2003a	54	51	28	959	0.66 [0.55, 0.76]	0.95 [0.93, 0.96]



Test 44. Age, Total hCG and SP1at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 44 Age, Total hCG and SP1at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	22	16	28	304	0.44 [0.30, 0.59]	0.95 [0.92, 0.97]

Test 45. Age, Total hCG and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 45 Age, Total hCG and Free ??hCG at 1:384 risk

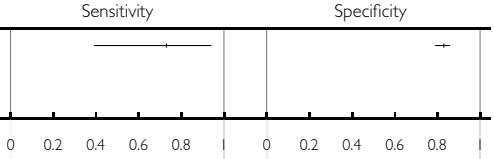
Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	9	69	2	431	0.82 [0.48, 0.98]	0.86 [0.83, 0.89]

Test 46. Age, Free ßhCG and uE3 at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 46 Age, Free ??hCG and uE3 at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	86	3	414	0.73 [0.39, 0.94]	0.83 [0.79, 0.86]

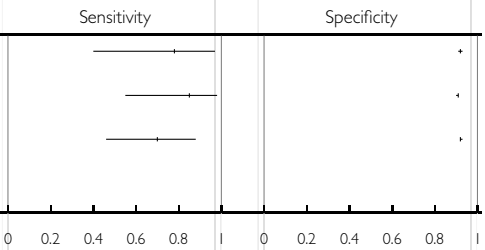


Test 47. Age, Free ßhCG and AFP at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 47 Age, Free ??hCG and AFP at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Brajenovic 1998	7	255	2	2924	0.78 [0.40, 0.97]	0.92 [0.91, 0.93]
Kadir 1999	11	418	2	3996	0.85 [0.55, 0.98]	0.91 [0.90, 0.91]
Rozenberg 2002	14	645	6	7632	0.70 [0.46, 0.88]	0.92 [0.92, 0.93]

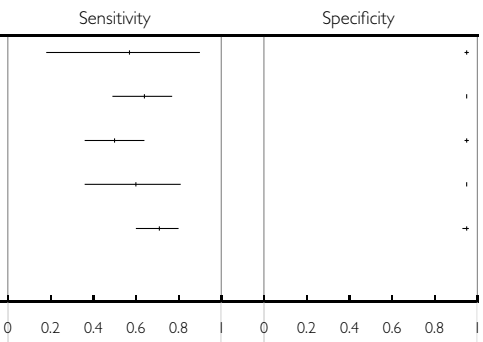


Test 48. Age, Free ßhCG and AFP at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 48 Age, Free ??hCG and AFP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Anandakumar 1999	4	60	3	1141	0.57 [0.18, 0.90]	0.95 [0.94, 0.96]
Hsu 1997a	30	411	17	7807	0.64 [0.49, 0.77]	0.95 [0.95, 0.95]
Knight 1998	26	253	26	4812	0.50 [0.36, 0.64]	0.95 [0.94, 0.96]
Rozenberg 2002	12	414	8	7863	0.60 [0.36, 0.81]	0.95 [0.95, 0.95]
Wald 2003a	58	51	24	959	0.71 [0.60, 0.80]	0.95 [0.93, 0.96]

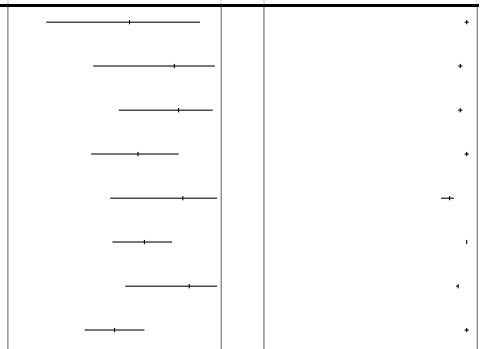


Test 49. Age, Free ßhCG and AFP at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 49 Age, Free ??hCG and AFP at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Anandakumar 1999	4	60	3	1141	0.57 [0.18, 0.90]	0.95 [0.94, 0.96]
Brajenovic 1998	7	255	2	2924	0.78 [0.40, 0.97]	0.92 [0.91, 0.93]
Chao 1999	12	804	3	9279	0.80 [0.52, 0.96]	0.92 [0.91, 0.93]
Extermann 1998	14	120	9	2396	0.61 [0.39, 0.80]	0.95 [0.94, 0.96]
Forest 1995	9	67	2	433	0.82 [0.48, 0.98]	0.87 [0.83, 0.89]
Hsu 1997a	30	411	17	7807	0.64 [0.49, 0.77]	0.95 [0.95, 0.95]
Kadir 1999	11	418	2	3996	0.85 [0.55, 0.98]	0.91 [0.90, 0.91]
Knight 1998	26	253	26	4812	0.50 [0.36, 0.64]	0.95 [0.94, 0.96]
Milunsky 1993	23	26	8	454	0.74 [0.55, 0.88]	0.95 [0.92, 0.96]
Rozenberg 2002	14	645	6	7632	0.70 [0.46, 0.88]	0.92 [0.92, 0.93]
Wald 2003a	58	51	24	959	0.71 [0.60, 0.80]	0.95 [0.93, 0.96]
Wenstrom 1997a	27	63	4	250	0.87 [0.70, 0.96]	0.80 [0.75, 0.84]

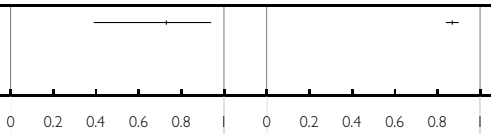


Test 50. Age, Free ßhCG and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 50 Age, Free ??hCG and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	64	3	436	0.73 [0.39, 0.94]	0.87 [0.84, 0.90]

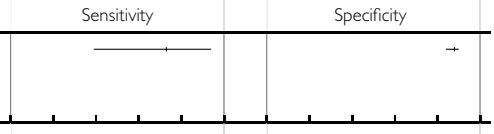


Test 51. Age, AFP and uE3 at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 51 Age, AFP and uE3 at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	8	62	3	438	0.73 [0.39, 0.94]	0.88 [0.84, 0.90]

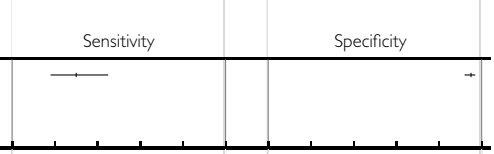


Test 52. Age, AFP and uE3 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 52 Age, AFP and uE3 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	15	16	35	304	0.30 [0.18, 0.45]	0.95 [0.92, 0.97]

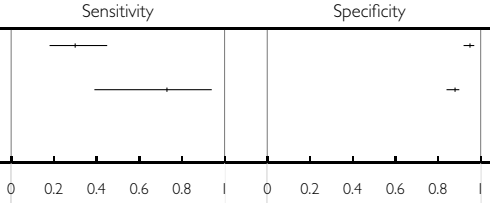


Test 53. Age, AFP and uE3 at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 53 Age, AFP and uE3 at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	15	16	35	304	0.30 [0.18, 0.45]	0.95 [0.92, 0.97]
Forest 1995	8	62	3	438	0.73 [0.39, 0.94]	0.88 [0.84, 0.90]

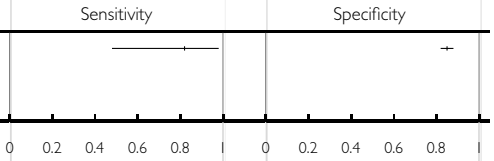


Test 54. Age, uE3 and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 54 Age, uE3 and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	9	75	2	425	0.82 [0.48, 0.98]	0.85 [0.82, 0.88]

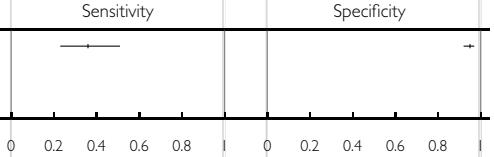


Test 55. Age, uE3 and SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 55 Age, uE3 and SP1 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	18	16	32	304	0.36 [0.23, 0.51]	0.95 [0.92, 0.97]

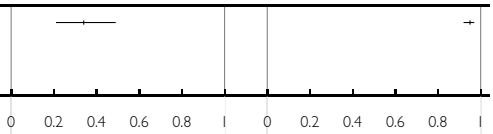


Test 56. Age, AFP and SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 56 Age, AFP and SP1 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	17	16	33	304	0.34 [0.21, 0.49]	0.95 [0.92, 0.97]

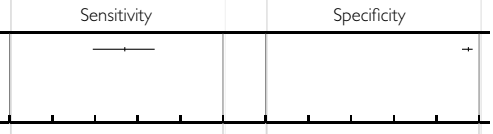


Test 57. Age, AFP and Hyperglycosylated hCG at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 57 Age, AFP and Hyperglycosylated hCG at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Talbot 2003	27	14	23	264	0.54 [0.39, 0.68]	0.95 [0.92, 0.97]

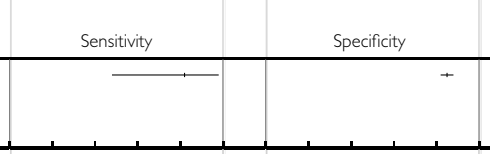


Test 58. Age, AFP and Free αhCG 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 58 Age, AFP and Free ??hCG 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	9	74	2	426	0.82 [0.48, 0.98]	0.85 [0.82, 0.88]

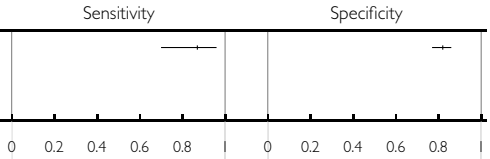


Test 59. Age, Total hCG, Free ßhCG and AFP at 1:266 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 59 Age, Total hCG, Free ??hCG and AFP at 1:266 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wenstrom 1997a	27	56	4	257	0.87 [0.70, 0.96]	0.82 [0.77, 0.86]

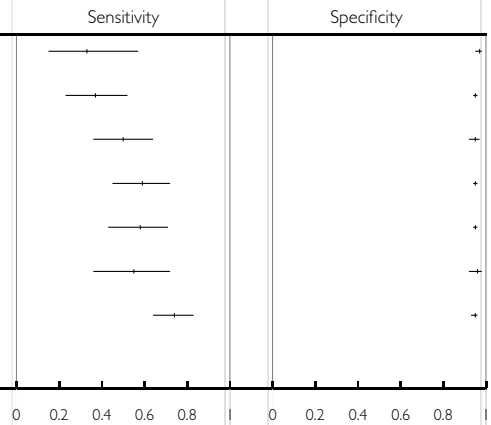


Test 60. Age, Total hCG, AFP and uE3 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 60 Age, Total hCG, AFP and uE3 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bahado-Singh 1999a	7	30	14	875	0.33 [0.15, 0.57]	0.97 [0.95, 0.98]
Bahado-Singh 2000	17	117	29	2228	0.37 [0.23, 0.52]	0.95 [0.94, 0.96]
Bartels 1994a	25	16	25	304	0.50 [0.36, 0.64]	0.95 [0.92, 0.97]
Haddow 1994	32	264	22	5018	0.59 [0.45, 0.72]	0.95 [0.94, 0.96]
Knight 1998	30	253	22	4812	0.58 [0.43, 0.71]	0.95 [0.94, 0.96]
Sancken 2003	18	8	15	180	0.55 [0.36, 0.72]	0.96 [0.92, 0.98]
Wald 2003a	61	51	21	959	0.74 [0.64, 0.83]	0.95 [0.93, 0.96]

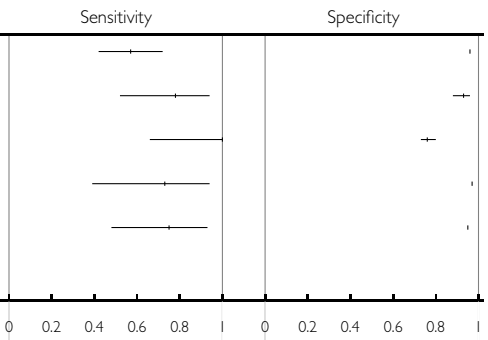


Test 61. Age, Total hCG, AFP and uE3 at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 61 Age, Total hCG, AFP and uE3 at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
David 1996	27	372	20	8939	0.57 [0.42, 0.72]	0.96 [0.96, 0.96]
Debieve 2000	14	15	4	185	0.78 [0.52, 0.94]	0.93 [0.88, 0.96]
Mancini 1991	9	170	0	552	1.00 [0.66, 1.00]	0.76 [0.73, 0.80]
Piggott 1994	8	203	3	6776	0.73 [0.39, 0.94]	0.97 [0.97, 0.97]
Ward 1999	12	673	4	12924	0.75 [0.48, 0.93]	0.95 [0.95, 0.95]

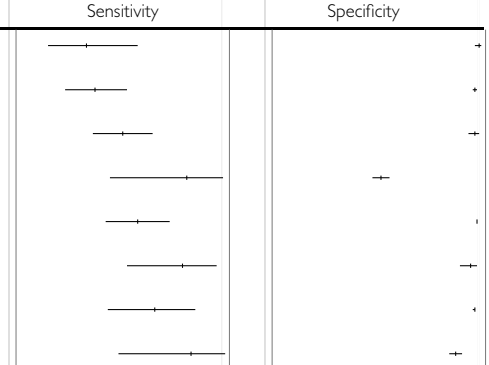


Test 62. Age, Total hCG, AFP and uE3 at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 62 Age, Total hCG, AFP and uE3 at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bahado-Singh 1999a	7	30	14	875	0.33 [0.15, 0.57]	0.97 [0.95, 0.98]
Bahado-Singh 2000	17	117	29	2228	0.37 [0.23, 0.52]	0.95 [0.94, 0.96]
Bartels 1994a	25	16	25	304	0.50 [0.36, 0.64]	0.95 [0.92, 0.97]
Bartels 1994b	8	315	2	330	0.80 [0.44, 0.97]	0.51 [0.47, 0.55]
David 1996	27	372	20	8939	0.57 [0.42, 0.72]	0.96 [0.96, 0.96]
Debieve 2000	14	15	4	185	0.78 [0.52, 0.94]	0.93 [0.88, 0.96]
Extermann 1998	15	137	8	2379	0.65 [0.43, 0.84]	0.95 [0.94, 0.95]
Forest 1995	9	68	2	432	0.82 [0.48, 0.98]	0.86 [0.83, 0.89]
Haddow 1994	48	1321	6	3961	0.89 [0.77, 0.96]	0.75 [0.74, 0.76]
Heyl 1990	12	19	4	66	0.75 [0.48, 0.93]	0.78 [0.67, 0.86]
Huderer-Duric 2000	10	852	2	1969	0.83 [0.52, 0.98]	0.70 [0.68, 0.71]
Kishida 2000	10	368	0	677	1.00 [0.69, 1.00]	0.65 [0.62, 0.68]
Knight 1998	30	253	22	4812	0.58 [0.43, 0.71]	0.95 [0.94, 0.96]



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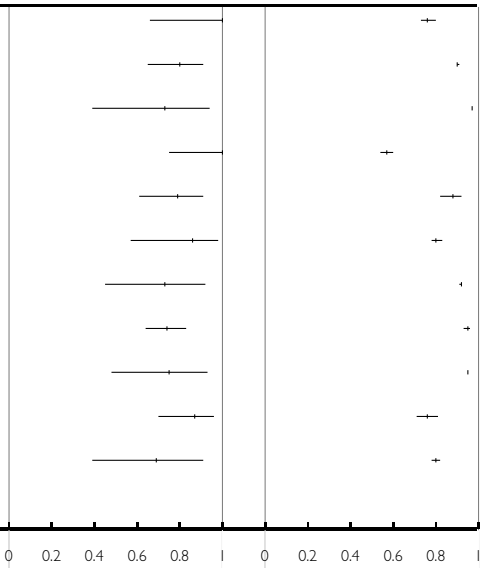
Study	TP	FP	FN	TN	Sensitivity	Specificity
Mancini 1991	9	170	0	552	1.00 [0.66, 1.00]	0.76 [0.73, 0.80]
Perona 1997	33	2031	8	18784	0.80 [0.65, 0.91]	0.90 [0.90, 0.91]
Piggott 1994	8	203	3	6776	0.73 [0.39, 0.94]	0.97 [0.97, 0.97]
Rosen 2002	13	424	0	569	1.00 [0.75, 1.00]	0.57 [0.54, 0.60]
Sancken 2003	26	23	7	165	0.79 [0.61, 0.91]	0.88 [0.82, 0.92]
Suzumori 1997	12	208	2	856	0.86 [0.57, 0.98]	0.80 [0.78, 0.83]
Verloes 1995	11	841	4	9594	0.73 [0.45, 0.92]	0.92 [0.91, 0.92]
Wald 2003a	61	51	21	959	0.74 [0.64, 0.83]	0.95 [0.93, 0.96]
Ward 1999	12	673	4	12924	0.75 [0.48, 0.93]	0.95 [0.95, 0.95]
Wenstrom 1997a	27	75	4	238	0.87 [0.70, 0.96]	0.76 [0.71, 0.81]
Wenstrom 1999	9	249	4	994	0.69 [0.39, 0.91]	0.80 [0.78, 0.82]

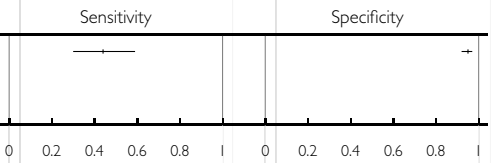


Test 63. Age, Total hCG, uE3 and SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 63 Age, Total hCG, uE3 and SP1 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	22	16	28	304	0.44 [0.30, 0.59]	0.95 [0.92, 0.97]

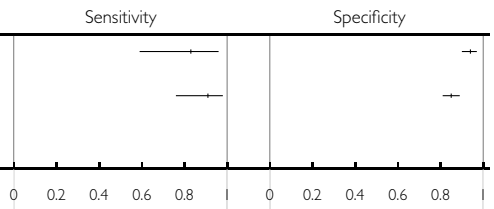


Test 64. Age, Total hCG, AFP and Inhibin A at 1:190 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 64 Age, Total hCG, AFP and Inhibin A at 1:190 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Debieve 2000	15	11	3	189	0.83 [0.59, 0.96]	0.95 [0.90, 0.97]
Wenstrom 1997	30	47	3	266	0.91 [0.76, 0.98]	0.85 [0.81, 0.89]



Test 65. Age, Total hCG, AFP and Inhibin A at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 65 Age, Total hCG, AFP and Inhibin A at 1:250 risk

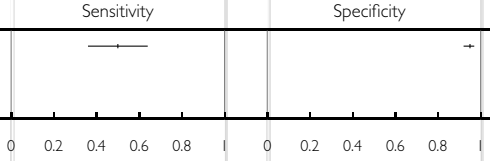
Study	TP	FP	FN	TN	Sensitivity	Specificity
Debieve 2000	16	13	2	187	0.89 [0.65, 0.99]	0.94 [0.89, 0.96]

Test 66. Age, Total hCG, AFP and SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 66 Age, Total hCG, AFP and SP1 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	25	16	25	304	0.50 [0.36, 0.64]	0.95 [0.92, 0.97]

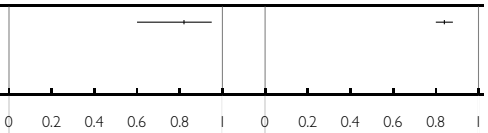


Test 67. Age, Total hCG, AFP and CA125 at 1:190 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 67 Age, Total hCG, AFP and CA125 at 1:190 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wenstrom 1997b	18	48	4	258	0.82 [0.60, 0.95]	0.84 [0.80, 0.88]

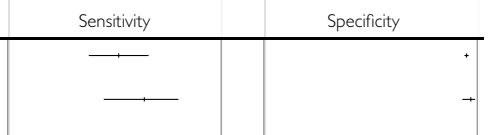


Test 68. Age, Free ßhCG, AFP and uE3 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 68 Age, Free ??hCG, AFP and uE3 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Knight 1998	27	253	25	4812	0.52 [0.38, 0.66]	0.95 [0.94, 0.96]
Sancken 2003	21	6	12	182	0.64 [0.45, 0.80]	0.97 [0.93, 0.99]
Wald 2003a	63	51	19	959	0.77 [0.66, 0.85]	0.95 [0.93, 0.96]



Test 69. Age, Free ßhCG, AFP and uE3 at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 69 Age, Free ??hCG, AFP and uE3 at 1:250 risk

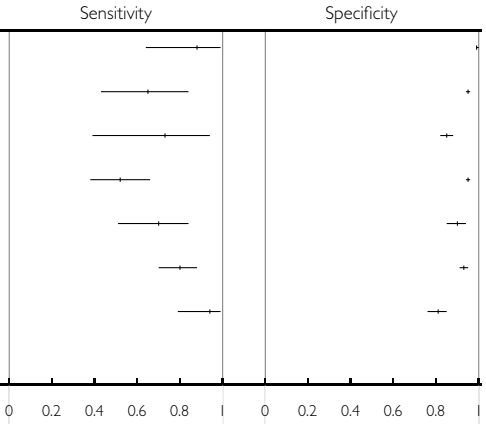
Study	TP	FP	FN	TN	Sensitivity	Specificity
Cioffi 2000	15	4	2	696	0.88 [0.64, 0.99]	0.99 [0.99, 1.00]
Wald 2003a	66	70	16	940	0.80 [0.70, 0.88]	0.93 [0.91, 0.95]

Test 70. Age, Free ßhCG, AFP and uE3 at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 70 Age, Free ??hCG, AFP and uE3 at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Cioffi 2000	15	4	2	696	0.88 [0.64, 0.99]	0.99 [0.99, 1.00]
Extermann 1998	15	123	8	2393	0.65 [0.43, 0.84]	0.95 [0.94, 0.96]
Forest 1995	8	73	3	427	0.73 [0.39, 0.94]	0.85 [0.82, 0.88]
Knight 1998	27	253	25	4812	0.52 [0.38, 0.66]	0.95 [0.94, 0.96]
Sancken 2003	23	18	10	170	0.70 [0.51, 0.84]	0.90 [0.85, 0.94]
Wald 2003a	66	70	16	940	0.80 [0.70, 0.88]	0.93 [0.91, 0.95]
Wenstrom 1997a	29	59	2	254	0.94 [0.79, 0.99]	0.81 [0.76, 0.85]

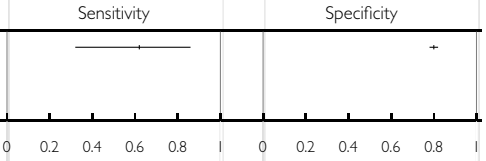


Test 71. Age, Free ßhCG, AFP and Inhibin A at 1:190 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 71 Age, Free ??hCG, AFP and Inhibin A at 1:190 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wenstrom 1999	8	249	5	994	0.62 [0.32, 0.86]	0.80 [0.78, 0.82]

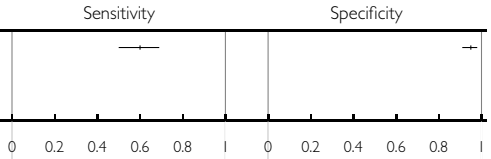


Test 72. Age, Free ßhCG, AFP and ProMBP at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 72 Age, Free ??hCG, AFP and ProMBP at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2004	64	11	43	216	0.60 [0.50, 0.69]	0.95 [0.91, 0.98]

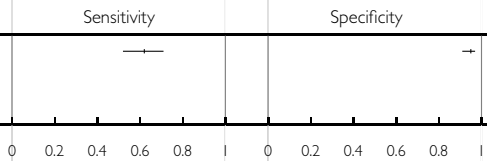


Test 73. Age, Free ßhCG, AFP and ProMBP at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 73 Age, Free ??hCG, AFP and ProMBP at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Christiansen 2004	66	12	41	215	0.62 [0.52, 0.71]	0.95 [0.91, 0.97]

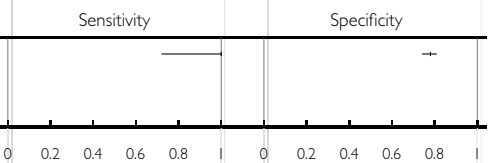


Test 74. Age, AFP, uE3 and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 74 Age, AFP, uE3 and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	11	111	0	389	1.00 [0.72, 1.00]	0.78 [0.74, 0.81]

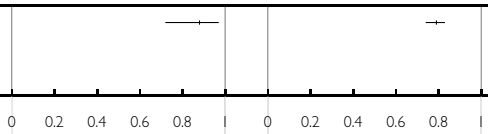


Test 75. Age, AFP, uE3 and Inhibin A at 1:233 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 75 Age, AFP, uE3 and Inhibin A at 1:233 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wenstrom 1997	29	66	4	247	0.88 [0.72, 0.97]	0.79 [0.74, 0.83]

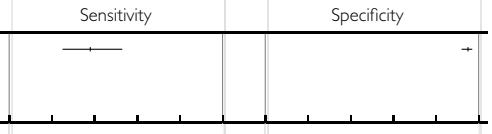


Test 76. Age, AFP, uE3 and SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 76 Age, AFP, uE3 and SP1 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	19	16	31	304	0.38 [0.25, 0.53]	0.95 [0.92, 0.97]

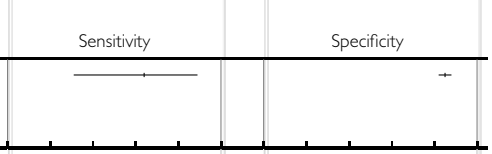


Test 77. Age, Total hCG, Free ßhCG, AFP and uE3 at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 77 Age, Total hCG, Free ??hCG, AFP and uE3 at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	7	74	4	426	0.64 [0.31, 0.89]	0.85 [0.82, 0.88]

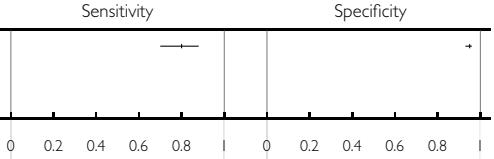


Test 78. Age, Total hCG, AFP, uE3 and Inhibin A at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 78 Age, Total hCG, AFP, uE3 and Inhibin A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	66	51	16	959	0.80 [0.70, 0.88]	0.95 [0.93, 0.96]

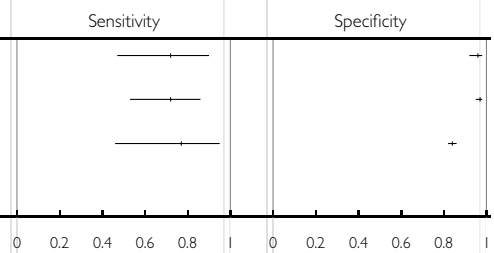


Test 79. Age, Total hCG, AFP, uE3 and Inhibin A at 1:150 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 79 Age, Total hCG, AFP, uE3 and Inhibin A at 1:150 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Debieve 2000	13	8	5	192	0.72 [0.47, 0.90]	0.96 [0.92, 0.98]
Palomaki 2006	23	15	9	493	0.72 [0.53, 0.86]	0.97 [0.95, 0.98]
Wenstrom 1999	10	199	3	1044	0.77 [0.46, 0.95]	0.84 [0.82, 0.86]

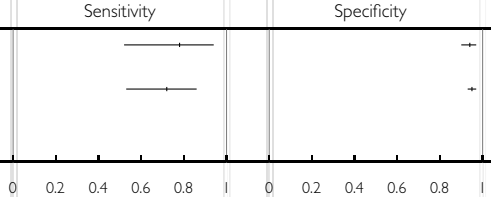


Test 80. Age, Total hCG, AFP, uE3 and Inhibin A at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 80 Age, Total hCG, AFP, uE3 and Inhibin A at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Debieve 2000	14	12	4	188	0.78 [0.52, 0.94]	0.94 [0.90, 0.97]
Palomaki 2006	23	25	9	483	0.72 [0.53, 0.86]	0.95 [0.93, 0.97]

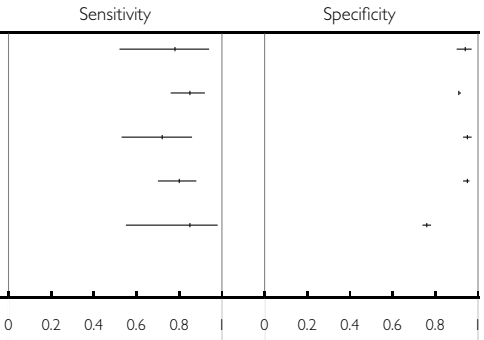


Test 81. Age, Total hCG, AFP, uE3 and Inhibin A at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 81 Age, Total hCG, AFP, uE3 and Inhibin A at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Debieve 2000	14	12	4	188	0.78 [0.52, 0.94]	0.94 [0.90, 0.97]
Malone 2005	74	2988	13	32161	0.85 [0.76, 0.92]	0.91 [0.91, 0.92]
Palomaki 2006	23	25	9	483	0.72 [0.53, 0.86]	0.95 [0.93, 0.97]
Wald 2003a	66	51	16	959	0.80 [0.70, 0.88]	0.95 [0.93, 0.96]
Wenstrom 1999	11	298	2	945	0.85 [0.55, 0.98]	0.76 [0.74, 0.78]

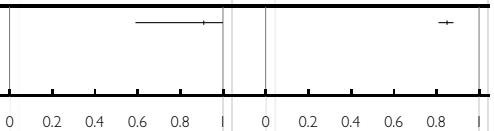


Test 82. Age, Total hCG, AFP, uE3 and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 82 Age, Total hCG, AFP, uE3 and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Forest 1995	10	77	1	423	0.91 [0.59, 1.00]	0.85 [0.81, 0.88]

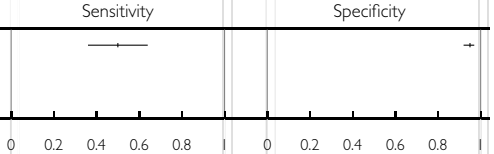


Test 83. Age, Total hCG, AFP, uE3 and SP1 at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 83 Age, Total hCG, AFP, uE3 and SP1 at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Bartels 1994a	25	16	25	304	0.50 [0.36, 0.64]	0.95 [0.92, 0.97]

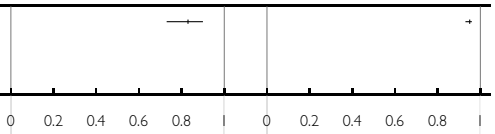


Test 84. Age, Free ßhCG, AFP, uE3 and Inhibin A at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 84 Age, Free ??hCG, AFP, uE3 and Inhibin A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	68	51	14	959	0.83 [0.73, 0.90]	0.95 [0.93, 0.96]

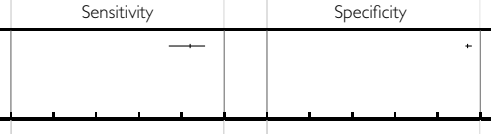


Test 85. Age, Free ßhCG, AFP, uE3 and Inhibin A at 1:250 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 85 Age, Free ??hCG, AFP, uE3 and Inhibin A at 1:250 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	69	58	13	952	0.84 [0.74, 0.91]	0.94 [0.93, 0.96]

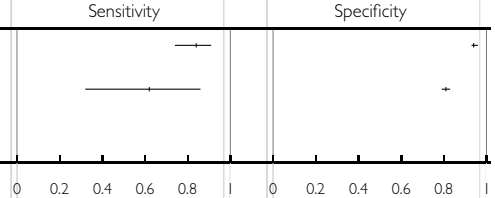


Test 86. Age, Free ßhCG, AFP, uE3 and Inhibin A at mixed cutpoints.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 86 Age, Free ??hCG, AFP, uE3 and Inhibin A at mixed cutpoints

Study	TP	FP	FN	TN	Sensitivity	Specificity
Wald 2003a	69	58	13	952	0.84 [0.74, 0.91]	0.94 [0.93, 0.96]
Wenstrom 1999	8	236	5	1007	0.62 [0.32, 0.86]	0.81 [0.79, 0.83]

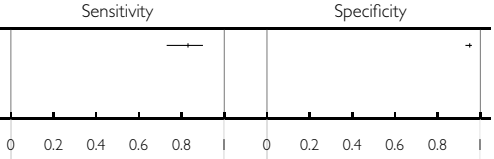


Test 87. Age, Total hCG, AFP, uE3, Inhibin A and PAPP-A at 5% FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 87 Age, Total hCG, AFP, uE3, Inhibin A and PAPP-A at 5% FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	68	51	14	959	0.83 [0.73, 0.90]	0.95 [0.93, 0.96]		

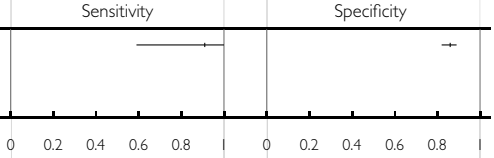


Test 88. Age, Total hCG, Free ßhCG, AFP, uE3 and Free αhCG at 1:384 risk.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 88 Age, Total hCG, Free ??hCG, AFP, uE3 and Free ??hCG at 1:384 risk

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Forest 1995	10	71	1	429	0.91 [0.59, 1.00]	0.86 [0.82, 0.89]		

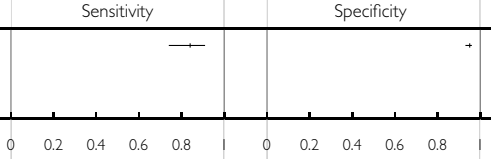


Test 89. Age, Free ßhCG, AFP, uE3, Inhibin A and PAPP-A at 5%FPR.

Review: Second trimester serum tests for Down's Syndrome screening

Test: 89 Age, Free ??hCG, AFP, uE3, Inhibin A and PAPP-A at 5%FPR

Study	TP	FP	FN	TN	Sensitivity	Specificity	Sensitivity	Specificity
Wald 2003a	69	51	13	959	0.84 [0.74, 0.91]	0.95 [0.93, 0.96]		



ADDITIONAL TABLES

Table 1. Direct comparisons of the diagnostic accuracy of the 12 test strategies

Ratio of DOR (95% CI); P value (studies)	Free ß hCG AFP uE3 Inhibin PAPPA (quintuple)	Total hCG AFP uE3 Inhibin PAPPA (quintuple)	Total hCG AFP Inhibin (triple)	Total hCG AFP uE3 Inhibin (quadruple)	Free ß hCG AFP uE3 Inhibin (quadruple)	Free ß hCG AFP uE3 (triple)	Total hCG AFP (double)	Free ß hCG AFP (double)
Total hCG AFP uE3 Inhibin PAPPA	1.1 (0.4,2.7) ; P=0.85 (K=1)							
Total hCG AFP Inhibin	-	-						
Total hCG AFP uE3 Inhibin	1.3 (0.5,3.2) ; P=0.58 (K=1)	1.2 (0.5,2.9) ; P=0.72 (K=1)	1. 6 (0.2,10.1) ; P=0.64 (K=1)					
Free ß hCG AFP uE3 Inhibin	1.1 (0.5,2.9) ; P=0.77 (K=1)	1.0 (0.4,2.6) ; P=0.92 (K=1)	-	0.8 (0.4,1.7) ; P=0.60 (K=2)				
Free ß hCG AFP uE3	1.8 (0.7,4.4) ; P=0.19 (K=1)	1.6 (0.7,4.0) ; P=0.26 (K=1)	-	1.4 (0.6,3.3) ; P=0.44 (K=1)	1.6 (0.7,3.8) ; P=0.31 (K=1)			
Total hCG AFP	2.8 (1.2,6.4) ; P=0.02 (K=1)	2.5 (1.1,5.8) ; P=0.03 (K=1)	4. 4 (0.8,25.7) ; P=0.10 (K=1)	2.3 (1.1,4.7) ; P=0.03 (K=2)	2.4 (1.0,5.6) ; P=0.04 (K=1)	1.2 (0.7,1.9) ; P=0.56 (K=3)		
Free ß hCG AFP	2.2 (0.9,5.2) ; P=0.07 (K=1)	2.0 (0.9,4.7) ; P=0.11 (K=1)		1.7 (0.7,3.9) ; P=0.21 (K=1)	1.9 (0.8,4.5) ; P=0.14 (K=1)	1.2 (0.8,1.9) ; P=0.34 (K=5)	1.0 (0.6,1.6) ; P=0.91 (K=4)	
Total hCG AFP uE3	1.8 (0.8,4.4) ; P=0.17 (K=1)	1.7 (0.7,3.9) ; P=0.24 (K=1)	2. 0 (0.3,12.7) ; P=0.47 (K=1)	1.2 (0.6,2.3) ; P=0.55 (K=3)	1.6 (0.8,3.2) ; P=0.22 (K=2)	1.0 (0.7,1.6) ; P=0.93 (K=6)	0.9 (0.6,1.3) ; P=0.66 (K=6)	0.9 (0.6,1.4) ; P=0.56 (K=5)
Free ß hCG	3.4 (1.5,7.9) ; P=0.004 (K=1)	3.1 (1.4,7.1) ; P=0.007 (K=1)	-	2.6 (1.2,5.9) ; P=0.02 (K=1)	3.0 (1.3,6.8) ; P=0.01 (K=1)	2.0 (1.2,3.3) ; P=0.008 (K=3)	1.7 (1.0,2.8) ; P=0.04 (K=3)	1.5 (1.0,2.3) ; P=0.06 (K=4)

Table 1. Direct comparisons of the diagnostic accuracy of the 12 test strategies (*Continued*)

Total hCG	4.8 (2.1,11.1); P=0.0002 (K=1)	4.4 (1.9,10.0); P=0.0004 (K=1)	-	3.7 (1.7,8.4); P=0.001 (K=1)	4.2 (1.8,9.6); P=0.0007 (K=1)	2.2 (1.3,3.6); P=0.003 (K=3)	1.9 (1.1,3.1); P=0.01 (K=3)	1.8 (1.1,3.0); P=0.02 (K=3)
AFP	7.5 (3.2,17.3); P=<0.0001 (K=1)	6.9 (3.0,15.7); P<0.0001 (K=1)	-	5.8 (2.6,13.1); P<0.0001 (K=1)	6.5 (2.8,15.1); P<0.0001 (K=1)	3.1 (1.5,6.2); P=0.002 (K=2)	2.2 (1.2,4.4); P=0.02 (K=2)	2.7 (1.6,4.5); P=0.0001 (K=3)
Ratio of DOR (95% CI); P-value (studies)	**Total hCG AFP uE3 (triple)**	**Free ß hCG (single)**	**Total hCG (single)**					
Free ß hCG	2.0 (1.2,3.4); P=0.005 (K=3)							
Total hCG	2.2 (1.4,3.7); P=0.002 (K=3)	1.1 (0.7,1.8); P=0.70 (K=3)						
AFP	2.9 (1.5,5.8); P=0.002 (K=2)	1.9 (1.1,3.0); P=0.01 (K=3)	1.1 (0.6,2.1); P=0.81 (K=2)					

Direct comparisons are made only using data from studies which compare each pair of tests on the same women. Relative DOR are computed by division of the DOR for the column by the DOR for the row. If the relative DOR is greater than one then the diagnostic accuracy of the test for the column is higher than that for the row, if less than one the diagnostic accuracy of the test in the row is higher than in the column. All test combinations include maternal age. - indicates that no comparative study is available.

Table 2. Indirect comparisons of the diagnostic accuracy of the 12 test strategies

Ratio of DOR (95% CI); P value		**Free ßhCG AFP uE3 Inhibin PAPPA (quintuple)**	**Total hCG AFP uE3 Inhibin PAPPA (quintuple)**	**Total hCG AFP Inhibin (triple)**	**Total hCG AFP uE3 Inhibin (quadruple)**	**Free ß hCG AFP uE3 Inhibin (quadruple)**	**Free ß hCG AFP uE3 (triple)**	**Total hCG AFP (double)**	**Free ß hCG AFP (double)**
	DOR (95% CI) Studies	*88 (35,224) k=1*	*80 (32,201) k=1*	*71 (23,220) k=2*	*50 (30,84) k=5*	*41 (18,94) k=2*	*34 (21,53) k=7*	*27 (19,39) k=15*	*26 (18,38) k=12*

Table 2. Indirect comparisons of the diagnostic accuracy of the 12 test strategies (*Continued*)

Total hCG AFP uE3 Inhibin PAPPA	*80 (32, 201) k=1*	1.1 (0.3,4.1); P=0.87							
Total hCG AFP Inhibin	*71 (23, 220) k=2*	1.2 (0.3,5.3); P=0.77	1.1 (0.3,4.8); P=0.88						
Total hCG AFP uE3 Inhibin	*50 (30,84) k=5*	1.8 (0.6,5.1); P=0.29	1.6 (0.6,4.6); P=0.39	1.4 (0.4,4.9); P=0.58					
Free ß hCG AFP uE3 Inhibin	*41 (18,84) k=2*	2.1 (0.6,7.4); P=0.23	1.9 (0.6,6.7); P=0.30	1.7 (0.4,7.0); P=0.44	1.2 (0.5,3.2); P=0.69				
Free ß hCG AFP uE3	*34 (21,53) k=7*	2.6 (0.9,7.4); P=0.06	2.4 (0.9,6.6); P=0.10	2.1 (0.6,7.2); P=0.23	1.5 (0.8,2.9); P=0.24	1.2 (0.5,3.2); P=0.67			
Total hCG AFP	*27 (19,39) k=15*	3.3 (1.2,8.7); P=0.02	2.9 (1.1,7.8); P=0.03	2.6 (0.8,8.6); P=0.11	1.8 (1.0,3.4); P=0.05	1.5 (0.6,3.7); P=0.37	1.2 (0.7,2.1); P=0.43		
Free ß hCG AFP	*26 (18,38) k=12*	3.4 (1.3,9.3); P=0.01	3.1 (1.2,8.3); P=0.03	2.8 (0.8,9.1); P=0.09	1.9 (1.0,3.6); P=0.04	1.6 (0.6,4.0); P=0.31	1.3 (0.7,2.3); P=0.35	1.1 (0.7,1.7); P=0.81	
Total hCG AFP uE3	*21 (16,28) k=24*	4.3 (1.6, 11.2); P=0.003	3.8 (1.5, 10.0); P=0.006	3.4 (1.1, 11.0); P=0.04	2.4 (1.4,4.3); P=0.003	2.0 (0.8,4.7); P=0.12	1.6 (1.0,2.7); P=0.06	1.3 (0.9,1.9); P=0.18	1.2 (0.8,1.9); P=0.33
Free ß hCG	*14 (8,24) k=4*	6.4 (2.2, 18.5); P=0.0005	5.8 (2.0, 16.6); P=0.001	5.2 (1.5, 18.2); P=0.01	3.6 (1.8,7.5); P=0.0004	3.0 (1.1,8.0); P=0.03	2.4 (1.3,4.7); P=0.007	2.0 (1.1,3.5); P=0.02	1.9 (1.0,3.4); P=0.04
Total hCG	*12 (8,20) k=4*	7.3 (2.6, 20.4); P=0.0002	6.6 (2.3, 18.4); P=0.0004	5.9 (1.7, 20.1); P=0.005	4.1 (2.1,8.2); P<0.0001	3.4 (1.3,8.7); P=0.01	2.8 (1.5,5.2); P=0.001	2.2 (1.3,3.8); P=0.003	2.1 (1.2,3.7); P=0.009
AFP	*8 (5,14) k=4*	10.8 (3.7, 31.4); P=<0.0001	9.7 (3.3, 28.1) P<0.0001	8.6 (2.4, 30.6); P=0.0008	6.1 (2.9, 12.8); P<0.0001	5.0 (1.8, 13.5); P=0.002	4.1 (2.1,8.0) P<0.0001	3.3 (1.8,6.0) P<0.0001	3.1 (1.7,5.8) P=0.0003
Ratio of DOR (95%CI); P-value		**Total hCG AFP uE3 (triple)**	**Free ßhCG (single)**	**Total hCG (single)**					

Table 2. Indirect comparisons of the diagnostic accuracy of the 12 test strategies (*Continued*)

	DOR (95%CI) Studies	*21 (16,28) k=24*	*14 (8,24) k=4*	*12 (8,20) k=4*					
Free ß hCG	*14 (8,24) k=4*	1.5 (0.9,2.6); P=0.14							
Total hCG	*12 (8,20) k=4*	1.7 (1.0,2.9); P=0.04	1.1 (0.6,2.2); P=0.71						
AFP	*8 (5,14) k=4*	2.5 (1.4,4.5); P=0.002	1.7 (0.8,3.4); P=0.15	1.5 (0.8,2.9); P=0.26					

Indirect comparisons are made using all available data. Relative DORs are computed by division of the DOR for the test in the column by the DOR for the test in the row. If the relative DOR is greater than one then the diagnostic accuracy of the test for the column is higher than that for the row, if less than one the diagnostic accuracy of the test in the row is higher than in the column. All test combinations include maternal age.

Table 3. Investigation of sources of heterogeneity

Test combination	**Relative DOR (95% CI)**	**P value**	**Sensitivity at 5% FPR (95% CI) (studies)**	
Effect of maternal age			**<= 35 years**	**> 35 years**
Free β hCG, AFP and age	0.56 (0.33, 0.96)	P=0.03	66.4 (58.8, 73.2) k=9	51.7 (39.1, 64.1) k=3
Total hCG, AFP, uE3 and age	0.43 (0.29, 0.63)	P< 0.0001	68.6 (62.3, 74.3) k=11	48.4 (40.7, 56.2) k=13
Total hCG, AFP and age	0.41 (0.12, 1.38)	P=0.15	69.1 (64.1, 73.7) k=13	54.2 (44.1, 64.1) k=2
Bias in both fitting and evaluating in derivation datasets			**Derivation dataset**	**Validation dataset**
Free β hCG, AFP and age	0.67 (0.40, 1.09)	P=0.11	67.0 (58.9, 74.5) k=6	57.2 (47.1, 66.6) k= 6
Total hCG, AFP, uE3 and age	1.48 (0.86, 2.56)	P=0.15	54.0 (43.2, 64.4) k=8	63.0 (54.6, 70.6) k=16
Total hCG, AFP and age	0.99 (0.64,1.52)	P=0.95	66.1 (59.2, 72.3) k=6	65.8 (59.3, 71.7) k=9

Table 4. Sensitivity analysis of maternal age effect

Correction made for missing false negatives in studies with delayed verification of test negatives	Free β hCG, AFP and age			Total hCG, AFP, uE3 and age			Total hCG, AFP and age		
	Relative DOR (P value)	Sensitivity(%) at 5%FPR		Relative DOR (P value)	Sensitivity(%) at 5%FPR		Relative DOR (P value)	Sensitivity(%) at 5%FPR	
		> 35yrs (n = 3)	<= 35yrs (n= 9)		> 35 yrs (n = 13)	<= 35 yrs (n = 11)		> 35yrs (n = 2)	<= 35yrs (n = 13)
No FN correction	ROR=0.56 (P=0.03)	51.7%	66.4%	ROR = 0.43 (P < 0.0001)	48.4%	68.6%	ROR=0.41 (P=0.15)	54.2%	69.1%
FN increased +10%	ROR = 0.61 (P=0.07)	51.7%	64.4%	ROR = 0.46 (P < 0.0001)	48.0%	66.4%	ROR=0.45 (P=0.14)	53.1%	67.1%
FN increased +20%	ROR = 0.66 (P=0.11)	51.6%	62.5%	ROR = 0.50 (P < 0.0001)	47.6%	64.4%	ROR=0.49 (P=0.14)	52.1%	65.3%
FN increased +30%	ROR=0.71 (P=0.18)	51.5%	60.7%	ROR = 0.54 (P < 0.0001)	47.2%	62.5%	ROR=0.53 (P=0.15)	51.1%	63.5%
FN increased +40%	ROR=0.75 (P=0.27)	51.5%	59.0%	ROR = 0.57 (P < 0.0001)	46.8%	60.7%	ROR=0.57 (P=0.16)	50.3%	61.8%
FN increased +50%	ROR=0.80 (P=0.39)	51.4%	57.4%	ROR = 0.61 (P = 0.01)	46.5%	59.0%	ROR=0.61 (P=0.18)	49.6%	60.2%

APPENDICES

Appendix 1. Search Strategy

Database: Ovid MEDLINE

---

1 exp Prenatal Diagnosis/
2 nuchal translucency.mp.
3 exp Pregnancy-Associated Plasma Protein-A/
4 pregnancy associated plasma protein a.mp.
5 papp-a.mp.
6 exp Chorionic Gonadotropin, beta Subunit, Human/
7 (b-hcg or bhcg).mp.
8 human chorionic gonadotropin.mp.
9 exp alpha-Fetoproteins/
10 alphafetoprotein$.mp.
11 alpha-fetoprotein$.mp.
12 afp.mp.
13 (unconjugated estriol or unconjugated oestriol).mp.
14 ue3.mp.
15 exp INHIBINS/
16 inhibin a.mp.
17 ultrasound.mp.
18 amniocentesis/
19 chorion$ vill$ sampling.mp.
20 Chorionic Villi-Sampling/
21 nasal bone.mp.
22 tricuspid regurgitation.mp.
23 ductus venosus.mp
24 marker$.mp.
25 screen$.mp.
26 detect$.mp.
27 accura$.mp.
28 predict$.mp.
29 ROC.mp.
30 ROC curve/
31 AUC.mp.
32 Area under curve/
33 exp false negative reactions/ or exp false positive reactions/
34 (false positive$ or false negative$).mp.
35 likelihood ratio$.mp.
36 sensitiv$.mp.
37 specific$.mp.
38 diagnos$.ti,ab.
39 "reproducibility of results".mp.
40 reference value$.mp.
41 reference standard$.mp.
42 exp Down Syndrome/
43 downs syndrome.mp.
44 down syndrome.mp.
45 trisomy 21.mp.
46 Aneuploidy/
47 aneuploidy.mp.

48 Mosaicism/
49 mosaicism.mp.
50 or/1-41
51 or/42-49
52 50 and 51
53 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).mp.
54 52 and 53
55 animal/ not (humans/ and animal/)
56 54 not 55
****

EMBASE via Dialog Datastar

1. PRENATAL-DIAGNOSIS#.DE.
2. FETUS-ECHOGRAPHY#.DE.
3. PREGNANCY-ASSOCIATED-PLASMA-PROTEIN-A#.DE.
4. CHORIONIC-GONADOTROPIN-BETA-SUBUNIT#.DE.
5. HCG.AB.
6. PAPP.AB.
7. ALPHA-FETOPROTEIN#.DE.
8. AFP.AB.
9. ALPHA ADJ FETOPROTEIN$
10. ALPHAFETOPROTEIN$
11. BETA ADJ HUMAN ADJ CHORIONIC ADJ GONADOTROPIN
12. PREGNANCY ADJ ASSOCIATED ADJ PLASMA ADJ PROTEIN
13. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).TI.
14. (UNCONJUGATED ADJ ESTRIOL OR UNCONJUGATED ADJ OESTRIOL).AB.
15. UE3
16. INHIBIN-A#.DE.
17. INHIBIN ADJ A
18. ULTRASOUND
19. AMNIOCENTESIS
20. CHORION-VILLUS-SAMPLING.DE.
21. NASAL ADJ BONE
22. TRICUSPID ADJ REGURGITATION
23. DUCTUS ADJ VENOSUS
24. MARKER OR MARKERS
25. SCREEN OR SCREENING
26. DETECT OR DETECTING OR DETECTION
27. FALSE ADJ POSITIVE$
28. FALSE ADJ NEGATIVE$
29. SENSITIVITY OR SENSITIVE OR SENSITIVITIES
30. SPECIFICITY OR SPECIFICITIES
31. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).TI.
32. (DIAGNOSE OR DIAGNOSIS OR DIAGNOSTIC OR DIAGNOSTICS OR DIAGNOSES OR DIAGNOSED).AB.
33. ROC.AB.
34. AUC.AB.
35. AREA-UNDER-THE-CURVE.DE.
36. ROC-CURVE.DE.
37. ACCURA$
38. PREDICT$
39. REPRODUCIBILITY.DE.

40. REFERENCE ADJ VALUE$
41. REFERENCE-VALUE.DE.
42. REFERENCE ADJ STANDARD$
43. DOWN-SYNDROME#.DE.
44. DOWN ADJ SYNDROME OR DOWNS ADJ SYNDROME
45. TRISOMY ADJ '21'
46. MOSAICISM
47. ANEUPLOIDY
48. ANTENATAL$ OR PRENATAL$ OR PREGNANCY OR PREGNANT OR TRIMESTER$ OR MATERNAL OR FETUS OR FOETUS OR FOETAL OR FETAL
49. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 37 OR 38 OR 39 OR 40 OR 42
50. 43 OR 44 OR 45 OR 46 OR 47
51. 48 AND 49 AND 50
52. HUMAN=YES
53. 51 AND 52

ADJ = adjacent AB = abstract
TI = title $ = truncation symbol DE = descriptor (similar to MeSH)
****

CINAHL via OVID

---

1 exp Prenatal Diagnosis/
2 nuchal translucency.mp.
3 pregnancy associated plasma protein.mp.
4 papp$.ti,ab.
5 exp Gonadotropins, chorionic/
6 (b-hcg or bhcg).mp.
7 human chorionic gonadotropin.mp.
8 exp alpha-Fetoproteins/
9 alphafetoprotein$.mp.
10 alpha-fetoprotein$.mp.
11 afp.mp.
12 (unconjugated estriol or unconjugated oestriol).mp.
13 ue3.mp.
14 inhibin$.mp.
15 ultrasound.mp.
16 amniocentesis/
17 chorion$ vill$ sampling.mp.
18 Chorionic Villi-Sampling/
19 nasal bone.mp.
20 tricuspid regurgitation.mp.
21 ductus venosus.mp.
22 marker$.mp.
23 screen$.mp.
24 detect$.mp.
25 accura$.mp.
26 predict$.mp.
27 ROC.mp.
28 ROC curve/
29 AUC.mp.
30 "area under curve".mp.
31 exp false negative reactions/ or exp false positive reactions/

32 (false positive$ or false negative$).mp.
33 likelihood ratio$.mp.
34 sensitiv$.mp.
35 specific$.mp.
36 diagnos$.ti,ab.
37 "reproducibility of results".mp.
38 reference value$.mp.
39 reference standard$.mp.
40 exp Down Syndrome/
41 downs syndrome.mp.
42 down syndrome.mp.
43 trisomy 21.mp.
44 aneuploidy.mp.
45 mosaicism.mp.
46 (antenatal$ or prenatal$ or trimester$ or pregnan$ or fetus or foetus or fetal or foetal).mp.
47 or/1-39
48 or/40-45
49 47 and 48 and 46
****

Search terms and instructions for Biosis

The following search terms were entered separately in standard search box (select 'Titles/subject/abstract' from the drop-down box on the right of the search box).

1. "reference standard*"
2. "reference value*"
3. "reproducibility of results"
4. diagnos*
5. sensitiv*
6. specific*
7. "likelihood ratio*"
8. "false negative*
9. "false positive"
10. "area under curve"
11. ROC
12. AUC
13. predict*
14. detect*
15. marker*
16. screen*
17. accura*
18. "ductus venosus"
19. "nasal bone"
20. "tricuspid regurgitation"
21. "chorion* vill* sampling"
22. amniocentesis
23. ultrasound
24. inhibin*
25. "unconjugaed oestriol"
26. "unconjugated estriol"
27. afp
28. "alpha fetoprotein*"
29. alphafetoprotein*

30. " bhcg"
31. "human chorionic gonadotrophin"
32. "papp a"
33. "pregnancy associated plasma protein"
34. "nuchal translucency"
35. foetal
36. fetal
37. foetus
38. foetal
39. prenatal*
40. antenatal*
41. pregnan*
42. maternal*
43. "trisomy 21"
44. mosaicism
45. "down* syndrome"

The search then used the history function to combine terms:
1-34 - combine using OR
35 - 42 - combine using OR
43 - 45 - combine using OR
The three sets were combined using AND

The combined search strategy had the form
(((((((al: "trisomy 21") or (al: (mosaicism))) or (al: "down* syndrome"))) and ((((((((((((((((((((((((((((((((al: "reference standard*") or (al: "reference value*")) or (al: "reproducibility of results")) or (al: (diagnos*))) or (al: (specific*))) or (al: (sensitiv*))) or (al: "likelihood ratio*")) or (al: "false negative*")) or (al: "false positive*")) or (al: "area under curve")) or (al: (auc))) or (al: (roc))) or (al: (predict*))) or (al: (accura*))) or (al: (detect*))) or (al: (screen*))) or (al: (marker*))) or (al: "ductus venosus")) or (al: "tricuspid regurgitation")) or (al: "nasal bone")) or (al: "chorion* vill* sampling")) or (al: (amniocentesis))) or (al: (ultrasound))) or (al: (inhibin*))) or (al: "unconjugated oestriol")) or (al: "unconjugated estriol")) or (al: (afp))) or (al: "alpha feto protein*")) or (al: "alpha fetoprotein*")) or (al: "b hcg")) or (al: "human chorionic gonadotropin")) or (al: "papp a")) or (al: "pregnancy associated plasma protein")) or (al: "nuchal translucency")))) and ((((((((((al: (foetal)) or (al: (fetal))) or (al: (foetus))) or (al: (fetus))) or (al: (pregnan*))) or (al: (trimester*))) or (al: (prenatal*))) or (al: (antenatal*))))))

****
The Database of Abstracts of Reviews of Effectiveness (DARE), National Research Register and Health Services Research Projects in Progress database
:
1. Down syndrome (MeSH)
2. down* next syndrome
3. trisomy
4. aneuploidy
5. mosaicism
6. OR/ 1-5

****
MEDION (http://www.mediondatabase.nl/)

ICPC code for pregnancy - 'W'.
****
The Database of Systematic Reviews and Meta-Analyses in Laboratory Medicine - download the database to a .pdf file and search for the following terms separately:

Down

Trisomy
Aneuploidy
Pregnant
Pregnancy
Pregnancies
Mosaicism
****

Appendix 2. Glossary of terms (adapted in part from the UK National Screening Committee Glossary)

Abnormal ductus venosus flow velocity	The ductus venosus is a vessel in the fetus which allows oxygenated blood from the placenta to bypass the fetal liver and flow straight to the heart. In conditions such as Down's syndrome the pressure in this vessel can be abnormally high
Absent nasal bone	Absence of the bone that forms the bridge of the nose, which may be detected at ultrasound scan during early pregnancy
Affected individuals	Those individuals who are affected by the disorder for which they are being screened
Amniocentesis	Amniocentesis is an invasive procedure which involves taking a small sample of the amniotic fluid (liquor) surrounding the baby, using a needle which goes through the abdominal wall into the uterus, and is usually performed after 15 weeks gestation
Chorionic villus sampling (CVS)	Chorionic villus sampling involves taking a sample of the placental tissue using a needle which goes through the abdominal wall and uterus or a cannula through the cervix. It is usually performed between 10 and 13 weeks gestation
Combined test	First trimester test (up to 13 + 6 weeks of pregnancy) based on combining nuchal translucency measurement with free beta-hCG, pregnancy-associated plasma protein A (PAPP-A) and the woman's age
Diagnostic accuracy	The amount of agreement between the information from the index test and the reference standard (see below)
Diagnostic test	A definitive test, performed after a positive screening test result that gives a diagnosis (i.e. yes or no)?
Double test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG ß either free beta-hCG or total hCG), together with the woman's age
First trimester	Pregnancy from conception up to 13 weeks and 6 days.
Iatrogenic	A disease or condition in a patient occurring as a result of treatment
Index test	A test or group of tests being evaluated in a systematic review

(*Continued*)

Integrated test	Measurements performed at different times of pregnancy combined into a single test result. Unless otherwise specified, 'integrated test' refers to the combination of nuchal translucency measurement and PAPP-A in the first trimester, with the quadruple test (see below) in the second
Mosaicism	This is a condition in which person has some cells containing a normal number of chromosomes, and some containing an abnormal number. The more abnormal cells there are, the greater the effect
Multiple of the median (MOM)	The serum test concentration for a pregnant woman divided by the average (median) for unaffected pregnancies in a defined population at the same stage of pregnancy
Quadruple test	Second trimester test (from 13 + 6 up to 24 weeks of pregnancy) based on the measurement of AFP, uE3, free beta-hCG (or total hCG), and inhibin-A together with the woman's age
Reference Standard	The best available method for establishing the presence or absence of the target disease or condition
Second trimester	Pregnancy from 14 weeks to 28 weeks gestation. Note that for the purposes of this Cochrane review, second trimester testing refers to the period of 14 to 24 weeks gestation
Tricuspid regurgitation	Leakiness of or backflow of blood through the tricuspid valve of the heart. The tricuspid valve separates the upper and lower chambers of the right side of the heart
Triple test	Second trimester test (from 14 up to 24 weeks of pregnancy) based on the measurement of AFP, unconjugated oestriol (uE3), and hCG (either total hCG or free beta-hCG) together with the woman's age
Trisomy	The presence of an extra chromosome resulting in three copies of a particular chromosome instead of the normal two
Translocation	Part of one chromosome is broken off and attached to another chromosome. This does not usually cause the individual any problems as they have a normal amount of chromosomes, but in an abnormal arrangement. It can be passed on as an extra chromosome to offspring, resulting in conditions such as Down's syndrome

HISTORY

Review first published: Issue 6, 2012

CONTRIBUTIONS OF AUTHORS

KA undertook the searches, applied eligibility criteria, extracted and entered data and wrote the first draft of the review.

JD supervised and planned the review, checked data extraction, supervised statistical analyses and wrote the second draft of the review.

BG checked data extraction and undertook statistical analyses.

JP applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

ZA applied eligibility criteria, provided senior clinical input, oversaw the review process, and approved the final draft of the review.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- University of Birmingham, UK.

Funding of the research time of JD and BG

External sources

- NIHR Health Technology Assessment Programme, UK.

Project grant - need to have reference number etc. Jim/Zarko can you add please?

- NIHR Health Technology Assessment Programme, UK.

Funding for the Cochrane Reviews of Diagnostic Test Accuracy Support Unit, based at the University of Birmingham (JD).

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The protocol intended to investigate several additional outcomes downstream from test accuracy, should they be reported in the test accuracy studies. When we attempted to extract this information however, it was found to be available in very few studies, and where such information was found it was difficult to extract meaningful data to allow for comparison between studies, as data were not reported in a universal manner. In several studies such outcomes were estimated rather than measured. Often they were not reported at all. The outcomes stated in the protocol which have not been included are: harms of testing; need for further testing; side effects of test; interventions and side effects; other abnormalities detected by testing; spontaneous miscarriage; miscarriage subsequent to invasive procedure, with or without normal karyotype; fetal karyotype; termination of pregnancy (prior to definitive testing or in a karyotypically normal pregnancy and following confirmation of Down's Syndrome or following detection of other chromosomal abnormalities); stillbirth; livebirth of affected and unaffected fetus; uptake of definitive testing by women.

The following refinements to the eligibility criteria were imposed to ensure that the quality of the included literature remained high. We excluded studies that identified fewer than five Down's syndrome pregnancies in their study population. We excluded studies that had less than 80% follow-up of participants.

In addition, the analytical strategy was informed by the volume of tests and studies included, and developed so that we focused on key tests and test combinations by a) only meta-analysed tests that were included in four or more papers or b) showed more than 70% sensitivity for more than 90% specificity. In addition a requirement that a minimum of 10 studies for a single test was required before subgroup analysis was undertaken. Consequently several possible sources of heterogeneity were not investigated due to lack of data.

NOTES

This review belongs to a suite of reviews examining antenatal screening for Down's syndrome which include four other titles: '*First trimester serum tests for Down's syndrome screening*'; '*First trimester serum and ultrasound tests for Down's syndrome screening*'; 'First and second trimester serum tests *with and without first trimester ultrasound tests for Down's syndrome screening*'; and '*Urine tests for Down's syndrome screening*'. The project as a whole has been much larger than initially anticipated, both in terms of size and statistical complexity. The initial search was completed in 2007. After identifying studies appropriate for inclusion, a significant amount of time has been devoted to data management and analysis.

The authors are conscious of the time lag from the latest literature search to publication, and the potential for the introduction of new second trimester serum tests in this time frame. The authors are also conscious of the potential for publication of new data pertaining to tests included in this review. The literature search has recently been updated and work is in progress with the other reviews in this suite, to bring these reviews up to date prior to publication. Following publication of the other reviews in this suite, we plan to update this review and then prepare an umbrella review, examining the overall best performing test combinations for antenatal Down's syndrome screening.

INDEX TERMS

Medical Subject Headings (MeSH)

Biological Markers [*blood]; Down Syndrome [*diagnosis]; Maternal Age; Pregnancy Trimester, Second [blood]; Prenatal Diagnosis [*methods]; Randomized Controlled Trials as Topic

MeSH check words

Adult; Female; Humans; Pregnancy