Non-Invasive Prenatal Screening

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Introduction

Non-invasive prenatal screening (NIPS) is addressed by this guideline.

Procedures addressed

The inclusion of any procedure code in this table does not imply that the code is under management or requires prior authorization. Refer to the specific Health Plan's procedure code list for management requirements.

Procedures addressed by this guideline	Procedure codes
Non-Invasive Prenatal Screening for Fetal Aneuploidy	81420
Non-Invasive Prenatal Screening for Fetal Aneuploidy with Risk Score	81507
Non-Invasive Prenatal Screening for Fetal Chromosomal Microdeletions	81422
Non-Invasive Prenatal Screening for Single-Gene Mutations	81105-81479
UNITY Fetal Risk Screen	0489U
Vasistera	0327U

Criteria

Introduction

Requests for non-invasive prenatal screening (NIPS) are reviewed using the following criteria.

Cell-free DNA-based prenatal screening for fetal aneuploidy

- Genetic Counseling:

NIPT

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  - Pre and post-test genetic counseling by an appropriate provider (as deemed by the Health Plan policy), AND
- Prenatal Screening:
  - Prenatal cell-free DNA screening for fetal aneuploidy (e.g. trisomy 13, 18, and 21) is medically necessary when all of the following criteria are met:
    - Singleton or twin pregnancy, AND
    - Gestational age within the window validated by the selected testing laboratory, AND
    - Rendering laboratory is a qualified provider of service per the Health Plan policy.
- Prenatal cell-free DNA screening is not medically necessary in the following circumstances:
  - A pregnancy in which a fetal demise has occurred
  - Triplet and higher-order multi-fetal gestation pregnancies
  - More than one prenatal cell-free DNA screen performed per pregnancy (exceptions for repeat screening will be considered on a case-by-case basis when requested due to initial results being unobtainable as a result of low fetal fraction)
  - When karyotyping, aneuploidy FISH, and/or cytogenomic microarray analysis (CMA) have already been performed on the pregnancy
- It is not medically necessary to perform maternal serum screening for aneuploidy and non-invasive prenatal screening (prenatal cell-free DNA screening) concurrently.
- Prenatal diagnosis by amniocentesis or CVS following NIPS is medically necessary when NIPS results are screen positive, inconclusive, or uninterpretable, or when additional information becomes available throughout the pregnancy that suggests additional risk factors.
- If non-invasive prenatal screening (prenatal cell-free DNA screening) has been successfully performed in the current pregnancy, other aneuploidy screening (by first or second trimester screening or integrated, step-wise sequential, or contingent sequential screening) is not medically necessary. Maternal serum screening for neural tube defects (AFP-only) is medically necessary.

Prenatal cell-free DNA screening for chromosome microdeletions

This test is considered Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.

- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

Prenatal cell-free DNA screening for single-gene mutations

For information regarding fetal Rh(D) genotyping using NIPS, please see the guideline *Human Platelet and Red Blood Cell Antigen Genotyping*, as that testing is not addressed here.

For all other indications, this test is Experimental, Investigational, or Unproven.

- Experimental, Investigational, or Unproven (E/I/U) refers to tests, or uses of tests, that have insufficient data to demonstrate an overall health benefit. This typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity) and significantly improves patient health outcomes (clinical utility). Such tests are also not generally accepted as the standard of care in the evaluation or management of a particular condition.
- In the case of laboratory testing, FDA approval or clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight. In addition, FDA approval or clearance often does not include an assessment of clinical utility.

Billing and Reimbursement

Introduction

This section outlines the billing requirements for tests addressed in this guideline. These requirements will be enforced during the case review process whenever appropriate. Examples of requirements may include specific coding scenarios, limits on allowable test combinations or frequency and/or information that must be provided on a claim for automated processing. Any claims submitted without the necessary information to allow for automated processing (e.g. ICD code, place of service, etc.) will not be reimbursable as billed. Any claim may require submission of medical records for post service review.

- Prenatal cell-free DNA screening is not reimbursable for pregnancies in which a fetal demise has occurred, or for triplet and other higher-order multiple gestations. These are defined by the presence of one of the diagnosis codes from Table: *ICD Codes Indicating Fetal Demise and Triplet or Higher-Order Multiple Gestations*.
- Screening for aneuploidy of the X and Y chromosomes and/or detection of less common trisomies, are not separately reimbursable under these coverage guidelines. Additional procedure codes billed with cell-free DNA screening for this purpose are not eligible for reimbursement.

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- Prenatal cell-free DNA screening for chromosome microdeletions (CPT: 81422) is not reimbursable.
- When prenatal cell-free DNA screening is otherwise reimbursable, the following limitations apply:
  - No more than one prenatal cell-free DNA screening is reimbursable per pregnancy, defined as no more than one paid prenatal cell-free DNA screen procedure code (e.g., 81420 or 81507) within 10 weeks.
  - Non-specific procedure codes (e.g. 81479, 81599) or any procedure codes that do not accurately describe the test methodology performed (e.g. 88271) are not eligible for reimbursement.

ICD Codes

ICD codes used for automated claims processing for this guideline.

Table: ICD Codes Indicating Fetal Demise and Triplet or Higher-Order Multiple Gestations

Codes and descriptions

Code or Range	Description
O30.1X	Triplet pregnancy
O30.2X	Quadruplet pregnancy
O31.00X0-O31.00X9	Papyraceous fetus, unspecified trimester
O31.01X0-O31.01X9	Papyraceous fetus, first trimester
O31.02X0-O31.02X9	Papyraceous fetus, second trimester
O31.03X0-O31.03X9	Papyraceous fetus, third trimester
O31.10X0-O31.10X9	Continuing pregnancy after spontaneous abortion of one fetus or more, unspecified trimester
O31.11X0-O31.11X9	Continuing pregnancy after spontaneous abortion of one fetus or more, first trimester
O31.12X0-O31.12X9	Continuing pregnancy after spontaneous abortion of one fetus or more, second trimester

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Code or Range	Description
O31.13X0-O31.13X9	Continuing pregnancy after spontaneous abortion of one fetus or more, third trimester
O31.20X0-O31.20X9	Continuing pregnancy after intrauterine death of one fetus or more, unspecified trimester
O31.21X0-O31.21X9	Continuing pregnancy after intrauterine death of one fetus or more, first trimester
O31.22X0-O31.22X9	Continuing pregnancy after intrauterine death of one fetus or more, second trimester
O31.23X0-O31.23X9	Continuing pregnancy after intrauterine death of one fetus or more, third trimester
O31.30X0-O31.30X9	Continuing pregnancy after elective fetal reduction of one fetus or more, unspecified trimester
O31.31X0-O31.31X9	Continuing pregnancy after elective fetal reduction of one fetus or more, first trimester
O31.32X0-O31.32X9	Continuing pregnancy after elective fetal reduction of one fetus or more, second trimester
O31.33X0-O31.33X9	Continuing pregnancy after elective fetal reduction of one fetus or more, third trimester
O31.8X13	Other complications specific to multiple gestation, first trimester, fetus 3
O31.8X14	Other complications specific to multiple gestation, first trimester, fetus 4
O31.8X15	Other complications specific to multiple gestation, first trimester, fetus 5
O31.8X23	Other complications specific to multiple gestation, second trimester, fetus 3

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Code or Range	Description
O31.8X24	Other complications specific to multiple gestation, second trimester, fetus 4
O31.8X25	Other complications specific to multiple gestation, second trimester, fetus 5
O31.8X33	Other complications specific to multiple gestation, third trimester, fetus 3
O31.8X34	Other complications specific to multiple gestation, third trimester, fetus 4
O31.8X35	Other complications specific to multiple gestation, third trimester, fetus 5
O31.8X93	Other complications specific to multiple gestation, unspecified trimester, fetus 3
O31.8X94	Other complications specific to multiple gestation, unspecified trimester, fetus 4
O31.8X95	Other complications specific to multiple gestation, unspecified trimester, fetus 5

What is a chromosome abnormality?

A chromosome abnormality is any difference in the structure, arrangement, or amount of genetic material packaged into the chromosomes.[1]

Humans typically have 23 pairs of chromosomes. Each chromosome has a characteristic appearance that should be the same in each person. Chromosome abnormalities can lead to a variety of developmental and reproductive disorders. Common chromosome abnormalities include Down syndrome (trisomy 21), trisomy 18, trisomy 13, Turner syndrome, and Klinefelter syndrome. Chromosome abnormalities occur in approximately 1 in 150 live births. A higher percentage of pregnancies are affected but lost during pregnancy.

About 6%-11% of stillbirths or neonatal deaths are associated with a chromosome abnormality.[2,3]

The risk of having a child with an extra chromosome, notably Down syndrome, increases as a woman gets older.[3] However, many babies with Down syndrome are born to women under 35 and the risk of having a child with other types of chromosome abnormalities, such as Turner syndrome or 22q11 deletion syndrome, is not related to maternal age. Therefore, prenatal screening for Down syndrome and certain other

chromosome abnormalities is now routinely offered to all pregnant women. As a result, prenatal diagnosis via amniocentesis or chorionic villus sampling (CVS) is now also an option for most pregnant women.

Test information

Introduction

Non-invasive prenatal screening (NIPS, also called prenatal cell-free DNA screening or cfDNA screening) is performed on a maternal plasma sample generally collected after 9 weeks' gestation.[4]

Methodology and Performance

Testing methodology relies on the presence of cell-free placental DNA in maternal circulation.[4] Approximately 10% of cell-free DNA in maternal circulation is of placental origin.[5]

Analysis of cell-free placental DNA is performed to identify pregnancies at increased risk for chromosomal aneuploidy. Detection rates for trisomies 21, 18, and 13 are greater than 98%, with false positive rates of less than 0.5%.[4]

Some laboratories also test for sex chromosome aneuploidies (such as Turner syndrome or Klinefelter syndrome) and rare chromosome microdeletion syndromes (such as 22q11 deletion syndrome or 1p36 microdeletion syndrome), with variable performance.

Each commercial or academic laboratory offering NIPS has a proprietary platform and bioinformatics pipeline.

Chromosome analysis via CVS and amniocentesis is also routinely available for diagnosis of fetal chromosome abnormalities in pregnancy.

Guidelines and evidence

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG, 2023) published a practice guideline regarding Non Invasive Prenatal Screening (NIPS) and recommended the following:[5]

- "ACMG recommends NIPS over traditional screening methods for all pregnant patients with singleton gestation for fetal trisomies 21, 18, and 13 (Strong recommendation based on high certainty of evidence)"

- "ACMG recommends NIPS over traditional methods for trisomy screening in twin gestations (Strong recommendation, based on high certainty of evidence)"
- Regarding sex chromosome aneuploidies (SCAs): "ACMG recommends that NIPS be offered to patients with a singleton gestation to screen for fetal SCA (Strong recommendation, based on high certainty of evidence)"
- "ACMG suggests that NIPS for 22q11.2 deletion syndrome be offered to all patients (Conditional recommendation, based on moderate certainty of evidence)"
- Regarding other copy number variants (CNVs): "At this time, there is insufficient evidence to recommend routine screening for CNVs other than 22q11.2 deletions (No recommendation, owing to lack of clinically relevant evidence and validation)"
- Regarding rare autosomal trisomies (RATs): "At this time, there is insufficient evidence to recommend or not recommend NIPS for the identification of RATs (No recommendation, owing to lack of clinically relevant evidence)"

The American College of Obstetricians and Gynecologists

The American College of Obstetricians and Gynecologists (ACOG, 2019; reaffirmed September 2023) issued a practice advisory on the use of cell-free DNA to screen for single-gene disorders and stated the following:[6]

- "The continued innovation in cell-free technology combined with the desire for a maternal blood test to predict the risk for fetal genetic disorders during a pregnancy has broadened the application of cell-free DNA screening beyond aneuploidy to single-gene disorders. Examples of single-gene disorders include various skeletal dysplasias, sickle cell disease and cystic fibrosis. Although this technology is available clinically and marketed as a single-gene disorder prenatal screening option for obstetric care providers to consider in their practice, often in presence of advanced paternal age, there has not been sufficient data to provide information regarding accuracy and positive and negative predictive value in the general population. For this reason, single-gene cell-free DNA screening is not currently recommended in pregnancy."

The American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine

In 2020, The American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal Fetal Medicine (SMFM) published a joint practice bulletin and stated the following:[7]

- "Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to all pregnant women regardless of maternal age or risk of chromosome abnormality." [Level A Recommendation: based on good and consistent scientific evidence]

- "If screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously." [Level A Recommendation: based on good and consistent scientific evidence]
- "Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing." [Level A Recommendation: based on good and consistent scientific evidence]
- "Cell-free DNA screening can be performed in twin pregnancies. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13." [Level B Recommendation: based on limited or inconsistent scientific evidence]

American Society of Human Genetics and European Society of Human Genetics

A 2015 joint statement by the American Society of Human Genetics (ASHG) and European Society of Human Genetics (ESHG) included the following recommendations:[8]

- "NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as cFTS. However, a positive NIPT result should not be regarded as a final diagnosis… Thus women should be advised to have a positive result confirmed through diagnostic testing, preferably by amniocentesis, if they are considering a possible termination of pregnancy."
- "Expanding NIPT-based prenatal screening to also report on sex chromosomal abnormalities and microdeletions not only raises ethical concerns related to information and counseling challenges but also risks reversing the important reduction in invasive testing achieved with implementation of NIPT for aneuploidy, and is therefore currently not recommended."

The International Society for Prenatal Diagnosis

The International Society for Prenatal Diagnosis (ISPD) first issued a position statement on NIPT in January 2011 and then updated its recommendations in April 2013 and again in April 2015. ISPD summarized:[9]

- "The following protocol options are currently considered appropriate:"
  - "cfDNA screening as a primary test offered to all pregnant women."
  - "cfDNA secondary to a high risk assessment based on serum and ultrasound screening protocols."

- "When cfDNA screening is extended to microdeletion and microduplication syndromes or rare trisomies the testing should be limited to clinically significant disorders or well-defined severe conditions."

The ISPD issued a position statement (2020) on cfDNA screening for Down syndrome in twin and triplet pregnancies. The statement compared cfDNA screening to other screening methods available for multiple gestation pregnancies, focusing on test characteristics. This approach is in contrast to other professional guidelines that compare the performance of cfDNA in twin pregnancies to that reported for cfDNA screening in singleton pregnancies. ISPD summarized recommendations for evidence-based practices:[10]

- "The use of first trimester cfDNA screening for the common autosomal trisomies is appropriate for twin pregnancies due to sufficient evidence showing high detection and low false positive rates with high predictive values. Moderate."
- "The finding of an increased risk on a cfDNA screening test in multiple pregnancies should be followed by counseling and an offer of diagnostic testing to confirm results. Strong."

The National Society of Genetic Counselors

The National Society of Genetic Counselors (NSGC, 2021) issued a position statement regarding the use of prenatal cell-free DNA screening:[11]

- "The National Society of Genetic Counselors believes that all pregnant patients, regardless of aneuploidy risk, should have access to prenatal aneuploidy screening using cell-free DNA (cfDNA)."
- "Patients who receive increased risk or inconclusive/atypical results should receive post-test genetic counseling with a knowledgeable healthcare provider, such as a genetic counselor. In such cases, confirmatory diagnostic testing may be indicated, and patients should be counseled that no irreversible actions should be taken based on the cfDNA screening alone."

Society of Obstetricians and Gynaecologists of Canada

The Society of Obstetricians and Gynaecologists of Canada (SOGC, 2017) stated: “Routine cfDNA screening for fetal microdeletions is not currently recommended (II-2B).”[12,13]

Selected Relevant Publications

Selected relevant publications pertaining to twin pregnancies, microdeletion testing, and single gene testing.

Multiple Gestation Pregnancies

The evidence base for NIPS in twin pregnancies suggested that NIPS may be useful as a screening test for common aneuploidies. However, well-designed clinical validity and clinical utility studies evaluating the performance of NIPS in triplet and higher order multifetal pregnancies in the general obstetric population are needed. A systematic evidence-based review published by the American College of Medical Genetics and Genomics (ACMG, 2022) included 7 studies in a meta-analysis evaluating the performance of cfDNA screening for aneuploidy in multi-fetal gestations.[14] The authors stated: "The results from our meta-analyses show NIPS performance in this population [twin gestations] are generally comparable to performance in singleton pregnancies for T21, T18, and T13. Results for other aneuploidies or microdeletions were less frequently reported and no firm conclusions can be drawn about the performance of NIPS for these outcomes. Very limited data is available on triplets or higher order multiple gestations."

Microdeletion Syndromes

The evidence base for the use of NIPS for microdeletion detection was of low quality.[14-37] Several systematic reviews have concluded that the evidence is insufficient to draw firm conclusions related to the use of NIPS for microdeletion testing. Diagnostic performance estimates were highly variable, there was insufficient utilization of reference testing in the available studies, and significant heterogeneity existed between studies due to differing NIPS methodologies, the number and type of deletions screened, and patient populations. Well-designed clinical validity studies with comprehensive reference test use in low-risk patient populations are needed along with clinical utility studies that evaluate pregnancy and postnatal outcomes. These studies should also allow for comparison of methodologies and the number and type of microdeletion/microduplication syndromes.

Single Gene Disorders

The strength of the available evidence for NIPS use in detecting single-gene disorders was low.[30,38-59] There are few clinical studies evaluating the performance of NIPS in detecting these disorders. The majority of available clinical studies have focused on targeted testing for common or familial variants. Sample failures and interpretation issues are common and related to a number of factors, including: low fetal fraction, difficulty distinguishing fetal from maternal variants, insufficient SNP numbers in the target region, and DNA recombination events. The evidence base is insufficient to permit definitive conclusions regarding the performance of NIPS to identify single-gene disorders. Large well-designed clinical validity and clinical

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utility studies evaluating NIPS for this indication in general obstetric and/or high-risk populations are needed.

Sex Chromosome Aneuploidies

The evidence base for NIPS use in SCA detection was of low quality.[34,60,61] Clinical performance data suggests that NIPS has high sensitivity and specificity for SCAs, but reported positive predictive values (PPVs) vary significantly based on subtype, test platform, and patient population. Accurate screening for Turner syndrome is difficult due to confined placental mosaicism and maternal somatic mosaicism, often leading to a low PPV and increased false positive results. Clinical utility data is limited and does not allow for assessment of the overall impact of SCA screening. Ethical concerns related to use of this screening include the challenges involved in provision of pre- and post-test parental counseling, the mild and highly variable phenotypic presentations of SCAs, increased rates of unnecessary invasive diagnostic testing, and a lack of consensus on therapeutic benefits for prenatally diagnosed patients.

Note:

This benefit/harm statement only applies to those jurisdictions that do not have Medicare guidance. Based upon the guidelines and evidence provided in the clinical policy, following EviCore's criteria for non-invasive prenatal screening will ensure that testing will be available to those members most likely to benefit from the information provided by the assay. For those not meeting criteria, it ensures alternate management strategies are considered. However, it is possible that some members who would benefit from the testing, but do not meet clinical criteria, will not receive an immediate approval for testing.

References

1. Gardner RJM, Sutherland GR. Chromosome Abnormalities and Genetic Counseling (Oxford Monographs on Medical Genetics, No 29). New York, NY: Oxford University Press; 2004.
2. Robinson A, Lindon MG. Clinical Genetics Handbook. 2nd ed. Cambridge, MA: Blackwell Scientific Publications; 1993.
3. ACOG Practice Bulletin No. 162, May 2016. Prenatal Diagnostic Testing for Genetic Disorders. *Obstet Gynecol*. 2016;127(5):e108-e122.
4. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 640: Cell-Free DNA Screening for Fetal Aneuploidy. *Obstet Gynecol*. 2015;126(3):e31-7.
5. Dungan JS, Klugman S, Darilek S, et al. Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population: An evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2023;25(2): 100336 doi: 10.1016/j.gim.2022.11.004 Available at: **https://www.nature.com/articles/gim201697 https://www.gimjournal.org/article/S1098-3600(22)01004-8/fulltext**
6. ACOG Practice Advisory. Cell-free DNA to screen for single-gene disorders. 2019 Feb. (Reaffirmed September 2023) Available at: **https://www.acog.org/clinical/clinical-guidance/practice-advisory/articles/2019/02/cell-free-dna-to-screen-for-single-gene-disorders**

7. ACOG and SMFM Practice Bulletin No. 226, October 2020. Screening for fetal chromosomal abnormalities. *Obstet Gynecol*. 2020;136(4):1-22.
8. Dondorp W, de Wert G, Bombard Y, et al. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. *Eur J Hum Genet*. 2015;23(11):1438-1450. doi: 10.1038/ejhg.2015.57
9. Benn P, Borell A, Chiu R, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. *Prenat Diagn*. 2015;35(8):725-34.
10. Palomaki G, Chiu R, Pertile M, et al. International Society for Prenatal Diagnosis Position Statement: cell free (cf)DNA screening for Down syndrome in multiple pregnancies. *Prenat Diagn*. October 2020; epub ahead of print. https://doi.org/10.1002/pd.58
11. National Society of Genetic Counselors Position Statements: Prenatal Cell-Free DNA Screening. Released 10-11-16, Revised April 2021. Available at: **https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/prenatal-cell-free-dna-screening-1**
12. Audibert F, De Bie I, Johnson JA, et al. No. 348-Joint SOGC-CCMG Guideline: Update on prenatal screening for fetal aneuploidy, fetal anomalies, and adverse pregnancy outcomes. *J Obstet Gynaecol Can*. 2017;39(9):805-817. doi: 10.1016/j.jogc.2017.01.032
13. Correction. *J Obstet Gynaecol Can*. 2018;40(8):1109. doi: 10.1016/j.jogc.2018.05.039
14. Rose NC, Barrie ES, Malinowski J, et al. ACMG Professional Practice and Guidelines Committee. Systematic evidence-based review: The application of noninvasive prenatal screening using cell-free DNA in general-risk pregnancies. *Genet Med*. 2022;24(7):1379-1391.
15. Noninvasive prenatal testing for trisomies 21, 18, and 13, sex chromosome aneuploidies, and microdeletions: a health technology assessment. *Ont Health Technol Assess Ser*. 2019;19(4):1-166. Available at: **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6395059/** .
16. Familiari A, Boito S, Rembouskos G, et al. Cell-free DNA analysis of maternal blood in prenatal screening for chromosomal microdeletions and microduplications: a systematic review. *Prenat Diagn*. 2021;41(10):1324-1331. doi: 10.1002/pd.5928
17. Wen L, Zhang Y, Gao J, et al. The predictive value of noninvasive prenatal screening for copy number variations: a cohort study and a systematic meta-analysis. *Expert Rev Mol Diagn*. 2023;23(8):713-722. doi: 10.1080/14737159.2023.2233415
18. Dar PE, Jacobsson B, Clifton R, et al. Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome. *Am J Obstet Gynecol*. 2022;227(1):79.e71-79.e11. doi: 10.1016/j.ajog.2022.01.002
19. Van Prooyen Schuurman L, Sistermans EA, Van Opstal D, et al. Clinical impact of additional findings detected by genome-wide non-invasive prenatal testing: Follow-up results of the TRIDENT-2 study. *Am J Hum Genet*. 2022;109(6):1140-1152. doi: 10.1016/j.ajhg.2022.04.018
20. Xue H, Yu A, Lin M, et al. Efficiency of expanded noninvasive prenatal testing in the detection of fetal subchromosomal microdeletion and microduplication in a cohort of 31,256 single pregnancies. *Sci Rep*. 2022;12(1). doi: 10.1038/s41598-022-24337-9
21. Chen Y, Lai Y, Xu F, et al. The application of expanded noninvasive prenatal screening for genome-wide chromosomal abnormalities and genetic counseling. *J Matern Fetal Neonatal Med*. 2021;34(16):2710-2716. doi: 10.1080/14767058.2021.1907333
22. Shi P, Wang Y, Liang H, et al. The potential of expanded noninvasive prenatal screening for detection of microdeletion and microduplication syndromes. *Prenat Diagn*. 2021;41(10):1332-1342. doi: 10.1002/pd.6002
23. Wang C, Tang J, Tong K, et al. Expanding the application of non-invasive prenatal testing in the detection of foetal chromosomal copy number variations. *BMC Med Genomics*. 2021;14(1):292. doi: 10.1186/s12920-021-01131-6
24. Faieta M, Falcone R, Duca S, et al. Test performance and clinical utility of expanded non-invasive prenatal test: Experience on 71,883 unselected routine cases from one single center. *Prenat Diagn*. 2024. doi: 10.1002/pd.6580
25. Gug M, Ratiu A, Andreescu N, et al. Approach and management of pregnancies with risk identified by non-invasive prenatal testing. *J Pers Med*. 2024;14(4):366. doi: 10.3390/jpm14040366
26. Hammer C, Pierson S, Acevedo A, et al. High positive predictive value 22q11.2 microdeletion screening by prenatal cell-free DNA testing that incorporates fetal fraction amplification. *Prenat Diagn*. 2024. doi: 10.1002/pd.6562

27. Li Y, Yang X, Zhang Y, et al. The detection efficacy of noninvasive prenatal genetic testing (NIPT) for sex chromosome abnormalities and copy number variation and its differentiation in pregnant women of different ages. *Heliyon*. 2024;10(2):e24155. doi: 10.1016/j.heliyon.2024.e24155
28. Pedrola Vidal L, Roselló Piera M, Martín-Grau C, et al. Prenatal genome-wide cell-free DNA screening: three years of clinical experience in a hospital prenatal diagnostic unit in Spain. *Genes*. 2024;15(5):568. doi: 10.3390/genes15050568
29. Wang C, Mei L, Wan Y, et al. Clinical value of positive CNVs results by NIPT without fetal ultrasonography-identified structural anomalies. *Mol Genet Genomic Med*. 2024;12(1):e2352. doi: 10.1002/mgg3.2352
30. Zhang J, Wu Y, Chen S, et al. Prospective prenatal cell-free DNA screening for genetic conditions of heterogenous etiologies. *Nat Med*. 2024;30(2):470-479. doi: 10.1038/s41591-023-02774-x
31. Cai M, Lin N, Chen X, et al. Non-invasive prenatal testing for the diagnosis of congenital abnormalities: Insights from a large multicenter study in southern China. *Braz J Med Biol Res*. 2023;56. doi: 10.1590/1414-431x2023e12506
32. Li C, Xiong M, Zhan Y, et al. Clinical potential of expanded noninvasive prenatal testing for detection of aneuploidies and microdeletion/microduplication syndromes. *Mol Diagn Ther*. 2023;27(6):769-779. doi: 10.1007/s40291-023-00674-x
33. Soster E, Tynan J, Gibbons C, et al. Laboratory performance of genome-wide cfDNA for copy number variants as compared to prenatal microarray. *Mol Cytogenet*. 2023;16(1). doi: 10.1186/s13039-023-00642-4
34. Tian W, Yuan Y, Yuan E, et al. Evaluation of the clinical utility of extended non-invasive prenatal testing in the detection of chromosomal aneuploidy and microdeletion/microduplication. *Eur J Med Res*. 2023;28(1). doi: 10.1186/s40001-023-01285-2
35. Yuan X, Yong W, Dai L, et al. The role of non-invasive prenatal testing and ultrasound in prenatal screening of fetal chromosomal abnormalities in singleton: a retrospective study. *Ann Transl Med*. 2023;11(2):111-111. doi: 10.21037/atm-22-6343
36. Zhang M, Tang J, Li J, et al. Value of noninvasive prenatal testing in the detection of rare fetal autosomal abnormalities. *Eur J Obstet Gynecol Reprod Biol*. 2023;284:5-11. doi: 10.1016/j.ejogrb.2023.03.002
37. Zhu S, Jia C, Hao S, et al. Evaluation of the clinical effects of non-invasive prenatal screening for diseases associated with aneuploidy and copy number variation. *Mol Genet Genomic Med*. 2023;11(9). doi: 10.1002/mgg3.2200
38. Camunas-Soler J, Lee H, Hudgins L, et al. Noninvasive prenatal diagnosis of single-gene disorders by use of droplet digital PCR. *Clin Chem*. 2018;64(2):336-345. doi: 10.1373/clinchem.2017.278101
39. Dello Russo C, Cesta A, Longo S, et al. Validation of extensive next-generation sequencing method for monogenic disorder analysis on cell-free fetal DNA: noninvasive prenatal diagnosis. *J Mol Diagn*. 2019;21(4):572-579. doi: 10.1016/j.jmoldx.2019.02.010
40. Guissart C, Dubucs C, Raynal C, et al. Non-invasive prenatal diagnosis (NIPD) of cystic fibrosis: an optimized protocol using MEMO fluorescent PCR to detect the p.Phe508del mutation. *J Cyst Fibros*. 2017;16(2):198-206. doi: 10.1016/j.jcf.2016.12.011
41. Zhang W, Lu S, Pu D, et al. Detection of fetal trisomy and single gene disease by massively parallel sequencing of extracellular vesicle DNA in maternal plasma: a proof-of-concept validation. *BMC Med Genomics*. 2019;12(1):151. doi: 10.1186/s12920-019-0590-8
42. Luo Y, Jia B, Yan K, et al. Pilot study of a novel multi-functional noninvasive prenatal test on fetus aneuploidy, copy number variation, and single-gene disorder screening. *Mol Genet Genomic Med*. 2019;7(4):e00597. doi: 10.1002/mgg3.597
43. Hoskovec J, Hardisty EE, Talati AN, et al. Maternal carrier screening with single-gene NIPS provides accurate fetal risk assessments for recessive conditions. *Genet Med*. 2023;25(2):100334. doi: 10.1016/j.gim.2022.10.014
44. Afzal M, Naeem MA, Ahmed S, et al. Noninvasive prenatal testing of beta-thalassemia for common Pakistani mutations: a comparative study using cell-free fetal DNA from maternal plasma and chorionic villus sampling. *Hematology.* 2022;27(1):353-359. doi: 10.1080/16078454.2022.2045052
45. Constantinou CG, Karitzi E, Byrou S, et al. Optimized droplet digital PCR assay on Cell-free DNA samples for non-invasive prenatal diagnosis: application to beta-thalassemia. *Clin Chem*. 2022;68(8):1053-1063. doi: 10.1093/clinchem/hvac076
46. D'Aversa E, Breveglieri G, Boutou E, et al. Droplet digital PCR for non-invasive prenatal detection of fetal single-gene point mutations in maternal plasma. *Int J Mol Sci*. 2022;23(5):2819. doi: 10.3390/ijms23052819

47. Hanxiao D, Luming S, Songchang C, et al. Noninvasive prenatal prediction of fetal haplotype with Spearman rank correlation analysis model. *Mol Genet Genomic Med*. 2022;10(8):e1988. doi: 10.1002/mgg3.1988
48. Pacault M, Verebi C, Lopez M, et al. Non-invasive prenatal diagnosis of single gene disorders by paternal mutation exclusion: 3 years of clinical experience. *BJOG*. 2022;129(11):1879-1886. doi: 10.1111/1471-0528.17201
49. Wu W, Zhou X, Jiang Z, et al. Noninvasive fetal genotyping of single nucleotide variants and linkage analysis for prenatal diagnosis of monogenic disorders. *Hum Genomics*. 2022;16(1). doi: 10.1186/s40246-022-00400-4
50. Xu LL, Yang D, Zhen L, et al. Impact of cell-free fetal DNA on early invasive prenatal diagnosis at a Chinese reference maternal medicine center. *J Matern Fetal Neonatal Med*. 2022;35(9):1764-1768. doi: 10.1080/14767058.2020.1769595
51. Yang XY, Meng Y, Wang YY, et al. Noninvasive prenatal diagnosis based on cell-free DNA for tuberous sclerosis: A pilot study. *Mol Genet Genomic Med.* 2022;10(7):e1952. doi: 10.1002/mgg3.1952
52. Gao S. Noninvasive detection of fetal genetic variations through polymorphic sites sequencing of maternal plasma DNA. *J Gene Med*. 2021;24(3):e3400.
53. Jiang F, Liu W, Zhang L, et al. Noninvasive prenatal testing for β-thalassemia by targeted nanopore sequencing combined with relative haplotype dosage (RHDO): a feasibility study. *Sci Rep.* 2021;11(1):5714. doi: 10.1038/s41598-021-85128-2
54. Yan H, Zhu X, Chen J, et al. Noninvasive prenatal sequencing for multiple Mendelian monogenic disorders among fetuses with skeletal dysplasia or increased nuchal translucency. *Prenat Diagn*. 2020;40(11):1459-1465. doi: 10.1002/pd.5792
55. Young E, Bowns B, Gerrish A, et al. Clinical service delivery of noninvasive prenatal diagnosis by relative haplotype dosage for single-gene disorders. *J Mol Diagn*. 2020;22(9):1151-1161. doi: 10.1016/j.jmoldx.2020.06.001
56. Kong L, Li S, Zhao Z, et al. Exploring factors impacting haplotype-based noninvasive prenatal diagnosis for single-gene recessive disorders. *Clin Genet*. 2024;105(1):52-61. doi: 10.1111/cge.14434
57. Pacault M, Verebi C, Champion M, et al. Non-invasive prenatal diagnosis of single gene disorders with enhanced relative haplotype dosage analysis for diagnostic implementation. *PLoS One*. 2023;18(4):e0280976. doi: 10.1371/journal.pone.0280976
58. Wynn J, Hoskovec J, Carter RD, et al. Performance of single-gene noninvasive prenatal testing for autosomal recessive conditions in a general population setting. *Prenat Diagn*. 2023;43(10):1344-1354. doi: 10.1002/pd.6427
59. Tsao DS, Silas S, Landry BP, et al. A novel high-throughput molecular counting method with single base-pair resolution enables accurate single-gene NIPT. *Sci Rep*. 2019;9(1). doi: 10.1038/s41598-019-50378-8
60. Loughry L, Pynaker C, White M, et al. State-wide increase in prenatal diagnosis of klinefelter syndrome on amniocentesis and chorionic villus sampling: Impact of non-invasive prenatal testing for sex chromosome conditions. *Prenat Diagn*. 2023;43(2):156-161. doi: 10.1002/pd.6103
61. Samango-Sprouse CA, Grati FR, Brooks M, et al. Incidence of sex chromosome aneuploidy in a prenatal population: 27-year longitudinal study in Northern Italy. *Ultrasound Obstet Gynecol*. 2023;62(2):266-272. doi: 10.1002/uog.26201
62. Johnston M, Warton C, Pertile MD, et al. Ethical issues associated with prenatal screening using non-invasive prenatal testing for sex chromosome aneuploidy. *Prenat Diagn*. 2023;43(2):226-234. doi: 10.1002/pd.6217

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