The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

Society for Maternal-Fetal Medicine

COMMITTEE OPINION

Number 640 • September 2015 *(This Committee Opinion Replaces Committee Opinion Number 545)*

Committee on Genetics
Society for Maternal–Fetal Medicine

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Cell-free DNA Screening for Fetal Aneuploidy

ABSTRACT: Noninvasive prenatal screening that uses cell-free DNA from the plasma of pregnant women offers tremendous potential as a screening method for fetal aneuploidy. A number of laboratories have validated different techniques for the use of cell-free DNA as a screening test for fetal aneuploidy. All tests have a high sensitivity and specificity for trisomy 18 and trisomy 21, regardless of which molecular technique is used. Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cell-free DNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. Patients should be counseled that cell-free DNA screening does not replace the precision obtained with diagnostic tests, such as chorionic villus sampling or amniocentesis and, therefore, is limited in its ability to identify all chromosome abnormalities. Cell-free DNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects. Patients who are undergoing cell-free DNA screening should be offered maternal serum alpha-fetoprotein screening or ultrasound evaluation for risk assessment. The cell-free DNA screening test should not be considered in isolation from other clinical findings and test results. Management decisions, including termination of the pregnancy, should not be based on the results of the cell-free DNA screening alone. Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy. Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.

Recommendations

- A discussion of the risks, benefits, and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing, should occur with all patients.
- Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.
- Although any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risk status, the patient choosing this testing should understand the limitations and benefits of this screening paradigm in the context of alternative screening and diagnostic options.
- The cell-free DNA test will screen for only the common trisomies and, if requested, sex chromosome composition.
- Given the potential for inaccurate results and to understand the type of trisomy for recurrence-risk counseling, a diagnostic test should be recommended for a patient who has a positive cell-free DNA test result.
- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed.
- Management decisions, including termination of the pregnancy, should not be based on the results of the cell-free DNA screening alone.

- Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cell-free DNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.
- Routine cell-free DNA screening for microdeletion syndromes should not be performed.
- Cell-free DNA screening is not recommended for women with multiple gestations.
- If a fetal structural anomaly is identified on ultrasound examination, diagnostic testing should be offered rather than cell-free DNA screening.
- Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy.
- Cell-free DNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects; patients who are undergoing cell-free DNA screening should be offered maternal serum alpha-fetoprotein screening or ultrasound evaluation for risk assessment.
- Patients may decline all screening or diagnostic testing for aneuploidy.

Introduction

Noninvasive prenatal screening that uses cell-free DNA from the plasma of pregnant women offers tremendous potential as a screening method for fetal aneuploidy. In 2011, cell-free DNA analysis became clinically available and the American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine recommended it as a screening option for women at increased risk of fetal aneuploidy. This population was defined as women 35 years or older, fetuses with ultrasonographic findings indicative of an increased risk of aneuploidy, women with a history of trisomy-affected offspring, a parent carrying a balanced robertsonian translocation with an increased risk of trisomy 13 or trisomy 21, and women with positive first-trimester or second-trimester screening test results. Given the increasing data available on its use as a screening test in the general obstetric population, this document was updated to review the advantages and limitations of the application of cell-free DNA screening in all pregnant women.

Circulating cell-free DNA of fetal origin comprises approximately 3–13% of the total cell-free maternal DNA after 10 weeks of gestation and is thought to be derived primarily from the placenta. The cell-free DNA test will screen for only the common trisomies and, if requested, sex chromosome composition. Testing can be performed starting as early as 9 weeks and until delivery. A number of laboratories have validated different techniques for the use of cell-free DNA as a screening test for fetal aneuploidy; all of the data rely on next-generation sequencing technologies and advanced bioinformatic analyses (1–7). All tests have a high sensitivity and specificity for trisomy 18 and trisomy 21, regardless of which molecular technique is used (Table 1). Sensitivities for trisomy 13 and sex chromosome abnormalities are somewhat lower, averaging 80–90%, but the specificity remains greater than 99% for each condition. Accuracy of sex determination generally exceeds 98% (1, 4–18). Regardless of which technology is used, results typically are available within 7–10 days of maternal sampling. The specificity for each screened condition usually is reported separately, so false-positive rates are cumulative and may approach 1%.

Laboratories report cell-free DNA test results in various ways. Some laboratories report aneuploidy risk as either "positive" or "negative," whereas others report the chance of aneuploidy. The laboratories that report the chance of aneuploidy most commonly use ">99%" as indicative of high risk and "<1/10,000" as indicative of low risk, although more intermediate results occasionally are reported. Neither of these reporting methods is as useful to obstetric providers and patients as a positive predictive value (the chance that the positive test result is a true positive) (see Table 1) or a residual risk (the chance that a negative test result is false). Given the importance of these data in providing accurate and understandable information to patients regarding screening test results, the American College of Obstetricians and Gynecologists and the Society for Maternal–Fetal Medicine encourage all laboratories to report results with positive predictive values and residual risk values for each aneuploidy tested.

The fetal fraction, the amount of the cell-free DNA in the maternal blood that is of fetal origin, is essential for accurate test results. Some laboratories require a fetal fraction of at least 4% for a reportable result. Other laboratories, however, do not measure or report the fetal fraction. The fetal fraction typically increases with advancing gestational age. Overall, the chance of screen failure ranges from approximately 1% to 8% and varies depending on the laboratory and the methodology used (1, 4, 5, 14). Results may not be obtained because of low fetal fraction in patients carrying aneuploid fetuses or those who are obese. For patients weighing more than 250 pounds, 10% or more may have a fetal fraction of less than 4% (19). Rates of aneuploidy as high as 23% (due to low fetal fraction or other unknown factors) have been reported for women who fail to receive an interpretable result from cell-free DNA testing. Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cell-free DNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. Although repeat screening can be performed, it may delay the diagnosis of aneuploidy, potentially limiting reproductive options, and only 50–60% of repeat screens will provide a result (14, 20).

Table 1. Cell-free DNA Test Performance Characteristics in Patients Who Receive an Interpretable Result*

			Age 25 years	Age 40 years
	Sensitivity (%)	Specificity (%)	PPV (%)	PPV (%)
Trisomy 21	99.3	99.8	33	87
Trisomy 18	97.4	99.8	13	68
Trisomy 13	91.6	99.9	9	57
Sex chromosome aneuploidy	91.0	99.6	--†	--

Abbreviation: PPV, positive predictive value.

*This table is modeled on 25- and 40-year-old patients based on aneuploidy prevalence at 16 weeks of gestation. Negative predictive values are not included in the table but are greater than 99% for all patient populations who receive a test result. Negative predictive values decrease when patients who do not receive a result are included. Test performance characteristics are derived from a summary of published reports and as assessed and compiled in published reviews.

†The positive and negative predictive values for the sex chromosome aneuploidies depend on the particular condition identified. In general, however, the PPV ranges from 20% to 40% for most of these conditions.

Applicability to clinical practice:

Positive predictive value (defined as true positives divided by true positives plus false positives) is directly related to the prevalence of the condition in the population screened. Based on the sensitivity and specificity of the test, when a population with an overall prevalence of 1/1,000 for trisomy 21 is screened, the positive predictive value of an abnormal result is 33%—only one in three women who get an abnormal result will have an affected fetus. If the prevalence is 1/75, the positive predictive value is 87%.

Data from Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2015;45:249–66; Porreco RP, Garite TJ, Maurel K, Marusiak B, Ehrich M, van den Boom D, et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. Obstetrix Collaborative Research Network. Am J Obstet Gynecol 2014;211:365.e1–365.12; Snijders RJ, Sebire NJ, Nicolaides KH. Maternal age and gestational age-specific risk for chromosomal defects. Fetal Diagn Ther 1995;10:356–67; Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound Obstet Gynecol 2013;42:15–33; *and* Verweij EJ, de Boer MA, Oepkes D. Non-invasive prenatal testing for trisomy 13: more harm than good? Ultrasound Obstet Gynecol 2014;44:112–4.

Use in the General Obstetric Population

Data on the performance of cell-free DNA testing in the general obstetric population have become available (1, 8, 11, 16, 17). The sensitivity and specificity in the general obstetric population are similar to the levels previously published for the aforementioned high-risk population. The positive predictive value, however, is lower in this population, given the lower prevalence of aneuploidy in the general obstetric population. That is, fewer women with a positive test result will actually have an affected fetus, and there will be more false-positive test results (Fig. 1).

Another limitation of cell-free DNA screening in the general obstetric population is that trisomies 13, 18, and 21 comprise a smaller proportion of the chromosome abnormalities found in the general obstetric population (21–23). Traditional serum analyte screening methods allow for higher detection rates of these other chromosome abnormalities as well as the risk of other adverse pregnancy outcomes. For example, a positive integrated screening test result may indirectly identify a fetus with an unbalanced rearrangement of a chromosome other than trisomies 13, 18, or 21. One study of women with abnormal traditional screening test results who had diagnostic testing estimated that up to 17% of clinically significant chromosomal abnormalities would not be detectable with most of the current cell-free DNA techniques (23). Given the performance of conventional screening methods and the limitations of cell-free DNA, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.

Testing for Other Genetic Conditions With Cell-free DNA

Although all laboratories that currently offer cell-free DNA screening for aneuploidy include trisomies 13, 18, and 21 as part of their standard panel, the approach to the sex chromosomes and other chromosome abnormalities vary. Some laboratories offer routine sex chromosome, microdeletion, and rare trisomy (eg, trisomy 16 or trisomy 22) assessment, whereas others require that sex chromosome and other assessments be requested in order for those results to be reported. Microdeletion syndromes occur sporadically or are due to other genetic

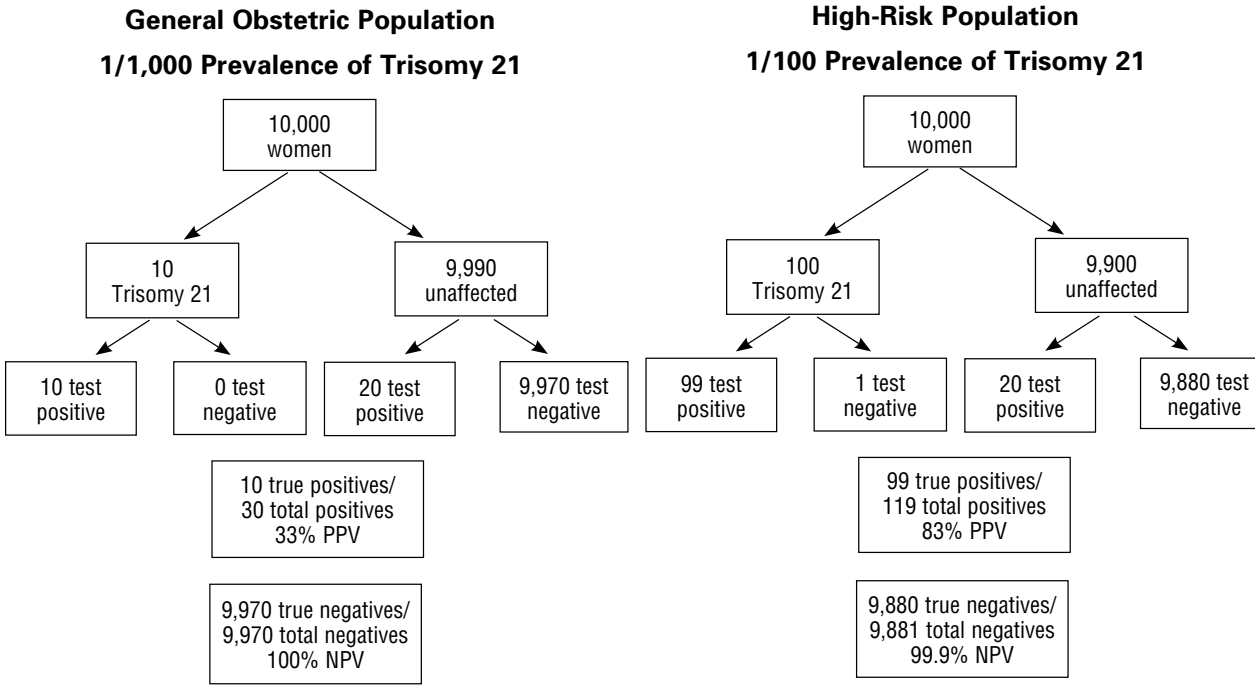

Fig.1. The importance of population prevalence on the predictive value for a screening test: an illustration with cell-free DNA. ⇦

Abbreviations: NPV, negative predictive value; PPV, positive predictive value

mechanisms (24, 25). Screening for these microdeletions has not been validated in clinical studies, and the true sensitivity and specificity of this screening test is uncertain. Routine cell-free DNA screening for microdeletion syndromes should not be performed.

Multiple Gestations

Regardless of the method, the accuracy of screening for aneuploidy is limited in multiple gestations. With any method based on maternal blood (serum analytes or DNA), only a single composite result for the entire gestation is provided, with no ability to distinguish a differential risk between fetuses. The data regarding the performance of cell-free DNA screening in twin gestations are limited (26, 27). Although preliminary findings suggest that this screening is accurate, larger prospective studies and published data are needed before this method can be recommended for multiple gestations. Cell-free DNA screening is not recommended for women with multiple gestations. There are no available data on higher-order multiples.

Counseling Patients About Their Options

Counseling regarding the limitations of cell-free DNA screening should include a discussion about how the screening method provides information regarding only trisomies 13, 18, and 21. If a sex chromosome analysis has been requested or is part of the standard panel, then this information should be conveyed as well. Some patients may request cell-free DNA screening to enable earlier sex identification. Patients should be counseled that cell-free DNA screening also assesses the risk of the aforementioned trisomies; if that information is not desired, the screening should not be performed.

Patients should be counseled that cell-free DNA screening does not replace the precision obtained with diagnostic tests, such as chorionic villus sampling or amniocentesis and, therefore, is limited in its ability to identify all chromosome abnormalities. Not only can there be false-positive test results, but a positive cell-free DNA test result for aneuploidy does not determine if the trisomy is due to a translocation, which affects the risk of recurrence. If a fetal structural anomaly is identified on ultrasound examination, diagnostic testing should be offered rather than cell-free DNA screening.

The cell-free DNA screening test should not be considered in isolation from other clinical findings and test results. Given the potential for inaccurate results and to understand the type of trisomy for recurrence-risk counseling, a diagnostic test should be recommended for a patient who has a positive cell-free DNA test result. Management decisions, including termination of the pregnancy, should not be based on the results of the

cell-free DNA screening alone. False-positive test results do occur and diagnostic testing with amniocentesis or chorionic villus sampling should be recommended before any pregnancy termination decision. Causes of false-positive test results have been reported, which include but are not limited to placental mosaicism, vanishing twins, and maternal malignancies (28–31).

Before offering cell-free DNA screening, counseling is recommended. The family history should be reviewed to determine if the patient should be offered other forms of screening or prenatal diagnosis for a particular disorder. In order to ensure accuracy and testing of the appropriate patient population, a baseline ultrasound examination also should be considered to confirm viability, the number of fetuses, and gestational dating, if not performed previously. Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy. A negative test result still carries a residual risk of one of the common trisomies and does not ensure that the fetus does not have another chromosome abnormality or genetic diagnosis. Cell-free DNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects. Patients who are undergoing cell-free DNA screening should be offered maternal serum alpha-fetoprotein screening or ultrasound evaluation for risk assessment. Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed. However, use of cell-free DNA screening as a follow-up test for patients with a positive traditional screening test result is reasonable for patients who want to avoid a diagnostic test. Given that the residual risk of a chromosomal abnormality with a normal cell-free DNA screening test result after an abnormal traditional screening test has been reported to be 2%, patients should be informed of this potential limitation (23).

Conclusion

A discussion of the risks, benefits, and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing, should occur with all patients. Such a discussion should include the advisability and applicability of cell-free DNA and other screening tests and the interpretation of test results, based on patient risk stratification. Although any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risk status, the patient choosing this testing should understand the limitations and benefits of this screening paradigm in the context of alternative screening and diagnostic options. Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population. The technology involved in cell-free DNA analysis and its application to prenatal screening for aneuploidy is a rapidly changing field. Therefore, any recommendations regarding its use in screening also will likely evolve quickly. It will be critical to remain abreast of this rapidly changing technology to provide patients with the most effective, accurate, and cost-conscious methods for aneuploidy screening.

For More Information

These resources are for information only and are not meant to be comprehensive. Referral to these resources does not imply the American College of Obstetricians and Gynecologists' endorsement of the organization, the organization's web site, or the content of the resource. The resources may change without notice.

ACOG has identified additional resources on topics related to this document that may be helpful for ob-gyns, other health care providers, and patients. You may view these resources at: www.acog.org/More-Info/cfDNA.

References

1. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group [published erratum appears in Obstet Gynecol 2012;120:957]. Obstet Gynecol 2012;119:890–901. [PubMed] [*Obstetrics & Gynecology*] ⇦
2. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7. [PubMed] [Full Text] ⇦
3. Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. Am J Hum Genet 1999;64:218–24. [PubMed] [Full Text] ⇦
4. Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;207:137.e1–137.e8. [PubMed] [Full Text] ⇦
5. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20. [PubMed] ⇦
6. Sparks AB, Wang ET, Struble CA, Barrett W, Stokowski R, McBride C, et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn 2012;32:3–9. [PubMed] [Full Text] ⇦
7. Zimmermann B, Hill M, Gemelos G, Demko Z, Banjevic M, Baner J, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. Prenat Diagn 2012;32:1233–41. [PubMed] [Full Text] ⇦
8. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, et al. DNA sequencing versus standard prenatal aneuploidy screening. CARE Study Group. N Engl J Med 2014;370:799–808. [PubMed] [Full Text] ⇦

9. Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2015;45:249–66. [PubMed] [Full Text] ⇦

10. Porreco RP, Garite TJ, Maurel K, Marusiak B, Ehrich M, van den Boom D, et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. Obstetrix Collaborative Research Network. Am J Obstet Gynecol 2014;211:365.e1–365.12. [PubMed] [Full Text] ⇦

11. Dar P, Curnow KJ, Gross SJ, Hall MP, Stosic M, Demko Z, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. Am J Obstet Gynecol 2014;211:527.e1–527.e17. [PubMed] [Full Text] ⇦

12. Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1–374.e6. [PubMed] [Full Text] ⇦

13. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med 2012;14:296–305.[PubMed] [Full Text] ⇦

14. Pergament E, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. Obstet Gynecol 2014;124:210–8. [PubMed] [*Obstetrics & Gynecology*] ⇦

15. Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;206:319.e1–319.e9. [PubMed] [Full Text] ⇦

16. Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, et al. Noninvasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies. Ultrasound Obstet Gynecol 2015;45:530–8. [PubMed] [Full Text] ⇦

17. Norton ME, Jacobsson B, Swamy GK, Laurent LC, Ranzini AC, Brar H, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med 2015;372:1589–97. [PubMed] [Full Text] ⇦

18. Bianchi DW, Parsa S, Bhatt S, Halks-Miller M, Kurtzman K, Sehnert AJ, et al. Fetal sex chromosome testing by maternal plasma DNA sequencing: clinical laboratory experience and biology. Obstet Gynecol 2015;125:375–82. [PubMed] [*Obstetrics & Gynecology*] ⇦

19. Ashoor G, Syngelaki A, Poon LC, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound Obstet Gynecol 2013;41:26–32. [PubMed] [Full Text] ⇦

20. Willems PJ, Dierickx H, Vandenakker E, Bekedam D, Segers N, Deboulle K, et al. The first 3,000 Non-Invasive Prenatal Tests (NIPT) with the Harmony test in Belgium and the Netherlands. Facts Views Vis Obgyn 2014;6:7–12. [PubMed] [Full Text] ⇦

21. Alamillo CM, Krantz D, Evans M, Fiddler M, Pergament E. Nearly a third of abnormalities found after first-trimester screening are different than expected: 10-year experience from a single center. Prenat Diagn 2013;33:251–6. [PubMed] ⇦

22. Gregg AR, Gross SJ, Best RG, Monaghan KG, Bajaj K, Skotko BG, et al. ACMG statement on noninvasive prenatal screening for fetal aneuploidy. Genet Med 2013;15:395–8. [PubMed] [Full Text] ⇦

23. Norton ME, Jelliffe-Pawlowski LL, Currier RJ. Chromosome abnormalities detected by current prenatal screening and noninvasive prenatal testing. Obstet Gynecol 2014;124:979–86. [PubMed] [*Obstetrics & Gynecology*] ⇦

24. Srinivasan A, Bianchi DW, Huang H, Sehnert AJ, Rava RP. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. Am J Hum Genet 2013;92:167–76. [PubMed] [Full Text] ⇦

25. Wapner RJ, Babiarz JE, Levy B, Stosic M, Zimmermann B, Sigurjonsson S, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. Am J Obstet Gynecol 2015;212:332.e1–332.e9. [PubMed] [Full Text] ⇦

26. Canick JA, Kloza EM, Lambert-Messerlian GM, Haddow JE, Ehrich M, van den Boom D, et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenat Diagn 2012;32:730–4. [PubMed] [Full Text] ⇦

27. Huang X, Zheng J, Chen M, Zhao Y, Zhang C, Liu L, et al. Noninvasive prenatal testing of trisomies 21 and 18 by massively parallel sequencing of maternal plasma DNA in twin pregnancies. Prenat Diagn 2014;34:335–40. [PubMed] [Full Text] ⇦

28. Curnow KJ, Wilkins-Haug L, Ryan A, Kirkizlar E, Stosic M, Hall MP, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. Am J Obstet Gynecol 2015;212:79.e1–79.e9. [PubMed] [Full Text] ⇦

29. Grati FR, Malvestiti F, Ferreira JC, Bajaj K, Gaetani E, Agrati C, et al. Fetoplacental mosaicism: potential implications for false-positive and false-negative noninvasive prenatal screening results. Genet Med 2014;16:620–4. [PubMed] [Full Text] ⇦

30. Hall AL, Drendel HM, Verbrugge JL, Reese AM, Schumacher KL, Griffith CB, et al. Positive cell-free fetal DNA testing for trisomy 13 reveals confined placental mosaicism. Genet Med 2013;15:729–32. [PubMed] [Full Text] ⇦

31. Osborne CM, Hardisty E, Devers P, Kaiser-Rogers K, Hayden MA, Goodnight W, et al. Discordant noninvasive prenatal testing results in a patient subsequently diagnosed with metastatic disease. Prenat Diagn 2013;33:609–11. [PubMed] [Full Text] ⇦

ISSN 1074-861X

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