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Prenatal Diagnosis:

Screening and Diagnostic Tools

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INTRODUCTION

Approximately 3% to 5% of pregnancies are complicated by birth defects or genetic disorders.¹ Chromosomal abnormalities are present in approximately 1 in 150 live births,² and congenital malformations remain the leading cause of infant death and a leading cause of childhood death.³ These chromosomal abnormalities include aneuploidy (defined as having one or more extra or missing chromosomes), translocations, duplications, and deletions.

The most common chromosomal disorder is trisomy 21 (Down syndrome), with an incidence of 1 per 800 live births.⁴ Trisomy 13 and 18 can also result in live births, though with a significantly lower incidence.^{2,4} Sex chromosome aneuploidies are less common than autosomal aneuploidies.⁴ The only known viable monosomy is monosomy X (Turner syndrome). Incidences are described in Table 1.

Risk of aneuploidy increases with maternal age (Table 2).^{2,4} Other factors also influence patients' risk in any given pregnancy, including the presence of birth defects or soft markers on ultrasound and past obstetric history, particularly if it is notable for a prior pregnancy affected by aneuploidy or another genetic disorder. A past family history of aneuploidy increases current pregnancy risk of aneuploidy, especially if a parent is a balanced robertsonian translocation carrier, though most cases are sporadic and secondary to chromosomal nondisjunction.

Patients report many different motivations for pursuing aneuploidy screening or prenatal diagnosis. Some may choose pregnancy termination if the defect is identified at an early enough gestational age. Others may choose to pursue screening or testing to allow them time

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to process the diagnosis and seek experienced clinicians who may be able to aid them in preparation for caring for an affected infant and to care for their child after delivery. Some birth defects, such as some neural tube defects, may be eligible for prenatal treatment with subsequently improved neonatal outcomes.⁵ All patients choosing to undergo screening or testing should receive counseling regarding risks, benefits, and limitations of their chosen testing plan from their health care provider or genetic counselor. It is important to note that aneuploidy screening and testing decisions are heavily value driven; a frank discussion of the benefits, risks, and limitations of tests is key in ensuring that care is appropriate for each patient's individual goals.

HISTORY OF SCREENING

Initial screening for birth defects was developed in the 1950s with ultrasound and has become increasingly prominent in obstetric care. Real-time gray-scale imaging became available in the 1970s and improved prenatal diagnosis by allowing for evaluation of pregnancies earlier in gestation. Aims of ultrasonography include determination of gestational age and fetal number, evaluation for malformations, testing of fetal well-being, and assistance with invasive diagnostic and therapeutic procedures.⁶ Amniocentesis, the first available prenatal chromosomal diagnostic testing option, was first described in the 1950s.⁷ Amniocentesis has become increasingly safe and is now used for several purposes, including genetic screening and infectious evaluations. Chorionic villus sampling (CVS) is another diagnostic test and can be performed earlier in gestation.

Subsequently, noninvasive tests, including serum analyte screening and cell-free DNA screening, were developed for purposes of screening for genetic abnormalities within a pregnancy.

In 2007, the American Congress of Obstetricians and Gynecologists (ACOG) released "ACOG Practice Bulletin No. 77," which recommended making aneuploidy screening or invasive testing available for all women, ideally at their first prenatal visit.⁸ This idea was revolutionary at the time, as previously only women who were considered to be at high risk had been offered these tests.

SCREENING TESTS

Most prenatal testing is intended for screening. These tests include serum screening, carrier screening, and ultrasound; the goals of these tests are to identify women with pregnancies at high risk of chromosomal abnormalities or birth defects. Although ultrasound can be diagnostic, such as in the case of open neural tube defect, serum screening is intended only to identify women with pregnancies at an increased risk. Numerous options for serum screening are available with varying test criteria and timing of employment (Table 3).⁴

FIRST-TRIMESTER SCREEN

The first-trimester screen is a commonly used screening test that includes a combination of serum screening and ultrasonographic examination of the nuchal translucency performed between 10 and 13 weeks 6 days' gestation. Serum markers, including free beta-human

chorionic gonadotropin (hCG) and pregnancy-associated plasma protein A, are collected with a capillary blood sample between 9 and 13 weeks 6 days' gestation. A risk estimate is then developed that incorporates maternal age, past pregnancy history, number of fetuses in the current gestation, weight, race, serum markers, and nuchal translucency measurement. Some risk estimators also incorporate presence or absence of visualized nasal bone. This risk estimate is then expressed as a ratio, such as 1 in 10. One in 300 is commonly used as the cutoff for a high-risk result, but the cutoff is laboratory dependent. The detection rate for trisomy 21 varies from 82% to 87% depending on the laboratory, using a 5% screen positive rate.⁴

A nuchal translucency of greater than 3 mm is significantly associated with both aneuploidy and structural malformations.^{4,9-12} In the initial observational study describing this phenomenon, 35% of patients with a nuchal translucency measurement greater than 3 mm subsequently had confirmed aneuploidy.⁹ A subsequent observational study confirmed increased prevalence of cardiac defects in patients with a nuchal translucency greater than 3.5 mm with chromosomally normal pregnancies.¹³ Risk of other anomalies, including single gene defects and central nervous system, cardiac, skeletal, and abdominal wall defects, is also significantly increased in these pregnancies.¹⁰ It is, therefore, recommended that any woman with a thickened nuchal translucency undergo a targeted ultrasound and be offered a fetal echocardiogram to assess for presence of other structural cardiac malformations regardless of whether aneuploidy is present or absent.⁴

Benefits of first-trimester screening include the early gestational age at which results are provided, allowing patients and providers time to interpret results and make decisions surrounding further pregnancy care, including pursuit of further diagnostic testing, genetic counseling, maternal fetal medicine consultation, or termination if desired. There are several drawbacks to this screen as well. This test relies on the availability of certified, experienced sonographers to perform the nuchal translucency measurement. It has been previously demonstrated that a measurement discrepancy of only 0.5 mm significantly decreases the sensitivity of this test.¹⁴ The test's improved sensitivity over the quadruple marker screen also varies with gestational age; the test has improved detection at 11 weeks, though performance characteristics are similar to the quadruple marker screen at 13 weeks.¹⁵

QUADRUPLE MARKER SCREEN

The quadruple marker screen, or the quad screen, is the initial serum screening test that became available in the 1990s. It is still commonly used today, particularly in patients who present for care after the first trimester, which comprises more than 25% of patients using public health clinics.¹⁶ The quad screen may be performed between 15 and 22 weeks' gestation and involves serum measurements of proteins secreted by the pregnancy, including hCG, alpha-fetoprotein (AFP), inhibin A, and unconjugated estriol. These protein measurements are combined with the patients' age, race, weight, number of fetuses in the current gestation, diabetes status, and gestational age to provide a risk estimate. Detection rate is slightly lower than that of the first-trimester screen, with a reported detection rate of 81% using a 5% screen positive rate.⁴

Advantages of the quad screen include its ability to screen for open neural tube defects in addition to aneuploidy. Serum AFP is secreted by the fetus and is present in the amniotic fluid and, therefore, also maternal serum. It also does not require a specially trained sonographer to perform and, thus, may be more readily available to some providers.

Several centers may offer variations on the quad screen, including the triple screen, which does not include inhibin measurements,¹⁷ or the penta screen, which also includes hyperglycosylated hCG.¹⁸ These tests do not seem to have improved test characteristics.

INTEGRATED, STEPWISE SEQUENTIAL, AND CONTINGENT SCREENING

Numerous screening modalities incorporate both a first-trimester screen and the quad screen. These modalities included integrated screening, the stepwise sequential screen, and the contingent screening. Integrated screening involves performing a first trimester screen, of which the results are not providing to the patient or provider, and subsequently performing a quad screen. All of these values are then incorporated into a single risk estimate to provide patient a comprehensive risk of her second trimester risk of aneuploidy. The detection rate is 96%, the highest of any available serum screens other than cell-free DNA, with a 5% screen positive rate. Downsides to this approach include its relatively late availability of results, limiting the time in which patients and their provider may have to make important decisions about future care.

Both the stepwise sequential screen and the contingent screen make first-trimester screening results available to patients. The stepwise sequential screen involves performing the first-trimester screen and the quad screen. Results are available to women after their first-trimester screen, allowing for earlier counseling and diagnosis for patients at high risk of aneuploidy. The contingent screen involves performing a first-trimester screen for all women, after which women are stratified into high-, medium-, and low-risk groups. The high-risk group is then offered a diagnostic test. The low-risk group has no further testing. The intermediate-risk group is offered quad screening. The detection rate varies between 80% and 94% for this screening method, with a 5% screen positive rate.

CELL-FREE FETAL DNA

Cell-free DNA, commonly referred to as noninvasive prenatal screening, became commercially available in 2011. This relatively new technology involves collecting a maternal serum sample, from which cell-free fragments of DNA from the pregnancy are isolated. This cell-free DNA is primarily placental in origin and is released from apoptotic trophoblasts. Fetal fraction increases with gestational age but is reliably greater than 10% as early as 10 weeks' gestation. Notably, fetal fraction of greater than 4% is required for reliable analysis. This cell-free DNA is then evaluated by one of 2 techniques (via massive parallel shotgun sequencing, targeted massive parallel sequencing, or interrogation of single nucleotide polymorphisms),¹⁹ depending on which laboratory is running the analysis. Results are typically reported with aneuploidy detected or no aneuploidy detected or as high- or low-risk for aneuploidy and with sex chromosome information if desired.

This screening test has the highest available detection rate of all available screening tests for trisomy 21 with a detection rate of 99% on a recently updated meta-analysis.²⁰ Detection rates for trisomy 18, 13, and sex chromosome abnormalities are significantly lower than for trisomy 21 (Table 4).²⁰ It is important to note that at present, cell-free DNA for aneuploidy screening is only recommended by the ACOG for women with high pretest risk of aneuploidy, as described in Box 1. It is also notable that the studies that provided the test characteristics described earlier excluded patients who did not have sufficient fetal fraction to provide a risk estimate. It has subsequently been found that an inconclusive result significantly increases aneuploidy risk, with low fetal fraction significantly associated with aneuploidy, particularly trisomy 13 and 18.²¹ Other factors that may influence fetal fraction include weight, with obese women having an increased risk of low fetal fraction, and lower gestational age.^{21,22}

This test should be clearly conveyed to patients as a screening test rather than a diagnostic test. The positive predictive value for trisomy 21 in the population for whom it is currently recommended is very high. However, positive predictive value depends on the prevalence of the disorder within the population. Therefore, the positive predictive value is expected to be significantly lower in an average-risk population. A recent retrospective cohort study out of 2 academic centers identified 105 patients with cell-free DNA results consistent with autosomal trisomies; of these, aneuploidy was only confirmed in 82% by karyotype, with the remainder of patients having normal antenatal or postnatal karyotype.²³ Previous studies have shown that patients' misunderstanding of this test is significant despite pretest counseling.²⁴ Notably, in the aforementioned cohort, 9 patients underwent termination of pregnancy without diagnostic confirmation of a chromosomal abnormality.²³ Any results should be interpreted with the aid of a genetic counselor in order to provide further guidance as to patients' individual risk. Calculators for individual risk estimates using cell-free DNA results are available through both the University of North Carolina's MomBaby Web site (available at med.unc.edu/obgyn/Patient_Care/specialty-services/maternal-fetalmedicine/mombaby/nips_calc.html; retrieved July 22, 2016) and the Perinatal Quality Foundation (available at perinatalquality.org; retrieved July 22, 2016). Ultrasound is recommended before testing to confirm fetal number and gestational age and to evaluate for presence of major anomalies identifiable in the first trimester, as this would alter a priori aneuploidy risk. In one retrospective cohort, 16% of patients were found to have ultrasound findings that altered counseling and recommendations regarding testing or screening modality, including incorrect pregnancy dating, embryonic or fetal demise, twin gestation or presence of an anomaly.²⁵ In those cases in which a cystic hygroma or anomaly is identified, patients may choose to undergo diagnostic testing rather than screening, allowing for earlier prenatal diagnosis.²⁵

Other benefits of cell-free DNA include its ability to accurately identify fetal sex with excellent accuracy and fetal Rh status in pregnancies at risk of Rh isoimmunization.²⁶ Food and Drug Administration–approved cell-free DNA technology for anti-Kell and other sources of isoimmunization is not yet available in the United States. Several laboratories have begun to report on other autosomal aneuploidies or for microdeletions; however, these tests are not currently validated and are not recommended at present.^{4,27} In fact, a retrospective analysis evaluating a small number of cases yielded 0% positive predictive

values for evaluated microdeletions; given low prevalence of microdeletion syndromes, positive predictive value for most microdeletions is not expected to surpass 10%.²⁸ Use of cell-free DNA has also not been widely studied in multiple gestations, and use is currently not recommended in this setting.²⁷

It is also worth noting that occasionally, cell-free DNA screening will reveal maternal chromosomal abnormalities or concerns, including maternal mosaicism or, rarely, maternal malignancies.²⁹ Patients should be counseled of this possibility before proceeding with screening. Maternal chromosomal abnormalities or malignancy may result in nonreportable or false-positive results. Other possible sources of false-positive results include vanishing twins or confined placental mosaicism. It is also worth noting that although cell-free DNA has the best detection rate for trisomy 21 of any screening modalities, sequential screening continues to have an improved detection rate for all chromosomal abnormalities over cell-free DNA, indicating that many other chromosomal abnormalities that may be identified with traditional serum screening may be missed with cell-free DNA.³⁰

ULTRASOUND ONLY

Ultrasound is now ubiquitous in pregnancy management. Nearly all women receive at least one ultrasonographic examination of their pregnancy during a routine obstetric care, and many receive more than one. The primary function of ultrasound and obstetric care is for confirmation of dating as well as surveillance for birth defects.

Many patients choose to pursue ultrasound screening only for evaluation of malformations or markers for aneuploidy, as second-trimester transabdominal ultrasonography performed between 18 and 23 weeks has become routine in prenatal care to evaluate for anatomic anomalies. Many patients also undergo first-trimester ultrasonography via either a transvaginal or transabdominal route to evaluate for viability, pregnancy number, and for evaluation of major anomalies that can be identified in the first trimester, such as anencephaly or cystic hygromas. Some anomalies have known associations with particular aneuploidies or chromosomal defects, increasing the likelihood of the presence of these conditions when identified.

DIAGNOSTIC TESTING

Diagnostic testing allows patients to know with as much certainty as possible whether their pregnancy may be affected by a particular genetic condition. The most common indication for diagnostic testing in the United States currently is advanced maternal age or maternal age of 35 years or older on the estimated date of delivery. Other common indications include positive aneuploidy screening results, known family history of genetic disorders, or anomalies identified on ultrasound. Although diagnostic testing is recommended by the ACOG to be available to all women, regardless of maternal age, patients should be counseled before proceeding on risk of pregnancy loss.

CHORIONIC VILLUS SAMPLING

CVS has decreased in frequency with the recent increased uptake of cell-free DNA screening. It remains the only diagnostic test available in the first trimester and allows for diagnostic analyses, including fluorescence in situ hybridization (FISH), karyotype, microarray, molecular testing, and gene sequencing. CVS is performed between 10 and 14 weeks' gestation. CVS has been performed before 9 weeks in the past, though this has shown to increase the risk of limb deformities and, therefore, is no longer recommended.

CVS may be performed via either transcervical or transabdominal approach. Via either approach, chorionic villi are collected for genetic evaluation under ultrasound guidance without entering the amniotic sac. CVS allows for earlier prenatal diagnosis, subsequently decreasing time of uncertainty and allowing for earlier (and, therefore, safer) pregnancy termination if desired. A disadvantage of CVS, however, is that approximately 1% to 2% of CVS results may reflect confined placental mosaicism rather than true fetal chromosomal abnormalities. Confined placental mosaicism may increase the risk of having a small-for-gestational-age infant.³¹ Pregnancy loss attributed to CVS is approximately 1 in 455 on the most recent estimates.^{32,33}

AMNIOCENTESIS

Amniocentesis, similar to CVS, has decreased in frequency with increased utilization of cell-free fetal DNA screening. It remains the only diagnostic test available in the second or third trimesters of pregnancy and may be performed at any gestational age after 15 weeks. Using this technique, a sterile needle is introduced into the amniotic sac under ultrasound guidance, and amniotic fluid is obtained and sent for testing. In addition to evaluation for genetic disorders, amniocentesis may also be used to evaluate for presence of intra-amniotic or fetal infection via culture or polymerase chain reaction or for neural tube defects by measuring amniotic fluid alpha-fetoprotein and acetylcholinesterase. Complications are more common at earlier gestational ages. Pregnancy loss attributed to amniocentesis is approximately 1 in 900 on most recent estimates.^{32,33}

CYTOGENETIC EVALUATIONS

Chromosome analysis from CVS and amniocentesis samples is the most reliably predictive method of identifying pregnancies affected by chromosomal disorders. However, some issues with cytogenetic testing have been identified that may limit the clinical utility of these methods.

Mosaicism refers to tissue that contains 2 or more distinct cell lines. It is thought to reflect true mosaicism when multiple colonies from multiple cultures reveal the same results. Pseudomosaicism refers to a single cell with a different genetic makeup than the others and is usually not clinically significant. Mosaicism may also arise in primary cell culture; when this occurs, it reflects pseudomosaicism rather than true mosaicism. Particular to CVS, confined placental mosaicism occurs in approximately 1% to 2% of pregnancies; although this does reflect true mosaicism, it carries different clinical concerns for the fetus than for other pregnancies. As confined placental mosaicism also causes false-positive cell-free DNA

results, amniocentesis is preferred over CVS for diagnostic testing in cases of positive cell-free DNA. With some trisomies, particularly trisomy 15, a diploid fetus often arises secondary to trisomy rescue, which does increase the risk of uniparental disomy and subsequently increases risk of Prader-Willi and Angelman syndrome. Cell culture failure also rarely occurs and is more common with sampling via CVS than with amniocentesis.

Multiple testing methodologies are available, designed to detect different types of genetic abnormalities. Large deletions and duplications may be identified with karyotype in more than 5 million base pairs, whereas small deletions and duplications may be identified with microarray technology at as small as a 50,000 base pair level.³⁴ FISH technology is available for identification of major autosomal aneuploidies or for selected deletions and duplications, such as DiGeorge syndrome. Single-gene disorders often require more targeted molecular approach to identify whether or not a particular mutation in a particular panel of genes is present or absent. As detection of aneuploidy is the most common indication for invasive testing, FISH is often the first test that is sent. This technology does not require cell culture; thus, results are often available within 48 hours. Despite that these results are obtained from a diagnostic procedure, these results should still be considered a screen and should be confirmed via karyotype given rare reports of both false-positive and false-negative results.³⁵ Microarray can also be performed on uncultured cells and, therefore, can also result in a more rapid turnaround time. Results can also be obtained from nonviable cells with this technique and, thus, may be more likely to result in cases of stillbirth. Given that microarray is able to detect both aneuploidy and smaller deletions and duplications with rapid turnaround, it is now recommended for evaluation of structural abnormalities as the initial testing strategy along with FISH, rather than conventional karyotype.³³

As availability and uptake of cytogenetic testing with microarray increases, increasing numbers of chromosomal abnormalities without known clinical consequences have been identified, which may increase parental anxiety when one of these variants of uncertain significance is identified. In these cases, parental studies are often considered to determine whether the variant is present in either parent. If so, it is more likely to be of little to no clinical significance. Given that 1.7% of structurally normal pregnancies without aneuploidy will have a variant of unknown significance detected,³⁶ patients opting for an amniocentesis with a normal anatomic survey should be counseled about the possibility of the finding of a variant of uncertain clinical significance with microarray testing.³³

PREIMPLANTATION GENETIC DIAGNOSIS

Preimplantation genetic diagnosis (PGD) is now widely available and may allow for even earlier detection of chromosomal abnormalities. This procedure is performed after in vitro fertilization (IVF) by manipulation of the embryo to either remove a polar body or to remove a single cell from the blastocyst. This procedure allows for detection of the abnormality before embryo transfer so that only unaffected embryos are transferred back. It is recommended that all pregnancies conceived with IVF/PGD be offered confirmatory testing with CVS or amniocentesis as false-negative reports are possible³⁷ with an anticipated negative predictive value of normal FISH of 81%.³⁸ The growing body of literature surrounding PGD illustrates minimal risk outside of the cost of this procedure.³³

SUMMARY

All women should be offered aneuploidy screening or diagnostic testing during pregnancy. Just as importantly, available options should be explained to patients and families in depth, most notably including the risks and benefits of each option, and how results might be reported. Patients who choose cell-free fetal DNA technology should be counseled that the test remains a screening test for aneuploidy at this time and that microdeletion testing continues to have poor positive predictive values due to the low prevalence of these disorders. It is not recommended that patients undergo more than one screening modality but rather that women who have positive screens and wish to pursue further testing be counseled on diagnostic testing with amniocentesis and CVS so as not to delay diagnosis. Amniocentesis and CVS are increasingly safe with low rates of pregnancy loss and should continue to be available to all women who desire diagnostic testing regardless of risk factors or presence or absence of anomalies.

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Box 1**Indications for cell-free DNA screening**

- Maternal age greater than 35 years at delivery
- Ultrasonographic findings indicating increased aneuploidy risk
- History of prior pregnancy affected by a trisomy
- Parental balanced robertsonian translocation increasing risk of trisomy 13 or 21
- High-risk first-trimester or second-trimester aneuploidy screening results

Data from Cell-free DNA screening for fetal aneuploidy. Committee Opinion No. 640. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2015; 126(3): e31–7.

KEY POINTS

- Aneuploidy screening should be offered to all women at their first prenatal visit.
- Cell-free fetal DNA screening is currently recommended for high-risk populations only and should be considered a screening test rather than a diagnostic test.
- Chorionic villus sampling and amniocentesis carry a small but potential risk of pregnancy loss but remain the only diagnostic methodologies available presently.
- Women should receive thorough pretest counseling regarding the risks and benefits of available options and should receive thorough posttest counseling with individualized interpretation of results.

Table 1

Incidence of common aneuploidies

Trisomy 21	1 in 800 live births
Trisomy 18	1 in 7500 live births
Trisomy 13	1 in 15,000 live births
Monosomy X (Turner syndrome)	1 in 5000 girls
Trisomy X	1 in 1000 girls
XXY (Klinefelter syndrome)	1 in 1000 boys
XYY	1 in 1000 boys

Data from Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson genetics in medicine. 7th edition. Philadelphia: Saunders/Elsevier; 2007.

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Table 2

Risk of aneuploidy by maternal age

Maternal Age at EDD (y)	Risk of Trisomy 21	Risk of Other Chromosomal Abnormality
20	1:1480	1:525
25	1:1340	1:475
30	1:940	1:384
35	1:353	1:178
40	1:85	1:62
45	1:35	1:18

Abbreviation: EDD, estimated date of delivery.

Adapted from Practice bulletin no. 163: screening for fetal aneuploidy. *Obstet Gynecol* 2016; 127(5): e124.

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Table 3

Characteristics of serum screening options for aneuploidy

Screening Test	Gestational Age at Screening (in wk)	Detection Rate for Trisomy 21 (%)	Screen Positive Rate (%)	Analytes and/or Measurements Obtained
First-trimester screen	10–13 [*]	82–87 ¹⁵	5	Nuchal translucency Papp-A hCG
Triple screen	15–22	69	5	hCG AFP uE3
Quad screen	15–22	81	5	hCG AFP uE3 DIA
Integrated	10–13 and 15–22	96	5	First-trimester screen, then quad screen
Sequential stepwise	10–13 and 15–22	95	5	First-trimester screen, then quad screen
Contingent screen	10–13 and 15–22	88–94	5	First-trimester screen, then quad screen
Cell-free DNA	Any age after 9 10 wk	99	0.5	Molecular evaluation of cell-free fetal DNA within maternal serum

Abbreviations: AFP, alpha-fetoprotein; DIA, dimeric inhibin-A; hCG, human chorionic gonadotropin; Papp-A, pregnancy-associated plasma protein A; uE3, unconjugated estriol.

^{*} Detection rate varies with gestational age, with improved detection at lower gestational ages.

Adapted from Practice bulletin no. 163: screening for fetal aneuploidy. *Obstet Gynecol* 2016; 127(5): e126.

Table 4

Estimated detection rate of cell-free DNA for aneuploidy and positive predictive value by maternal age

	Pooled Detection Rate²⁰ (%)	PPV at 25 y of Age^a (%)	PPV at 35 y of Age^a (%)	PPV at 45 y of Age^a (%)
Trisomy 21	99.2	51	79	98
Trisomy 18	96.3	15	39	90
Trisomy 13	91.7	7	21	Data insufficient to calculate
Monosomy X	90.3	41	41	41

Abbreviation: PPV, positive predictive value.

^aPredictive values calculated via the Perinatal Quality Foundation calculator. Available at perinatalquality.org; retrieved July 22, 2016.

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