Cell-Free Fetal DNA Testing

Policy Number: MATERNITY 025.35
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➲ Instructions for Use

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Related Policies

- Chromosome Microarray Testing (Non-Oncology Conditions)
- Preimplantation Genetic Testing and Related Services

Coverage Rationale

Cell-Free Fetal DNA testing using maternal plasma to determine fetal genotype is proven and medically necessary when the individual undergoing testing is alloimmunized or at risk for alloimmunization due to maternal RhD status or the presence of red cell antigen antibodies, and all of the following:

- Paternal genotyping shows heterozygosity for RhD or paternal RhD status is unknown
- Indicated invasive diagnostic testing [e.g., amniocentesis, chorionic villus sampling (CVS)] for fetal genotyping has been offered and declined

Due to insufficient evidence of efficacy, Cell-Free Fetal DNA testing using maternal plasma is considered unproven and not medically necessary for indications beyond screening for trisomies 21, 18, 13, and sex chromosome Aneuploidy. This includes but is not limited to the following:

- Testing for the sole purpose of determining Twin Zygosity
- Genome-wide or exome-wide screening (e.g., MaterniT® Genome)
- Cell-Free Fetal DNA expanded panel testing (panels that include testing beyond trisomies 21, 18, 13, and sex chromosome Aneuploidy)
- Screening for the following:
  - Microdeletions/microduplications/copy number variations (CNVs)
  - Single gene disorders (e.g., Vistara™, PreSeek™)
  - Fetal antigen status other than RhD

Genetic Counseling

Genetic counseling is strongly recommended prior to fetal screening or prenatal diagnosis in order to inform persons being tested about the advantages and limitations of the test as applied to a unique person.

Medical Records Documentation Used for Reviews

Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. Medical records documentation may be required to assess whether the member meets the clinical criteria for coverage but does not guarantee coverage of the service requested; refer to the protocol titled Medical Records Documentation Used for Reviews.

Definitions

Aneuploidy: A normal human cell has 23 pairs of chromosomes. An abnormal number of chromosomes in a human cell is called Aneuploidy. This includes trisomy, where there is an extra chromosome present, or monosomy, where a chromosome is missing. Aneuploidy can impact any of the chromosomes, including sex chromosomes. Down syndrome (trisomy 21) is a common Aneuploidy. Patau syndrome (trisomy 13) and Edwards syndrome (Trisomy 18) are other notable aneuploidies. [American College of Obstetricians and Gynecologists (ACOG) Dictionary, 2025]

Cell Free Fetal DNA (cffDNA or cfDNA): Small fragments of fetal DNA from the placenta that move freely in the pregnant individual's blood. These fragments can be analyzed via a noninvasive prenatal screening test. (ACOG Dictionary, 2025)

Comparative Genomic Hybridization (CGH): CGH is a technology that can be used for the detection of genomic copy number variations (CNVs). Tests can use a variety of probes or Single Nucleotide Polymorphisms (SNPs) to provide copy number and gene differentiating information. All platforms share in common that individual and reference DNA are labelled with dyes or fluorescing probes and hybridized on the array. A scanner then measures differences in intensity between the probes, and the data is expressed as having greater or less intensity than the reference DNA. (South et al., 2013)

Non-Invasive Prenatal Testing/Screening (NIPT/NIPS): A common term used to describe different types of analysis of cffDNA. (Allyse and Wick, 2018)

Twin Zygosity: Zygosity refers to the type of conception. Dizygotic (nonidentical, fraternal) twins result from multiple ovulations with (near) synchronous fertilization of two separate ova by two separate sperm cells. Dizygotic twins thus share the same genetic relationship as nontwin siblings and share approximately 50% of genes. Monozygotic twins (so-called identical twins) are generated by division of a zygote that originated from the fertilization of one single ovum by one single sperm cell. (De Paepe, 2023)

Whole Genome Sequencing (WGS): WGS determines the sequence of the entire DNA in a person, or a tissue type, such as a tumor, which includes the protein making (coding) as well as non-coding DNA elements. (MedlinePlus 2021c)

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies may apply.

CPT Code	Description
0060U	Twin zygosity, genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood
0488U	Obstetrics (fetal antigen noninvasive prenatal test), cell-free DNA sequence analysis for detection of fetal presence or absence of 1 or more of the Rh, C, c, D, E, Duffy (Fya), or Kell (K) antigen in alloimmunized pregnancies, reported as selected antigen(s) detected or not detected
0489U	Obstetrics (single-gene noninvasive prenatal test), cell-free DNA sequence analysis of 1 or more targets (e.g., CFTR, SMN1, HBB, HBA1, HBA2) to identify paternally inherited pathogenic variants, and relative mutation-dosage analysis based on molecular counts to determine fetal inheritance of maternal mutation, algorithm reported as a fetal risk score for the condition (e.g., cystic fibrosis, spinal muscular atrophy, beta hemoglobinopathies [including sickle cell disease], alpha thalassemia)
0494U	Red blood cell antigen (fetal RhD gene analysis), next-generation sequencing of circulating cell-free DNA (cfDNA) of blood in pregnant individuals known to be RhD negative, reported as positive or negative
0536U	Red blood cell antigen (fetal RhD), PCR analysis of exon 4 of RHD gene and housekeeping control gene GAPDH from whole blood in pregnant individuals at 10+ weeks gestation known to be RhD negative, reported as fetal RhD status

CPT Code	Description
81422	Fetal chromosomal microdeletion(s) genomic sequence analysis (e.g., DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood
81479	Unlisted molecular pathology procedure

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Description of Services

During pregnancy, cell-free DNA (cfDNA) from the placenta circulates in a pregnant individual's blood. Fetal cfDNA from this blood can be screened for aneuploidies and other genetic anomalies, with testing offered as early as 10 weeks gestation. Available tests use different methodologies and algorithms for data analysis. These tests may identify women with an increased risk of having a child with a genetic disorder, but they cannot conclusively diagnose, confirm, or exclude the possibility of a genetic condition. Only conventional prenatal diagnosis [i.e., chorionic villus sampling (CVS) or amniocentesis] can definitively diagnose fetal genetic conditions. (ACOG, 2020)

Clinical Evidence

RhD Genotyping

Gilstrop et al. (2025) conducted a clinical validation study to assess the performance of a next-generation sequencing (NGS)-based, prenatal cell-free DNA (cfDNA) test for fetal RhD in pregnant individuals with an RhD-negative phenotype. Key inclusion criteria included passing quality metrics for samples, available previous prenatal cfDNA results for fetal aneuploidy, documented RhD-negative serologic results for the pregnant person, available newborn RhD serology results, maternal genotype results identifying participant as an RhD deletion or RhD-CE-D hybrid genotype, and identification of the pregnancy as singleton or monozygotic twin based on single nucleotide polymorphism (SNP)-based testing. Demographic and clinical data were included to evaluate how well the results would apply to the U.S. population. Maternal and fetal RhD genotypes were assessed using prospective cfDNA NGS. Investigators were blinded to the fetal RhD status during the analysis. The cohort was comprised of 655 pregnant individuals representing a diverse distribution of races and ethnicities within the RhD-negative U.S. population. The reported results showed no false-negative results; all 356 fetuses were accurately identified as RhD positive (sensitivity 100%, 95% CI, 98.9-100%). Among the 297 RhD-negative fetuses, 295 were correctly identified as RhD negative (specificity 99.3%, 95% CI, 97.6-99.8%). For fetuses with a negative RhD phenotype, the cfDNA test was able to accurately identify three that carried the fetal RhD pseudogene (RHDΨ) genotype. Of note, cfDNA fetal RhD was not reported in two cases. According to the authors, the assay demonstrated high-performance, achieving 100% sensitivity, 99.3% specificity, a positive predictive value (PPV) of 98.4%, and a negative predictive value (NPV) of almost 100% beginning at nine weeks of gestation. They suggest that implementing this test could conserve the U.S. RhD immune globulin supply by decreasing the need for unnecessary blood products in up to 40% of RhD-negative pregnant individuals. The study's strengths include the following: the cohort was large and racially diverse, serologic truth was available for all newborns, and the three most common RhD variants were evaluated. A limitation is that potential sampling bias existed due to the non-random cohort of individuals with previous SNP-based cfDNA screening. In addition, the study was funded by, and the majority of authors were associated with, the manufacturer (Natera) of the test under study, creating additional potential for bias. Despite these limitations, the authors assert that their results support the use of prenatal cfDNA screening as highly sensitive and specific tool for assessing fetal RhD status in a diverse U.S. population.

In a 2024 Hayes Clinical Utility Evaluation, the use of cfDNA testing for RhD genotypes to direct treatment with anti-D immunoglobulin prophylaxis in RhD-negative non-alloimmunized pregnancies was assessed. Nine applicable studies meeting inclusion criteria were identified and analyzed. Overall, this low-quality body of evidence indicated that the number of unnecessary anti-D prophylaxis injections were reduced when cfDNA RhD testing was performed. Based on this data, clinical utility is inferred (Hayes utility score = 3/probable) due to the widely established test accuracy and the availability of safe and effective treatment. Existing clinical practice guidelines and/or position statements weakly support the use of cfDNA testing in these cases.

In a 2021 systematic review and meta-analysis, Alshehri & Jackson evaluated the application of cfDNA for fetal RhD genotyping in conjunction with quantitative maternal alloantibody analysis for early diagnosis of pregnancies at risk of hemolytic disease of fetus and newborn (HDFN). A total of 19 studies from January 2006 to April 2020 were included in the analysis. The researchers found that cfDNA testing was highly sensitive and specific (as early as 11 weeks gestation) with regard to early RhD genotyping with a preference for high-throughput platforms and feel that this evidence supports the inclusion of cfDNA testing along with maternal alloantibody quantitation in routine pregnancy management. They note that knowledge of parental ethnicity is key for correct interpretation of cfDNA results and quantitative screening results

and that cfDNA testing would lead to less anxiety and inconvenience for pregnant individuals. The authors conclude that future large-scale studies evaluating cfDNA non-RhD genotyping including varying ethnic groups and with the presence of clinically significant alloantibodies are needed.

A 2020 Ontario Health Technology Assessment (HTA) evaluated the accuracy, clinical utility, and cost-effectiveness of noninvasive fetal RhD blood group genotyping for RhD-negative pregnant individuals. The evaluation included a literature search which identified six systematic reviews addressing test accuracy and 11 studies addressing clinical utility. Test accuracy was found to be high across all the systematic reviews and indicated that implementation of fetal cfDNA testing for RhD genotype could lead to avoidance of unneeded RhIG prophylaxis (GRADE: Low), good compliance with targeted RHIG prophylaxis (GRADE: very low), and high uptake of genotyping (GRADE: low). In addition, alloimmunization may not increase with the use of fetal cfDNA RhD genotyping for targeting prenatal RHIG prophylaxis, and unnecessary monitoring and invasive procedures in alloimmunized pregnant individuals may be reduced (both GRADE: very low). The HTA concluded that overall, fetal cfDNA testing for fetal RhD blood group genotyping is an accurate test to detect RhD incompatibility and help steer management of RhD-negative pregnancies, but only low to very low-quality evidence was identified to indicate that fetal cfDNA testing for RhD genotype would lead to the avoidance of unnecessary RhIG prophylaxis, high compliance with targeted RhIG programs and high uptake of genotyping. Studies by Yang et al. (2019), and Mackie et al. (2017), discussed in evidence below, and Saramago et al. (2018), previously discussed in this policy, were included in this HTA.

A prospective cohort, systematic review and meta-analysis was performed by Yang et al. (2019, included in the 2020 Ontario HTA discussed above) to assess the diagnostic accuracy of high-throughput NIPT for fetal RhD status in RhD-negative women not known to be sensitized to the RhD antigen. Databases scanned for this meta-analysis included MEDLINE, EMBASE and Science Citation Index and were searched through February 2016. Included for review were 3921 identified studies. The study population included RhD-negative pregnant women known to not be sensitized to the RhD antigen and the index test was high-throughput cfDNA on maternal plasma. Serological cord blood testing at birth was considered the reference standard and eligible studies were required to report diagnostic accuracy data including true positive, false positive, true negative, and false negative absolute numbers. Diagnostic accuracy of NIPT varied by gestational age with data suggesting that NIPT was consistently accurate any time after the first trimester. The false negative rate (those incorrectly classified as RhD negative) was 0.34% (95% CI 0.15-0.76) and the false positive rate (incorrectly classified as RhD positive) was 3.86% (95% CI 2.54-5.82). Because this study is a meta-analysis, the authors described the risk of bias in the original articles and several of the included studies were deemed to be high-risk for bias due to the selected populations and the reference standards. The authors concluded that the use of NIPT for fetal RhD screening in all RhD-negative women is possible. Results would significantly reduce the need for unnecessary prenatal anti-D prophylaxis, while marginally increasing the risk of sensitization due to false negative results.

Manfroi et al. (2018) performed fetal RhD genotyping with polymerase chain reaction (PCR) using cfDNA from maternal plasma to determine the diagnostic accuracy of non-invasive fetal genotyping at different gestational ages. A commercial multiple-exon assay was used to determine the accuracy of fetal RhD genotyping. Samples from RhD negative women (n = 367) with RhD positive partners or partners with unknown RhD phenotype were collected between 24-28 weeks gestation; due to lack of available first trimester samples the analysis was restricted to 24-28 weeks during which fetal genotyping is usually performed for prenatal RhIG administration. Neonatal results were provided for 284 pregnancies. The reported sensitivity and specificity were 100% and 97.5%, respectively. Diagnostic accuracy was 96.1% including 9/284 inconclusive results. The low number of early gestational age samples is a weakness of the study, and the authors attribute a false negative result to this. The authors concluded that cfDNA for RhD genotyping is an accurate and reliable tool for fetal immunoprophylaxis.

Saramago et al. (2018) conducted an HTA of the use of cfDNA to determine fetal RhD status. The authors searched MEDLINE and other databases, from inception to February 2016, for studies of high-throughput prenatal cfDNA tests of maternal plasma to determine fetal RhD status in RhD-negative pregnant women who were not known to be sensitized to the RhD antigen. Inclusion criteria for all reviews included pregnant women who were RhD negative and not known to be sensitized to the RhD antigen. For examining diagnostic accuracy, inclusion criteria were prospective cohort studies reporting absolute numbers, and for clinical effectiveness, studies that used high throughput NIPT in which anti-D prophylaxis was given and clinical outcomes were reported. The inclusion criteria for implementation outcomes were any publication that reported issues related to the implementation of, or practical advice relating to, NIPT. Eight studies were included in the diagnostic accuracy review, seven studies were included in the clinical effectiveness review and 12 studies were included in the review of implementation. The meta-analysis found that women in the studies were at least 11 weeks gestation or later, and mostly Caucasian with singleton pregnancies. The false negative rate (at risk of sensitization) was 0.34%, and the false positive rate (receiving unnecessary anti-D prophylaxis) was 3.86%. Clinical outcome data was limited to confirm the true sensitization rate, but there was no evidence of potential adverse effects. The authors

concluded that there was limited data on clinical effectiveness of NIPT for fetal RhD status, and more studies were needed for non-Caucasians and multiple gestations.

Screening for Rare Aneuploidies

The use of cfDNA for determination of aneuploidies other than trisomies 13, 18, 21, and sex chromosome aneuploidies is still in the early stages of development. Evidence at this time is limited and has shown a high number of false positive results.

A total of 33,079 pregnant individuals were included in a retrospective analysis of prenatal diagnostic results and pregnancy outcomes of cases where high-risk of rare autosomal trisomies (RATs) was detected via cfDNA prenatal testing (Yan et al., 2025). The study, which took place at a reproductive medical facility in in China, identified 66 cases where the fetus was found to be high-risk for RATs, for a detection rate (DR) of 0.20% (66/33,079). Of the 66 identified cases, seven individuals opted not to undergo amniocentesis. The prenatal diagnostic procedures did not confirm corresponding RATs in any of the remaining 59 cases where amniocentesis was performed. Five individuals were lost to study follow-up and one individual terminated her pregnancy for personal reasons. Full term pregnancy/birth was achieved by 50 of the 60 individuals with reported outcomes (83.33%), while 10 (16.67%) experienced some type of adverse outcome (preterm birth, miscarriage, intrauterine growth restriction, fetal abnormality related to placental hemangioma, hydronephrosis, diagnosed chromosomal abnormality). The authors note that because most fetuses with RAT do not survive beyond 12 weeks gestation, RATs identified by cfDNA testing after 12 weeks are typically indicative of placental mosaicism and not fetal trisomy. The presence of trisomic cells in the placenta can, however, disrupt normal placental development which can lead to adverse pregnancy outcomes. They recommend further study examining pregnancy outcomes in cases of RATs.

A retrospective study was conducted by Yang et al. (2025) to evaluate the clinical performance of expanded non-invasive prenatal testing (NIPT-plus) and compare its effectiveness screening for chromosomal aneuploidies with that of standard non-invasive prenatal testing (NIPT). The study included screening results, confirmatory invasive testing results, and follow-up data from pregnant women who underwent either NIPT (6792 cases) or NIPT-Plus (5237 cases). The researchers calculated the PPV, sensitivity, specificity, and other indicators for different types of chromosomal abnormalities in NIPT and NIPT-plus. In the NIPT-plus samples, the average number of unique reads was 5.26 times greater than in the standard NIPT samples. No significant difference in the PPV or positive rate between NIPT-plus and NIPT was identified for screening chromosomal aneuploidies. The high-risk group had a greater PPV when compared with the low-risk group, but in the NIPT-plus group, no significant difference in the PPV between the low-risk and high-risk groups was discovered. Pregnant individuals in the study had a higher rate of confirmatory invasive testing for common trisomies, sex chromosome abnormalities (SCAs), and copy number variations (CNVs) than for rare autosomal aneuploidies (RAAs). The rate of pregnancy termination was higher for common trisomies, RAAs, and CNVs than for SCAs. Both NIPT and NIPT-plus screening had a sensitivity of 100% and a specificity of over 0.99 for common trisomies and SCAs with no detected cases of false negatives, indicating that both tests are effective in screening for the common trisomies and SCAs, even though NIPT-plus has approximately five times more sequencing data than standard NIPT. The authors concluded that these findings demonstrate that both NIPT and NIPT-plus are effective in screening for common trisomies, SCAs, and RAAs in different groups of pregnant individuals, and although NIPT plus can effectively screen for pathogenic CNVs due to the increased amount of sequencing performed, it does not improve detection of common trisomies, SCAs, and RAAs when compared with standard NIPT. Noted limitations of this study include the following: 1) It is too early to determine if a newborn has SCAs or CNVs three months postpartum; symptoms of pathogenic CNVs and SCAs may not appear until childhood, and 2) the study excluded screening-positive instances without confirmatory invasive testing results as well as those lost to follow-up, which could impact the accuracy of the results and conclusions of this study. In addition, the software used for analysis of CNVs was limited to only “pathogenic” or “likely pathogenic” which did not allow for the comprehensive evaluation of CNV detection efficiency.

In a recent systematic review and meta-analysis, Konya et al. (2024) assessed the specificity and accuracy of genome-wide cfDNA prenatal testing for RATs and structural chromosome abnormalities (StrCAs) to help determine clinical utility for this testing. Both the screening accuracy and pregnancy outcomes of cases where testing revealed rare chromosomal abnormalities were analyzed. A total of 17 studies were included, with an overall population of 740,076 cfDNA fetal tests performed. Of the 740,076 tests, 1738 of these were positive for RATs. Two methodologies were used to determine true positive cases: the first was a confirmed methodology where only cases that were verified by diagnostic genetic testing were considered true positives with definitive diagnosis; the second was an extended methodology where, in addition to cases confirmed by genetic testing, intrauterine fetal death and termination of pregnancy due to abnormality confirmed by ultrasound were also considered true positives (no definitive diagnosis was made in these cases but it was likely the fetus was affected). Pooled PPV using the confirmed method was 0.07. Using the extended method, the pooled PPV was 0.13. The pooled false positive rate among all prenatal cfDNA tests was 0.0020. Heterogeneity was high (I^2 = 95%). Many cases of RAT identified by cfDNA testing were not confirmed in the fetus; this is most often due to placental mosaicism,

which also increases risk of adverse pregnancy outcomes and warrants increased prenatal monitoring. The highest rates of true positives were found for trisomies 16, 22, and 2 using the confirmed method. Using the extended method, the highest rates of true positives were found for trisomies 15, 16, and 22. Overall, the meta-analysis revealed frequency data related to rare chromosomal abnormalities, test-positive rates, and accurate PPV for each chromosomal abnormality (this varied widely between chromosomes), which may assist clinicians with pre- and post-test counseling and decision-making. The authors assert that their findings indicate that prenatal screening for rare chromosome abnormalities with cfDNA can lead to early identification of individuals who may be candidates for verification of fetal status via invasive diagnostic testing. Further investigation of the clinical utility of screening for rare chromosomal abnormalities is required before this testing can be incorporated into standard practice. Publications by Zhang et al. (2023) and Van Opstal et al. (2018), discussed below, and Scott et al. (2018), Wan et al. (2018), Pertile et al. (2017), and Fiorentino et al. (2017), previously discussed in this policy, were included in this systematic review and meta-analysis.

Another recent systematic review and meta-analysis (Acreman et al., 2023) sought to determine the PPV of cfDNA screening for RATs. This analysis included 31 studies with 1703 pregnant individuals tested. This evaluation found pooled PPV for the diagnosis of RATs to be 11.46% (95% confidence interval [CI], 7.80-15.65). Heterogeneity was high (I^2 = 82%). When the analysis was restricted to five studies with low risk of bias, pooled PPV was 9.13%. No assessment of sensitivity and specificity was performed because the majority of studies only ordered confirmatory testing for individuals whose results showed them to be high-risk. The researchers maintain that these findings provide helpful information for clinicians and also for pregnant individuals who may be considering expanded testing for conditions beyond the common trisomies, while noting that this testing is not currently recommended by professional societies. They further indicate that in situations where there is a strong clinical suspicion of aneuploidy (e.g., abnormal ultrasound), cfDNA testing is considerably inferior to invasive diagnostic testing. Publications by Van Opstal et al. (2018), discussed below, and van der Meij et al. (2019), Xue et al. (2019), Brison et al. (2018), Scott et al. (2018), Wan et al. (2018), and Pertile et al. (2017), previously discussed in this policy, were included in this systematic review and meta-analysis.

To evaluate the application of NIPT for screening rare autosomal abnormalities, a study was conducted on 81,518 pregnant individuals who had undergone NIPT at a Chinese hospital between May 2018 and March 2022 (Zhang et al., 2023). Samples deemed high risk were evaluated with amniotic fluid karyotyping and chromosome microarray analysis (CMA) with pregnancy outcomes recorded. NIPT identified 292 cases (0.36%) of rare autosomal abnormalities in the cohort. One hundred-forty of these (0.17%) were RATs. Of the 140 pregnancies with RATs detected, 102 individuals agreed to invasive testing for confirmation. Five of these individuals were found to have a true positive (PPV = 4.90%). CNVs were identified in 152 samples (0.19%); 95 individuals agreed to CMA testing which confirmed true positive in 29 pregnancies (PPV = 30.53%). Clinical information was acquired in 81 of the 97 cases with false-positive RATs results. Of these, 37 (45.68%) had adverse perinatal outcomes including a higher incidence of small for gestational age babies, intrauterine growth retardation and preterm birth. Based on these results, the authors indicate that NIPT is not recommend for screening for RATs.

Hayes (2021, updated 2024) published a Clinical Utility Evaluation addressing the use of cfDNA screening for fetal RATs in singleton and twin pregnancies. The report asserts that the use of this screening in singleton pregnancies leads to confirmatory testing in some women, but few of the women with confirmed RAAs used the final diagnostic results for pregnancy management decisions. Of those who underwent confirmatory diagnostics based on the RAA cfDNA screening, more than 50% of the cases were found to be false positives. Overall, the published evidence regarding use of RAA fetal testing in singleton pregnancy is very low in quality and insufficient to come to any conclusions regarding clinical utility at this time. With regard to fetal RAA testing in twin pregnancies, there were no identified peer-review studies that assessed clinical utility in individuals with twin pregnancies; evidence is thus insufficient to draw conclusions related to the clinical utility of this RAA testing in twin pregnancies.

Van Opstal et al. (2018) reported on the presence of rare trisomies and other abnormalities found by the Trial by Dutch laboratories for Evaluation of Noninvasive Prenatal Testing (TRIDENT) study. The TRIDENT study was a trial where NIPT was offered as an alternative to pregnant women considering invasive prenatal diagnosis between April 2014 and April 2015. NIPT testing was performed using whole-genome shallow massively parallel shotgun sequencing. Of 3306 enrolled cases, 753 were analyzed only for chromosomes 21, 13 and 18. The remaining 2553 were analyzed for all chromosomes and for segmental subchromosomal abnormalities. Results were reported in 2527 cases. In 78, a common trisomy was found, and follow up information was reported elsewhere. Forty-one cases of another type of chromosome abnormality were identified. One case of reported trisomy 8 was terminated at a private clinic before any follow up was available. In the remaining 40 cases, 10 were confirmed to be true positives. These included two cases of trisomy 9, which were confirmed to be mosaic in the fetus. Both resulted in live births with multiple congenital anomalies. One case of a dual trisomy 15 and trisomy 22 was reported, and fetal tissue confirmed a mosaic trisomy 15. The pregnancy resulted in a live birth with no identifiable anomalies. One trisomy 22 was identified, confirmed as a mosaic trisomy 22. The pregnancy had multiple anomalies and was terminated. Six of the 10 cases were genomic imbalances that included dup 2p, del 6q,

del80/dup8q, del 9p, del 12q, and del 18p. All were confirmed through amniocentesis. In 22 of the 40 positive cases, placental testing confirmed that confined placental mosaicism was the likely cause of the NIPT results, and in this group, there were 10 infants with some impact, ranging from small for gestational age to multiple congenital anomalies. The authors concluded that genome-wide screening for NIPT results in identification of chromosomal aberrations other than trisomy 13, 18, or 21 in about one-third of screen positive results, and this information is important for pregnancy management.

Other Screening Using Cell-Free DNA (cfDNA)

The evidence is insufficient to support the use of other prenatal screening using cfDNA, such as screening for microdeletions and CNVs, and/or the use of cfDNA for prenatal exome and genome sequencing. Further validation studies are needed to determine the sensitivity, specificity, and positive predictive value of cfDNA used for these purposes.

In a study to assess the clinical performance of whole genome sequencing (WGS)-based cfDNA screening including 22q11.2 detection, Hammer et al. (2024) analyzed 380,000 pregnant individuals with both average and high-risk pregnancies. The cfDNA screening test (Prequel, Myriad Genetics, Inc.), which incorporates the amplification of fetal fraction (FF), was administered to 379,428 participants meeting study eligibility requirements. Of all participants, 76 received a positive result on the screening test for a de novo 22q11.2 microdeletion; this equates to a screen-positive rate of one in 4992. Twenty-two of the 76 had diagnostic test results available and all 22 of these were confirmed as true positives, yielding a PPV of 100% (95% CI 84.6%–100%). In addition, ultrasound results for pregnancies that screened positive were consistent with findings associated with 22q.11.2 deletion syndrome. Because the performance of the test is dependent on FF, the distribution of FF in the screen-positive and screen-negative groups was compared to determine whether test performance could have been different between the two groups; no significant difference was found, indicating that the test performed similarly between the two groups. Because 22q11.2 deletion syndrome is a rare finding, this study did not assess sensitivity, specificity, or NPV. The authors suggest that the prevalence and severity of 22q11.2 deletion syndrome warrant screening with a cfDNA assay using FF amplification for all pregnant individuals. Although the study participants were diverse in ethnicities, body mass index (BMI), and testing indications, they were commercially ascertained so there is uncertainty regarding whether the cohort is representative of a general pregnancy population. In addition, although outcomes were obtained for 74 screen-positive cases, clinical information was incomplete for 17 of those. Lastly, the high PPV in this study may be related to the fact that diagnostic testing was performed primarily for those who had ultrasound findings that suggested possible 22q11.2 deletion syndrome. Further investigation exploring test sensitivity, specificity, and NPV as well as detailed outcome information across all participants is needed.

In another study leveraging the use of prenatal genome-wide cfDNA, Soster et al. (2024) detailed the outcomes of a small cohort of individuals with screen-positive results for trisomy 20. These individuals represented a subset of participants from larger cohort where screening was done for RAAs (Mossfield et al., 2022). In all, 10 cases with screen positive results for trisomy 20, nine of which had diagnostic information available, were reviewed. The one case without diagnostic information was a pregnancy that ended in fetal demise. Of the nine cases where diagnostic information was available, all nine had normal diagnostic results after amniocentesis. One case had a mosaic partial duplication rather than a full trisomy 20 which could explain the assay showing trisomy 20. Only one case in the cohort had placental testing; the result was discordant with the cfDNA testing result. Confined placental mosaicism could not be ruled out for the rest of the cases in the cohort due to the lack of placental testing. Half of the 10 cases had adverse pregnancy outcomes, suggesting that the possible presence of underlying confined placental mosaicism or mosaic/full fetal trisomy 20, but this could not be confirmed. This cohort was made up of mainly individuals of advanced maternal age. The authors recommend further study, including larger cohorts and participants of average-risk.

Liu et al. (2024) performed a retrospective review investigating the use of noninvasive prenatal screening (NIPS) to detect CNVs in fetuses with ultrasound soft markers (USMs) in pregnancies not involving advanced maternal age (AMA). The study included pregnancies with isolated USMs in the second-trimester from January 2020 to December 2022 at West China Second University Hospital, Sichuan University. NIPS was performed in the Department of Medical Genetics. The results included 6647 pregnant women screened using the Berry Genomics NIPS algorithm. Those with positive NIPS results underwent amniocentesis for prenatal diagnosis. The results were analyzed and compared among different USMs. A total of 96 pregnancies were positive for fetal chromosome anomalies including 37 aneuploidies and 59 CNVs. The PPVs for trisomy 21, trisomy 18, trisomy 13, and sex chromosome aneuploidies were 66.67%, 80.00%, 0%, and 30.43%, respectively. NIPS sensitivity for aneuploidies was 100%. For CNVs, the PPV was 35.59% with a false positive rate of 0.57%. Six pathogenic CNVs (P CNVs) were identified, with two detected by NIPS and four missed (three below the NIPS resolution limit and one false negative). Aneuploidies were more common in fetuses with absent or hypoplastic nasal bone, while P CNVs were more frequent in fetuses with aberrant right subclavian artery (ARSA). Study limitations included the following: 8.23% of pregnant women were lost to follow-up, making it difficult to accurately assess the sensitivity and specificity of NIPS for detecting CNVs due to unclear clinical outcomes; 45 fetuses had birth defects, but 43

did not undergo postpartum CMA, preventing confirmation of pathogenic CNVs; the number of USMs varied across categories, with only seven cases of mild ventriculomegaly but over 5000 cases of echogenic intracardiac focus (EIF), potentially leading to selection bias. Per the authors, future research goals will include focus on re-establishing contact with parents of fetuses lost to follow-up to determine presence or absence of birth defects, and the identification of more cases with both NIPS-derived significant CNVs and postpartum CMA results to better assess NIPS accuracy for CNVs. The researchers state that their results indicate that while NIPS is highly sensitive for detecting common aneuploidies/SCAs and has moderate PPVs for CNVs in non-AMA pregnant women with fetal USMs, it appears to have limited ability to detect P CNVs. The authors strongly recommend additional prenatal diagnosis when NIPS indicates CNVs and advise against using NIPS for CNV screening in non-AMA pregnant women with fetal USMs, particularly in fetuses with ARSA.

Maya et al. (2023) sought to evaluate the theoretical value-add of two types of cfDNA screening expansions in pregnancies with no evidence of major structural abnormalities over standard cfDNA testing (13, 18, 21, X and Y) and also assess them in terms of the added value of CMA in a retrospective cohort study. The study was based on the CMA results of pregnant individuals with normal ultrasounds who had undergone amniocentesis between January 2013 and February 2022. Of the 8605 pregnancies assessed, 1.4% (n = 122) had clinically significant CMA results. Standard cfDNA testing would have theoretically identified 36.1% of these. In addition to aneuploidies detectable with standard cfDNA testing, three cases detectable with expanded cfDNA testing (including commonly found microdeletions) and nine cases detectable with genome-wide cfDNA screening (excluding common microdeletions) were identified in the overall cohort. The researchers assert that of the clinically significant CMA findings, standard cfDNA screening would miss 63.9% and genome-wide cfDNA screening would miss 54.1%. cfDNA screening expanded to include detection of microdeletions would increase value over standard cfDNA testing by approximately 0.035% and genome-wide cfDNA screening including large CNVs would result in an increase in value of approximately 0.14%; these results are far lower than the value-add of CMA (0.91%).

Several recent studies have explored the accuracy of cfDNA testing for the identification of microdeletion/microduplication syndromes (MMS) and nonsyndromic CNVs. Yang et al. (2022) assessed 19,068 singleton pregnancies that had been screened with cfDNA testing using high-throughput sequencing. Of 170 individuals whose testing revealed abnormalities, 113 (66.5%) opted for invasive testing. PPV of CNV sequencing for all types of CNVs detected in the study was 35.4% (61.5% for pathogenic MMSs and 27.6% for nonsyndromic CNVs). Although performance for MMSs was relatively high, the low PPV for nonsyndromic CNVs led the authors to conclude that the use of expanded cfDNA testing would likely increase unnecessary invasive tests and potentially lead to inappropriate terminations of pregnancy.

In a prospective study assessing performance of expanded NIPT (Zou et al., 2023), the PPV of the NIPT expanded test for CNVs was found to be 51.72%. Using a clinically available genome-wide cfDNA assay (Sequenom) to test 701 pregnant individuals, Soster et al. (2023) found that when CNVs evaluated were at least 7Mb and the test was specific to specific microdeletions, sensitivity was 93.8%, specificity was 97.3%, PPV was 63.8% and NPV was 99.7% when compared to microarray testing. However, when out-of-scope CNVs were included as false negatives, the sensitivity fell to 63.8%. The authors of this study indicated that microarray testing via amniocentesis provides the most accurate and thorough assessment of fetal CNVs, but genome-wide cfDNA testing may be an option for individuals who decline or are otherwise unable to undergo invasive diagnostic testing, though diagnostic testing would still be required to confirm screen-positive results. Further study is recommended to explore potential clinical utility and assess the impact of reducing the size threshold of CNVs.

The accuracy of expanded NIPT (NIPT-plus) in the detection of clinically significant fetal CNVs was the subject of a prospective analysis of 31,260 singleton pregnancies from June 2017 to December 2020 (Xue et al., 2022). Of the 31,260 pregnant individuals who underwent NIPT-plus testing at a single hospital in Fuzhou, China, results were obtained in 31,256. High risk of clinically significant CNVs was detected in 221 individuals (0.71%). Of these, 18 refused further evaluation. Two-hundred three underwent invasive testing for prenatal diagnosis revealing 78 true positive cases and 125 false positive cases. Overall PPV was 38.42% and false positive rate was 0.40%. Where known microdeletion/microduplication syndromes were identified (n = 27), the PPVs were as follows: DiGeorge syndrome, 75%; 22q11.22 microduplication syndrome, 80%; Prader-Willi syndrome, 50%; and cri-du-chat syndrome, 50%. The remaining significant fetal CNVs (n = 175) had a combined PPV of 46.5% for CNVs greater than 10 Mb and 28.57% for CNVs of 10 Mb or less. Overall the results indicate that NIPT screening had relatively high performance for identification of 22q11.2 microduplication syndrome and DiGeorge syndrome in this study, but low/moderate detection for other clinically significant CNVs. Further high-quality studies with larger and more diverse populations, increased depth of sequencing and improved algorithms are needed.

A 2022 (updated 2025) Hayes Clinical Utility Evaluation addressed cfDNA screening for fetal chromosome CNVs in individuals with twin or singleton pregnancies. There were no peer-reviewed studies evaluating clinical utility of cfDNA

screening for fetal CNVs in individuals with twin pregnancy identified. Although some evidence suggests that use of cfDNA screening for fetal CNVs in singleton pregnancies may lead to confirmatory diagnostic testing in some women, it is unknown if additional CNV testing will impact the rate of confirmatory diagnostic testing from common aneuploidy cfDNA screening. Among individuals who underwent confirmatory diagnostic testing based on the CNV cfDNA result, there was a high rate of false positive results. The current published evidence is of low quality and currently does not support conclusions regarding clinical utility.

Results of a multicenter, prospective observational study designed to assess the performance of SNP-based cfDNA screening for detection of 22q11.2 deletion syndrome were published by Dar et al. (2022). The study also assessed prevalence of 22q11.2 deletion syndrome and the performance of an updated cfDNA algorithm which the researchers blinded to the pregnancy outcome. Enrollees from 21 centers in six countries participated, undergoing SNP based cfDNA screening specific to 22q11.2 deletion syndrome. Either prenatal or newborn DNA samples were requested in all cases, so that genetic confirmation using chromosomal microarrays could be performed. A total of 20,887 individuals were enrolled and a genetic outcome was available for 87.6% (18,289). Twelve 22q11.2 deletion syndrome cases were confirmed in this cohort (including five nested deletions), which yielded a prevalence of one in 1524. Of the total cohort, cfDNA screening identified 17,976 cases as low risk for 22q11.2 deletion syndrome and 38 cases as high risk, with 275 cases non-reportable. Ultimately, nine of 12 cases of 22q11.2 were identified, equating to a sensitivity of 75%, a specificity of 99.84% and a PPV of 23.7%. NPV was 99.98%. No cases with non-reportable outcome resulted in a diagnosis of 22q11.2 deletion syndrome. The updated algorithm identified 10 of 12 cases and led to lower false positive rate and an increased PPV of 52.6%. The authors concluded that cfDNA screening for 22q11.2 deletion syndrome can detect most affected cases with a low false positive rate and has the ability to detect smaller, nested deletions. However, the overall confirmed number of cases of 22q11.2 deletion syndrome in this study was low, liming the ability to accurately calculate PPV as stratified by risk factors. In addition, estimates of DRs for rare conditions are associated with wide CIs and finally, there were varied indications for testing and prevalence rates may not reflect risk in the average population. In addition, the study was funded by Natera, the maker of a test including 22q11.2 microdeletion screening.

Cui et al. (2019) evaluated the clinical utility of NIPT for the detection of CNVs by reporting on 161 pregnancies with ultrasound findings and negative NIPT results for chromosomal aneuploidy. Fetal CNVs were diagnosed by CNV sequencing; fetal and parental karyotypes were obtained by G-banding. NIPT revealed 11 CNVs ≥ 1Mb in nine samples, including two CNVs in each one of two separate samples. CNV sequencing on amniotic fluid was performed for 137 samples and 24 samples of fetal tissue. Fetal karyotypes were obtained for 78 cases and seven cases were diagnosed as abnormal. The sensitivity and specificity of NIPT for detecting CNV > 1Mb were 83.33% and 99.34%, respectively. The PPV and NPV were 90.91% and 98.68%, respectively. The sensitivity and specificity for CNVs 1Mb-5Mb was higher than for those ≥ 5Mb. The authors claimed that NIPT can be performed for pregnancies with structural fetal anomalies for CNV detection, however due to the residual chromosomal aneuploidy risks for pregnancies with soft ultrasound markers, women with structural ultrasound anomalies should be offered invasive procedures for diagnosing CNVs. This study is difficult to generalize to the average screening population, as only pregnancies with ultrasound anomalies and negative NIPT results were selected for analysis. Future studies are needed for NIPT and CNV detection.

DiGeorge syndrome, also known as velocardiofacial syndrome or 22q11 deletion syndrome, is one of the most common microdeletion syndromes with an incidence of 1 in 3,000-6,000 births. Affected individuals have a wide array of clinical manifestations, including congenital heart defects, immune dysfunction, hypocalcemia, mild-to-severe learning disabilities, and an increased risk of mental health disorders. Ravi et al. (2018) reported on the clinical validity of an SNP based NIPT assay to detect fetal 22q11.2 deletions during pregnancy. Women from six prenatal centers were enrolled in the study and underwent invasive prenatal diagnosis for a variety of reasons. At the time of blood draw, information about gestational age, maternal age and weight, and time between the invasive procedure and blood draw were collected. Samples from participants that were less than nine weeks gestation, had a fetal demise, had atypical 22q distal deletions on invasive testing, or had equivocal invasive test results were excluded. Participants with inconclusive or no call NIPT results were also excluded and no redraws were requested. The study was internally blinded but ultimately included 10 subjects with confirmed fetal 22q11.2 deletions and 390 with unaffected pregnancies. The mean age was 28, and the gestational age averaged 21 weeks for affected pregnancies and 12.8 weeks for unaffected pregnancies. Samples were tested at Natera using a massively multiplexed PCR (mmPCR) amplification targeting SNPs covering chromosomes 13, 18, 21, 22, X, and Y. The target set contained 13,926 distinct genetic loci, including 1351 SNPs spanning a 2.91 Mb section of the 22q11.2 region that constitutes approximately 87% of all deletions detected in individuals with the 22q11.2 deletion syndrome. Risk status for 22q11.2 deletion was assigned as high- or low-risk, or risk unchanged/no call. High-risk calls with maternally deleted haplotypes were sequenced at a higher depth of read to confirm high-risk status. For cases with FF of 2.8–6.5%, the sample was evaluated only for the presence or absence of the paternally-inherited haplotype. Of the ten affected pregnancies, nine were identified as test positive, or high risk. Of the 390 unaffected samples, one false positive was found. Overall, the study found the sensitivity to be 90%, the specificity to be 99.7%, and based on a prevalence of 22q11.2 deletions of 1 in 1442 in pregnancy, the estimated PPV was 19.6%.

Gross et al. (2016) evaluated the ability of a SNP based NIPT test to detect 22q11 deletion syndrome in a commercial lab. A retrospective analysis was performed for 21,948 consecutive samples for fetal aneuploidy and microdeletion screening received over a 6-month period from February to August 2014. Demographic information received included indication for testing, gestational age, maternal date of birth, maternal weight, and whether the mother was a known microdeletion carrier. A paternal sample was requested but not required, and 5912 (26.9%) cases included a paternal sample. Prior to analysis of 22q11.2, the standard panel testing for aneuploidy at chromosomes 13, 18, 21, X, and Y was conducted; samples that failed quality control at this step were not evaluated for 22q11.2. This region was analyzed by 672 SNPs targeting the 2.91-megabase (Mb) loci associated with the 22q11.2 deletion syndrome. Fetal results were predicted based on the pattern of SNPs, FF, and paternal results when available. Ninety-five cases were reported as high risk. Of these, 84 had some outcome data available. Invasive testing was performed in 48 cases, and 11 had post-natal testing; testing was declined by the remaining participants. Of those with follow up diagnostic testing, 11 were true positives, and 50 were false positives. Seventy-seven high risk participants had ultrasound data available. Of these, 26 had anomalies observable on ultrasound of which nine were true positives. There were three pregnancy terminations related to screening results of 22q11.2 deletion, two of which were confirmed as true positive. The authors conclude that the availability of genetic counseling and other resources to manage high risk 22q11.2 cases is an important aspect of this screening test.

While individually rare, subchromosomal abnormalities occur in 1.6% of pregnancies. Helgeson et al. (2015) reported on the development of an algorithm to be applied to cell-free fetal DNA (cfDNA) testing to support identifying 5pdel, 22q11del, 15qdel, 1p36del, 4pdel, 11qdel, and 8qdel in routine testing. Low coverage whole genome massively parallel sequencing was used to analyze cffDNA and used a statistical method to search for consistently under-represented regions followed by a decision tree to differentiate whole-chromosome events from regional deletions. A cohort of 175,393 high risk pregnancies was used to test the algorithm. Samples were collected from October 2013 to October 2014. Fifty-five cases were screen positive for subchromosomal events. Outcome data was available for 53 cases. Chromosome microarray or FISH confirmed the findings in 41 (77%) cases. Nine cases did not have confirmational testing but had clinical features on ultrasound consistent with the deletion. Three cases were false positives. The false negative rate and sensitivity were not conclusively determined.

Fetal Antigen Testing (Other Than for RhD Status)

There is a lack of evidence in the peer-reviewed literature supporting use of cfDNA testing to determine fetal antigen status (other than RhD) for pregnant individuals who are alloimmunized for specific antigens. Additional study, including high quality trials focused on impact to clinical outcomes, is needed.

Rego et al. (2025) conducted a multicenter prospective cohort study to evaluate the accuracy of NGS-based quantitative cfDNA analysis for fetal antigen genotyping in individuals with alloimmunized pregnancies. The study included 156 individuals with alloimmunized pregnancies undergoing clinical fetal antigen cfDNA analysis between 10 0/7 and 37 0/7 weeks of gestation at 120 clinical sites. After delivery, neonatal buccal swabs were collected between 0 and 270 days of life and were sent to an outside independent laboratory for antigen genotyping. The laboratory was blinded to the fetal cfDNA results. Concordance between cfDNA analysis results and neonatal genotype was determined for 465 antigen calls for the following antigens: K1 (n = 143), E (124), C (60), Fy a (50), c (47), and D(RhD) (41). The 465 calls included 145 in which the fetus was antigen positive and 320 in which the fetus was antigen negative, resulting in 100% sensitivity, specificity, and accuracy. The authors concluded that cfDNA analysis is highly sensitive and specific for determining fetal antigen genotype as early as 10 weeks of gestation in individuals with alloimmunized pregnancies. They determined that with 100% accuracy and no need for paternal testing or invasive procedures, the assay has the potential to provide informative results about fetal risk to more alloimmunized individuals when compared with the traditional approach and can streamline clinical management while improving equitable access to care. Noted limitations include overrepresentation of individuals identifying as White, although the no-call rate for the assay did not differ across varying races and ethnicities. In addition, many authors had affiliations with the manufacturer of the test under study, creating potential for bias.

Alford et al. (2023) used quantitative counting template (QCT) technology in the development of an NGS-based fetal cfDNA screening test that was then used to identify RhD, C, c, K (Kell), and Fy^a (Duffy) fetal antigen genotypes present in the blood of pregnant individuals in an ethnically diverse population within the U.S. The use of QCT was leveraged to allow for highly specific and sensitive quantification and identification of paternally derived fetal antigen alleles in cfDNA. In an analytical validation including 1061 preclinical samples, use of the test to determine fetal antigen status was found to have sensitivity of 100% (95% CI 99–100%) and specificity of 100% (95% CI 99–100%). An independent evaluation of two duplicate plasma samples was performed for 1683 clinical samples which showed precision of 99.9%. The “no results” rate (in clinical practice) was 0% for 711 RhD-negative non-alloimmunized pregnant individuals and 0.1% for 769 cases where the individual was alloimmunized. Clinical validation demonstrated that fetal cfDNA testing results were 100% concordant with neonatal antigen genotype serology results in a corresponding 23 RhD-negative pregnancies and 93 antigen evaluations in 30 alloimmunized pregnant individuals. The researchers concluded that NGS-based fetal

antigen cfDNA testing has the potential to detect more fetuses at risk for hemolytic disease than the standard practice which relies on paternal genotyping and invasive diagnostics, thereby limiting results due to lack of adherence or faulty attribution of paternity. The authors proposed that integration of fetal cfDNA testing for the detection of fetal antigens into care for both alloimmunized and RhD-negative non-alloimmunized pregnant individuals could increase efficiency in prenatal care and potentially reduce unneeded treatment and supervision. While this data shows promise, additional high-quality studies measuring the impact of this testing on clinical outcomes is needed. In addition, the majority of authors of this study are noted to be affiliated with a fetal cfDNA test manufacturer, creating risk of bias.

In a 2023 publication, Clausen and van der Schoot focused on the use of fetal cfDNA testing for blood group antigen genotyping as a diagnostic tool for the predication of hemolytic disease of the fetus and newborn in pregnancies where the pregnant individual was immunized. The authors indicate that these noninvasive tests have demonstrated high performance accuracy and predict that the use of cfDNA fetal blood group antigen genotyping will soon be expanded in clinical practice. Anticipated challenges include the use this testing in mixed ethnic populations and the need for improvement of care in many low-income countries around the world.

Single Gene Disorders

The use of cfDNA testing to evaluate single gene disorders is in the early stages of development. The evidence available is limited and contains small sample sizes. Further studies with larger numbers of participants are needed to determine the clinical utility of this approach.

In the largest clinical validation of carrier screening with reflex to single gene noninvasive prenatal testing (sgNIPT) in a general population setting to date, Wynn et al. (2023) endeavored to strengthen the evidence supporting the use of single gene cfDNA testing to assess the fetal risk of autosomal recessive conditions. Specifically, clinical performance of carrier screening with reflex to sgNIPT was evaluated for four conditions: cystic fibrosis, spinal muscular atrophy, alpha thalassemias, and beta hemoglobinopathies. Pregnancy outcome records of individuals who had undergone this testing were reviewed and a comparison of neonatal outcomes to the fetal risk predicted by the sgNIPT test was performed. A total of 42,067 pregnant individuals from 811 unique practices throughout 45 states and Puerto Rico underwent carrier screening. Of these, 7538 carriers (17.9%) reflexed to sgNIPT. Fetal/neonatal outcomes were obtained for 528 individuals, including 25 impacted pregnancies. High concordance was found between sgNIPT results and neonatal/fetal outcomes. The sgNIPT assay was found to have a sensitivity of 96% (95% CI: 79.65%–99.90%), specificity of 95.2% (95% CI: 92.98%–96.92%), average PPV of 50.0% (95% CI:35.23%–64.77%), and NPV of 99.8% (95% CI: 98.84%–99.99%). Overall performance of carrier screening with reflex to sgNIPT was determined to have a sensitivity of 92.4% and a specificity of 99.9%. These are not impacted by partner carrier screening or misattributed paternity, in contrast to the traditional carrier screening workflow, for which sensitivity is 35% and maximum PPV is 25%. Based on the results above, the authors assert that carrier screening with reflex to sgNIPT has good performance in a general population and should be considered as first line testing in many situations, including cases where biological partner sample is not available. They indicate that the test used was able to identify high-risk pregnancies related to autosomal recessive conditions with high sensitivity and specificity and has the potential to increase access to actionable health information. Noted limitations included collection of outcomes that relied on individual and provider reporting; of the over 42,000 individuals initially screened, outcomes were received for 528, including only 25 affected neonates. In addition, the authors of this study had affiliations with a test manufacturer who provided financial support for this study, creating a potential for bias.

Adams et al. (2023) conducted a clinical pilot study seeking to determine utility of single gene non-invasive prenatal screening (NIPS-SGD) in a group of high-risk pregnant individuals. The NIPS-SGD panel screened for pathogenic variants in 30 genes. Pregnant individuals qualifying for study participation had one or more of the following indications: (1) sperm age ≥ 40 years, (2) nuchal translucency ≥ 3.5 mm, (3) fetal anomaly, or (4) family history of a condition included for assessment in the panel. Participants were offered concurrent diagnostic testing. A total of 228 participants completed NIPS-SGD testing and of these, eight (3.5%) had a positive result. No false positive or negative results were identified in 78 participants who underwent diagnostic testing. Ultimately, 41 of the participating individuals received a molecular diagnosis, but 34 of these (82.9%) were outside the scope of the NIPS-SGD test. Positive results from the NIPS-SGD testing impacted medical management for five individuals. The researchers concluded that NIPS-SGD has the potential to detect prenatal diagnoses earlier, which may lead to better monitoring and focused genetic assessment, but diagnostic testing is still preferred when clinically indicated. Additional high-quality validation studies are needed to establish the value-add for NIPS-SGD before this testing can be implemented broadly.

To assess the performance of carrier screening for cystic fibrosis, hemoglobinopathies and spinal muscular atrophy with reflex single-gene noninvasive prenatal screening (sgNIPS), Hoskovec et al. (2023) conducted a study on an unselected population of 9,151 pregnant individuals in the United States. Screening for carrier status of noted conditions was performed, and 1,669 samples (18.2%) were found to be heterozygous for at least one pathogenic variant and reflexed to

sgNIPS. The results of sgNIPS were compared with outcomes identified via parent surveys or provider reports for 201 newborns. Overall, informative results were obtained for 98.7% of pregnancies including either negative carrier report or for those identified as heterozygous for a pathogenic variant, a sgNIPS result. In the subgroup with outcomes information, the NPV of sgNIPS was found to be 99.4% (95% CI = 96.0%-99.9%) and the average PPV of sgNIPS was 48.3% (95% CI = 36.1%-60.1%). A key factor was the use of personalized PPVs which accurately reflected the proportion of impacted pregnancies in each PPV range; all pregnancies where sgNIPS fetal risk was found to be > 9/10 (90% PPV) were indeed affected. The authors concluded that prenatal screening with sgNIPS is an option that can provide accurate fetal risk without a paternal screening test and results can be used for counseling and pregnancy management. The study had limitations, including the low number of outcomes collected and a relatively high rate of no-call results (1.3%), all of whom were heterozygous for a pathogenic variant. In addition, outcomes were determined via newborn screening and not through molecular diagnosis, which is the gold standard. Further research including larger cohorts and more complete collection of outcomes, as well as studies that focus on the impact of carrier screening with sgNIPS on clinical practice are needed.

Young et al. (2020) performed a retrospective analysis for non-invasive prenatal diagnosis (NIPD) single-gene testing for pregnancies at risk for cystic fibrosis (CF), spinal muscular atrophy (SMA) and X-linked Duchenne/Becker muscular dystrophies (DMD/BMD) by utilizing the relative haplotype dosage (RHDO) method. RHDO uses a capture-based targeted enhancement, followed by massive parallel sequencing and analysis by relative haplotype dosage. The requirements for NIPD using RHDO include known family history of disorder, confirmed molecular diagnosis, and necessary reference samples when possible. RHDO allows both paternal and maternal inheritance to be determined by measuring allelic imbalance between two haplotypes in cfDNA with phasing conducted through SNP sequencing; multiple single gene disorders can be performed during the same sequencing run and the same assay can be used for all families at risk for a particular condition which eliminates preliminary work-up prior to pregnancy. RHDO for DMD/BMD was performed in at-risk pregnancies following confirmation of a male fetus by cfDNA and required maternal haplotype with a male reference sample (previous affected, previous unaffected, other male relative affected, or unaffected maternal grandfather) providing the mutated or normal haplotype. Due to the 12% chance for DMD/BMD gene recombination, RHDO was performed 5'-3' and 3'-5'. For CF/SMA, maternal and paternal haplotypes in addition to a reference child (i.e., previously affected child, an unaffected non-carrier child) or a carrier child if parents had different mutations were used for phasing. From September 2016 to October 2019, 152 at-risk pregnancies were referred to the West Midlands Regional Genetics Laboratory. Follow-up genetic testing was performed for 70 of the 146 pregnancies for which a diagnostic result was issued. In all cases, follow-up testing confirmed the RHDO result and no discrepancies were reported demonstrating 100% concordance. For an additional 39 cases no postnatal discrepancies were reported as of the time of study publication. The authors concluded that NIPD by RHDO can be performed clinically for both autosomal recessive and X-linked disorders with a high sensitivity and specificity. However, this study is difficult to generalize to a non-selected population as the families selected needed to have a known family history of the disorder. In addition, this study had a lack of follow up data for many cases. In conclusion, the authors emphasized the importance of prenatal counseling for individuals undergoing NIPD for single gene disorders and having access to NIPT for routine aneuploidy screening at 10 weeks of pregnancy. Further testing is needed to validate this method for clinical use.

Zhang et al. (2019) developed and reported a clinical validation experience with a novel method for non-invasive prenatal sequencing for a panel of causative genes for frequently occurring monogenic, dominant disorders. Maternal cfDNA was barcoded and enriched and analyzed by NGS for target regions of 30 genes. Low level fetal variants were then determined by a statistical analysis adjusted for NGS read count and FF. Likely pathogenic and pathogenic variants were confirmed by a secondary amplicon-based cfDNA test. Clinical testing was performed on 422 pregnancies, with or without ultrasound findings. Of these 422 cases, 390 had negative testing and 32 had positive results. Follow-up testing on cases was limited and only included 233 of the 422 original cases. The researchers stated that this study revealed 20 true-positives, 127 true-negatives, zero false-positives and zero false-negatives. A significant limitation of this study was the lack of follow up data for many cases therefore the clinical sensitivity and specificity is limited to only cases with outcomes. The authors concluded that by using this novel NIPT NGS method, a large number of dominant, monogenic disorders can be identified however additional validation studies are needed.

Xiong et al. (2018) conducted a feasibility study on individuals of Southeast Asian descent to determine if targeted sequencing and relative mutation dosage can be used to correctly identify maternal beta-thalassemia mutations in cffDNA. Samples were collected from 49 couples at risk to have a child with beta-thalassemia, and genomic DNA was evaluated from the parents, cfDNA and either amniocentesis or CVS. Common *HBB* mutations were targeted using nested PCR. Relative mutation dosage was used to determine if the fetus had the wild type allele or the maternal carrier allele. Forty-eight of the samples were able to be classified using cffDNA (98%). The correct result was obtained in 44 of the 48 cases (91.7%), and there was one false positive and three false negatives. The overall sensitivity was 87.5% and the specificity was 95.8% for the inheritance of the maternal allele.

Clinical Practice Guidelines

American College of Medical Genetics and Genomics (ACMG)

AMCG published a guideline (Dungan et al., 2023) addressing the use of prenatal cfDNA screening for fetal chromosome abnormalities in general-risk populations. The guideline was largely based on the results of the 2022 ACMG systematic review (Rose et al.,) and used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Evidence to Decision framework to establish recommendations. The evidence reliably demonstrated better accuracy of prenatal cfDNA screening in comparison with traditional screening techniques for trisomies 13, 18, and 21 in both singleton and twin gestation pregnancies and noted that the identification of RATs and other microdeletion syndromes with prenatal cfDNA screening is "an emerging area of interest." Specific recommendations are as follows:

- Prenatal cfDNA screening for fetal trisomies 13, 18, and 21 is recommended over traditional screening methods for all pregnant patients with singleton gestation pregnancies. (Strong recommendation based on high certainty of evidence)
- Prenatal cfDNA screening for fetal trisomies is recommended over traditional screening methods for twin gestation pregnancies. (Strong recommendation based on high certainty of evidence)
- Prenatal cfDNA screening should be offered to individuals with single gestation pregnancies to assess for fetal SCA. (Strong recommendation, based on high certainty of evidence)
- ACMG suggests offering prenatal cfDNA screening for 22q11.2 deletion syndrome to all pregnant individuals. (Conditional recommendation, based on moderate certainty of evidence)
- Currently, there is insufficient evidence for the recommendation of routine screening for CNVs other than 22q11.2 deletions. (No recommendation, owing to lack of clinically relevant evidence and validation)
- Currently, there is insufficient evidence to either recommend, or not recommend, prenatal cfDNA screening for the identification of RATS. (No recommendation, owing to lack of clinically relevant evidence)

In addition to the above recommendations, the ACMG guideline indicates that the most frequent explanation for no-call results in prenatal cfDNA screening is insufficient FF. Low FF has been linked to varying adverse pregnancy outcomes, but definitive rates of pregnancy complications and standard monitoring practices have not been determined. ACMG also notes that certain pregnancy factors can interfere with the performance of prenatal cfDNA screening; vanishing twin syndrome is known example.

American College of Obstetricians and Gynecologists (ACOG)

ACOG's NIPT Summary of Recommendations includes the following:

- Prenatal genetic screening options including serum screening with or without nuchal translucency ultrasound or cfDNA screening and diagnostic testing with chorionic villus sampling (CVS) or amniocentesis should be discussed and offered to all pregnant individuals regardless of age or risk of chromosomal abnormality.
- If screening is accepted, individuals should have one prenatal screening approach; multiple screening tests should not be performed simultaneously.
- cfDNA is the most sensitive and specific screening test for the most common fetal aneuploidies. However the potential for false-positive and false-negative results exists. Importantly, cfDNA testing is not equivalent to diagnostic testing.
- Individuals whose screening tests are positive for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with opportunity for further diagnostic testing to confirm results.
- Individuals whose screening tests are negative should be informed that although this result substantially decreases their risk of the targeted aneuploidy, it does not ensure that the fetus is unaffected. Other genetic disorders that are not part of the screening/testing should be reviewed.
- In cases of cfDNA screening test results that are not reported by the laboratory or are uninterpretable (a no-call test result), individuals should be informed that test failure is associated with an increased risk of aneuploidy. They should undergo further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.
- cfDNA screening used as follow-up for individuals with a screen positive serum analyte screening test result is an option for individuals who want to avoid invasive diagnostic testing. Individuals must be informed that this approach could delay a definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.
- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be part of pretest counseling performed for individuals with multiple gestations. Overall, performance of screening for trisomy 21 by cfDNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. As such, it is difficult to determine an accurate DR for trisomy 18 and 13.
- Prenatal screening and prenatal diagnosis should be offered to all individuals regardless of previous preimplantation genetic testing, as preimplantation genetic testing is not uniformly accurate.
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, a significant risk of an inaccurate test result exists when serum-based aneuploidy screening or fetal cfDNA screening is used. In these cases, individuals should be counseled and diagnostic testing should be offered.

- When unusual or multiple aneuploidies are detected by cfDNA, affected individuals should be referred for genetic counseling and maternal–fetal medicine consultation. (ACOG, 2025)

In 2024, ACOG published a Clinical Practice Update to Practice Bulletin 192, Management of Alloimmunization in Pregnancy. The update addresses paternal and fetal genotyping in the management of pregnancy alloimmunization, stating that fetal antigen genotyping is recommended in situations where the paternal genotype is heterozygous or unknown. The Clinical Practice Update makes the following recommendations:

- "Paternal RHD zygosity testing using genotypic analysis is recommended for RhD alloimmunization risk assessment. It may be reasonable to defer or discontinue fetal surveillance for anemia in the setting of paternal genotyping that is RHD homozygous negative.
- Because cfDNA testing possesses performance characteristics that appear comparable with those of molecular testing, while avoiding the rare complications and costs associated with diagnostic genetic testing, it is reasonable to use it as an alternative tool for fetal RHD testing among alloimmunized patients with potentially at-risk pregnancies who decline amniocentesis.
- cfDNA for the assessment of selected non-RhD red blood cell antigens may be considered for pregnant patients declining amniocentesis, after weighing cost, access, and the encouraging-yet-limited data supporting its use."

An ACOG practice advisory recognizes the emerging technology and availability of cfDNA screening for single-gene disorders but emphasized that there is insufficient evidence to demonstrate accuracy and positive and NPV for general population use (ACOG, 2019; reaffirmed 2023). For this reason, ACOG does not recommend single gene cfDNA screening in pregnancy.

ACOG's Practice Bulletin 181 addressing the prevention of RhD alloimmunization (2017) notes that cfDNA testing is being used to determine fetal RhD status and select candidates for antenatal anti-D immune globulin prophylaxis and studies have shown high rates of sensitivity and specificity for fetal RhD status determination in the first trimester, however at current costs, noninvasive assessment of fetal RhD status is not recommended for routine use at this time.

American College of Obstetricians and Gynecologists (ACOG)/Society for Maternal-Fetal Medicine (SMFM)

Pregnancy at 35 years of age or older was the focus of Obstetric Care Consensus number 11, developed jointly by ACOG and SMFM (2023). In this consensus ACOG and SMFM recommend that prenatal genetic screening options (serum screening with or without nuchal translucency ultrasound or cfDNA screening) as well as diagnostic testing (chorionic villus sampling or amniocentesis) be discussed and offered to all pregnant individuals regardless of their risk of chromosomal abnormality or age. Each individual has the right to either pursue or decline genetic screening and diagnostic testing (GRADE 1A; strong recommendation, high-quality evidence).

In Practice Bulletin 231, ACOG and SMFM (2021) address prenatal screening for fetal chromosome abnormalities in multi fetal gestation pregnancies indicating that all women with multifetal gestations, regardless of age, are candidates for screening for fetal chromosome abnormalities, however, no method of fetal chromosome abnormality screening including serum samples will be as accurate in twin gestations as it is in singleton gestations. The bulletin asserts that it is important to include this information in the counseling process for patients with multiple gestation pregnancies. No data exists for serum screening for high-order multiple gestations (e.g., triplets or quadruplets) Analyzing risk vs. benefit for screening/diagnostic testing in individuals carrying multiple fetuses is complex. cfDNA screening can be performed in twin pregnancies, however because there is a smaller number of reported affected cases than in singleton pregnancies, it is challenging to determine accurate DR for trisomy 18 and 13. Since twin fetuses in a single pregnancy each contribute variable amounts of cfDNA, it's possible that a fetus with a chromosomal abnormality would contribute less fetal DNA, thereby masking the aneuploid test result. Though recent studies have suggested the sensitivity for trisomy 21 with cfDNA in twin pregnancies may be similar to singletons, there has been a higher rate of test failure in twins.

ACOG and SMFM addressed screening for fetal chromosomal abnormalities in ACOG Practice Bulletin Number 226. (2020)

Level A recommendations (based on good and consistent scientific evidence) regarding cfDNA include:

- Prenatal genetic screening (serum screening with or without nuchal translucency ultrasound or cfDNA screening) and diagnostic testing should be offered to all pregnant women regardless of maternal age or risk for chromosome abnormality.
- If screening is accepted, patients should only have one screening performed and not multiple screening tests performed simultaneously.

- cfDNA 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, cfDNA testing is not equivalent to diagnostic testing.
- Patients with positive screening should have genetic counseling, comprehensive ultrasound and be offered diagnostic testing.
- Patients whose cfDNA are not reportable are at increased risk for chromosomal aneuploidy and should be offered genetic counseling, comprehensive ultrasound and diagnostic testing.

Level B recommendations (based on limited or inconsistent scientific evidence) regarding cfDNA include:
- The use of cfDNA as follow-up for patients with a screen positive serum-analyte test results is an option for patients who want to avoid diagnostic testing.
- In situations of isolated, soft ultrasound markers and no prior screening has been performed cfDNA, quad screen or diagnostic testing should be offered.
- cfDNA screening can be performed for twin pregnancies. Overall performance of screening for trisomy 21 by cfDNA 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 DR for trisomy 18 and 13.
- Prenatal screening and prenatal diagnosis should be offered to all pregnant individuals regardless of previous preimplantation genetic testing.

Level C recommendations (primarily based on consensus/expert opinion) regarding cfDNA include:
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cfDNA is used. In these situations, the pregnant individual should be counseled on this information and offered diagnostic testing.
- Patients with unusual or multiple aneuploidies detected by cfDNA should be referred for genetic counseling.

In addition, it is noted in this bulletin that although screening for a limited number of microdeletions with cfDNA is available, this testing has not been clinically validated and is not recommended. With regard to CNV testing, the practice bulletin states there is currently no genetic screening test available to comprehensively screen for all CNVs. Another type of testing offered by some laboratories includes genome-wide cfDNA screening for large deletions and duplications, analyzing the whole genome to potentially detect abnormalities larger than what cfDNA microdeletion screening can accomplish. This type of testing is not recommended by ACOG/SMFM as it has not been clinically validated and accuracy of detection/false-positive rates have not been determined. Lastly, the bulletin notes that “although repeat screening may be considered in the setting of a sample drawn at an early gestational age or a specific concern regarding sample characteristics, because repeat sampling delays a diagnostic test, it is not advised if screening results are consistent with sonographic anomalies, or if a patient is at a gestational age at which the delay may compromise their reproductive options.”

American Society of Human Genetics (ASHG)/European Society of Human Genetics (ESHG)

In a joint statement, ASHG and ESHG present different scenarios for NIPT-based screening for common autosomal aneuploidies. The statement suggests that trade-offs involved in these scenarios should be assessed in light of the aim of screening, the balance of benefits and burdens for pregnant women and their partners. The statement includes the following recommendations:
- NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as combined first-trimester screening. However, a positive NIPT result should not be regarded as a final diagnosis: false positives occur for a variety of reasons. Women should be advised to have a positive result confirmed through diagnostic testing if they are considering a possible termination of pregnancy.
- Expanding NIPT-based prenatal screening to also report on sex chromosomal abnormalities and microdeletions is not recommended.

When the test is assessed in terms of its predictive value, the low prevalence of the relevant conditions in the target population must be taken into consideration. For instance, the PPV in a general risk population can result in a higher rate of false alarms because of the lower prevalence of aneuploidies in this population. (Dondorp et al., 2015)

International Society for Prenatal Diagnosis (ISPD)

To update information regarding current technologies, implementation practices and clinical experiences, the ISPD published a new position statement on the use of NIPT for fetal chromosomal conditions in 2023. The consensus position offered by the ISPD is summarized as follows:

- NIPT is the most accurate screening test for the common autosomal aneuploidies (trisomies 21, 13 and 18) in unselected singleton populations, and those at known increased probability. It can be offered in primary or contingent screening models with context-specific considerations in local health policy influencing decisions and implementation models.
- False-positive results occur with NIPT. Therefore, ISPD strongly recommends that all patients with a high chance of NIPT result have genetic counseling and are offered diagnostic testing, particularly if the termination of pregnancy is being considered.
- Fetal fraction is an important quality control metric, but substantial variation exists between laboratories and test methodologies. Laboratories should perform their own internal validation of their limit of detection and threshold for 'no call' results.
- Providers (laboratory and clinicians) should have established clinical pathways for the management of patients with a "no call" result. This may include detailed ultrasound, offer of repeat NIPT, alternative screening test, and/or diagnostic testing.
- If technically relevant, protocols for the identification and disclosure of suspected malignancy should be developed by laboratories.
- NIPT for SCA is sufficiently accurate to be offered alongside autosomal aneuploidy screening with specific pretest counseling and consent. However, other societal, economic, cultural and ethical factors may need to be considered in health policy decisions regarding population-based screening for the sex chromosomes.
- There is insufficient data to assess the performance and clinical utility of routine NIPT for RATs, sub-chromosomal imbalances and microdeletion/duplication syndromes. Further research is required to evaluate these applications of NIPT, but if offered as part of local practice there should be protocols in place to manage high-risk results and detailed platform-specific counseling available both pre- and post-testing.
- At least one early first trimester scan for dating, diagnosis of multiple pregnancy, and confirmation of fetal viability should be offered before performing NIPT.
- Fetuses with ultrasound abnormalities, including NT measurement ≥ 3.5 mm, should be offered diagnostic testing and evaluation with chromosomal microarray regardless of the prior NIPT result.
- The ethical implementation of NIPT requires attention to provision of quality pre-testing counseling, equity of access, and access to appropriate downstream clinical services.
- All stakeholders, including healthcare consumers, should be involved in determining local implementation models and future directions for NIPT. (Hui et al., 2023)

National Institute for Health and Care Excellence (NICE)

A 2016 (updated 2021) NICE guideline (DG25) recommends the use of high-throughput cfDNA testing for fetal RhD genotype to guide decisions regarding antenatal prophylaxis with anti-D immunoglobulin (dependent on a cost threshold) to reduce unnecessary use of blood products in pregnant woman. This recommendation was based on a comprehensive review of studies that assessed diagnostic accuracy, assessment of clinical outcomes, evaluation of implementation issues, and cost effectiveness.

National Society of Genetic Counselors (NSGC)

In a 2021 position statement, the NSGC states its belief that all pregnant individuals, regardless of aneuploidy risk, should have access to prenatal screening using cfDNA. They recommend that healthcare providers present cfDNA for aneuploidy as a topic within the context of other prenatal screening and testing options, including the option of pursuing diagnostic testing as a first-line approach or declining any screening or testing altogether. Discussions should also include individual preferences, values and needs as well as the limitations and the benefits of genetic screening with cfDNA. They further recommend careful consideration of the test's PPV, particularly in rare disorders.

U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

Laboratories that perform DNA-based tests are regulated by the FDA under the Clinical Laboratory Improvement Amendments. Refer to the following website for more information: https://www.fda.gov/medical-devices/ivd-regulatory-assistance/clinical-laboratory-improvement-amendments-clia. (Accessed March 3, 2025)

A list of nucleic acid-based tests that have been cleared or approved by the FDA Center for Devices and Radiological Health is available at: https://www.fda.gov/medical-devices/in-vitro-diagnostics/nucleic-acid-based-tests. (Accessed March 4, 2025)

References

The foregoing Oxford policy has been adapted from an existing UnitedHealthcare national policy that was researched, developed and approved by UnitedHealthcare Medical Technology Assessment Committee. [2025T0560HH]

Acreman ML, Bussolaro S, Raymond YC, et al. The predictive value of prenatal cell-free DNA testing for rare autosomal trisomies: a systematic review and meta-analysis. Am J Obstet Gynecol. 2023 Mar;228(3):292-305.e6.

Adams S, Llorin H, Maher O, et al. Single gene non-invasive prenatal screening for autosomal dominant conditions in a high-risk cohort. Prenat Diagn. 2023 Aug;43(9):1110-1119.

Alford B, Landry BP, Hou S, et al. Validation of a non-invasive prenatal test for fetal RhD, C, c, E, K and Fya antigens. Sci Rep. 2023 Aug 7;13(1):12786.

Allyse MA, Wick MJ. Noninvasive Prenatal Genetic Screening Using Cell-free DNA. JAMA. 2018 Aug 14;320(6):591-592.

Alshehri AA, Jackson DE. Non-invasive prenatal fetal blood group genotype and its application in the management of hemolytic disease of fetus and newborn: systematic review and meta-analysis. Transfus Med Rev. 2021 Apr;35(2):85-94.

American College of Obstetricians and Gynecologists (ACOG). Clinical Practice Update. Paternal and fetal genotyping in the management of alloimmunization in pregnancy. Available at: https://journals.lww.com/greenjournal/abstract/2024/08000/acog_clinical_practice_update__paternal_and_fetal.34.aspx. Accessed February 20, 2025.

American College of Obstetricians and Gynecologists (ACOG) Dictionary. 2024. Available at: https://www.acog.org/womens-health/dictionary. Accessed March 4, 2025.

American College of Obstetricians and Gynecologists (ACOG). FAQ 165. Prenatal genetic screening tests. Last updated October 2020; reviewed November 2023. Available at: https://www.acog.org/womens-health/faqs/prenatal-genetic-screening-tests. Accessed February 28, 2025.

American College of Obstetricians and Gynecologists (ACOG). NIPT summary of recommendations. 2025. Available at: https://www.acog.org/advocacy/policy-priorities/non-invasive-prenatal-testing/current-acog-guidance. Accessed March 4, 2025.

American College of Obstetricians and Gynecologists' Committee on Clinical Consensus-Obstetrics; Gantt A; Society for Maternal-Fetal Medicine; Metz TD, Kuller JA, Louis JM; Society for Maternal-Fetal Medicine; Cahill AG, Turrentine MA; American College of Obstetricians and Gynecologists. Obstetric Care Consensus #11, Pregnancy at age 35 years or older. Am J Obstet Gynecol. 2023 Mar;228(3):B25-B40.

American College of Obstetricians and Gynecologists (ACOG). Practice Advisory: Cell-free DNA to screen for single-gene disorders. February 2019; 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. Accessed March 4, 2025.

American College of Obstetricians and Gynecologists (ACOG). Practice Bulletin No. 181. Prevention of RhD alloimmunization. Obstet Gynecol. 2017 August;130(2):e57-70. Reaffirmed 2024.

American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics; Committee on Genetics; Society for Maternal-Fetal Medicine. Screening for fetal chromosomal abnormalities: ACOG practice bulletin, number 226. Obstet Gynecol. 2020 Oct;136(4):e48-e69.

American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics, Society for Maternal-Fetal Medicine. Multifetal gestations: twin, triplet, and higher-order multifetal pregnancies: ACOG Practice Bulletin, Number 231. Obstet Gynecol. 2021 Jun 1;137(6):e145-e162.

Brison N, Neofytou M, Dehaspe L, et al. Predicting fetoplacental chromosomal mosaicism during non-invasive prenatal testing. Prenat Diagn. 2018 Mar;38(4):258-266.

Clausen FB, van der Schoot CE. Noninvasive fetal blood group antigen genotyping. Blood Transfus. 2024 Jan 29. Epub ahead of print.

Cui W, Liu X, Zhang Y, et al. Evaluation of non-invasive prenatal testing to detect chromosomal aberrations in a Chinese cohort. J Cell Mol Med. 2019; 23:7873-7878.

Dar P, Jacobsson B, Clifton R, et al. Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome. Am J Obstet Gynecol. 2022 Jan 13:S0002-9378(22)00006-0.

De Paepe, M. Creasy and Resnik's Maternal-Fetal Medicine. St. Louis, MO: Elsevier c2023. Chapter 5, Multiple Gestation: The Biology of Twinning; p. 72.

Dondorp W, de Wert G, Bombard Y, et al.; European Society of Human Genetics.; American Society of Human Genetics. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Eur J Hum Genet. 2015 Nov;23(11):1438-50.

Dungan JS, Klugman S, Darilek S, et al.; ACMG Board of Directors. 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 Feb;25(2):100336.

Fiorentino F, Bono S, Pizzuti F, et al. The clinical utility of genome-wide noninvasive prenatal screening. Prenat Diagn. 2017 Jun;37(6):593-601.

Gilstrop Thompson M, Xu W, Moore B, et al. Clinical validation of a prenatal cell-free DNA screening test for fetal RHD in a large U.S. cohort. Obstet Gynecol. 2025 Feb 1;145(2):211-216.

Gross SJ, Stosic M, McDonald-McGinn DM, et al. Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. Ultrasound Obstet Gynecol. 2016 Feb;47(2):177-183.

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 Jul;44(8):925-935.

Hayes, Inc. Clinical Utility Evaluation. Cell-free DNA (cfDNA) (formerly NIPS, NIPT) screening for fetal chromosomal copy number variants. Hayes, Inc.; February 23, 2022,updated February 19, 2025.

Hayes, Inc. Clinical Utility Evaluation. Cell-free DNA (cfDNA) [formerly NIPS, NIPT] screening for fetal rare autosomal trisomies. Hayes, Inc.; December 2021, updated December 9, 2024.

Hayes, Inc. Clinical Utility Evaluation. Fetal RhD noninvasive prenatal testing. Hayes, Inc.; December 16, 2024.

Helgeson J, Wardrop J, Boomer T, et al. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. Prenat Diagn. 2015;35:999-1004.

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 Feb;25(2):100334.

Hui L, Ellis K, Mayen D, et al. Position statement from the International Society for Prenatal Diagnosis on the use of non-invasive prenatal testing for the detection of fetal chromosomal conditions in singleton pregnancies. Prenat Diagn. 2023 Jun;43(7):814-828.

Konya M, Czimbalmos A, Loczi L, et al. Genome-wide, non-invasive prenatal testing for rare chromosomal abnormalities: a systematic review and meta-analysis of diagnostic test accuracy. PLoS One. 2024 Nov 5;19(11):e0308008.

Liu Y, Liu S, Liu J, et al. Identification of copy number variations among fetuses with isolated ultrasound soft markers in pregnant women not of advanced maternal age. Orphanet J Rare Dis. 2024 Feb 10;19(1):56.

Mackie FL, Hemming K, Allen S, et al. The accuracy of cell-free fetal DNA-based noninvasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. BJOG. 2017 Jan;124(1):32-46.

Manfroi S, Calisesi C, Fagiani P, et al. Prenatal non-invasive foetal RHD genotyping: diagnostic accuracy of a test as a guide for appropriate administration of antenatal anti-D immunoprophylaxis. Blood Tranfus 2018 Nov; 16(6):514-524.

Maya I, Salzer Sheelo L, Brabbing-Goldstein D, et al. Clinical utility of expanded non-invasive prenatal screening compared with chromosomal microarray analysis in over 8000 pregnancies without major structural anomaly. Ultrasound Obstet Gynecol. 2023 Jun;61(6):698-704.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); [updated 2020 Jun 24]. What are whole exome sequencing and whole genome sequencing? [updated 2021c Jul 28; reviewed 2021 Jul 28]. Available at: https://medlineplus.gov/genetics/understanding/testing/sequencing/. Accessed March 4, 2025.

Mossfield T, Soster E, Menezes M, et al.; Global Expanded NIPT Consortium. Multisite assessment of the impact of cell-free DNA-based screening for rare autosomal aneuploidies on pregnancy management and outcomes. Front Genet. 2022 Aug 29;13:975987.

National Institute for Health and Care Excellence (NICE). High-throughput non-invasive prenatal testing for fetal RHD genotype. Diagnostics Guidance (DG25). November 9, 2016 (updated 2021).

National Society of Genetic Counselors (NSGC). Position statement. Prenatal cell-free DNA screening. April 2021. Available at: https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/prenatal-cell-free-dna-screening-1. Accessed March 4, 2025.

Ontario Health (Quality). Noninvasive Fetal RhD Blood Group Genotyping: A Health Technology Assessment. Ont Health Technol Assess Ser. 2020 Nov 2;20(15):1-160.

Pertile MD, Halks-Miller M, Flowers N, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. Sci Transl Med. 2017 Aug 30;9(405).

Ravi H, McNeill G, Goel S, et al. Validation of a SNP-based noninvasive prenatal test to detect the fetal 22q11.2 deletion in maternal plasma samples. PLoS One. 2018 Feb 23;13(2):e0193476.

Rego S, Ashimi Balogun O, Emanuel K, et al. Cell-free DNA analysis for the determination of fetal red blood cell antigen genotype in individuals with alloimmunized pregnancies. Obstet Gynecol. 2024 Oct 1;144(4):436-443.

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 Jul;24(7):1379-1391.

Saramago P, Yang H, Llewellyn A, et al. High-throughput noninvasive prenatal testing for fetal rhesus D status in RhD-negative women not known to be sensitised to the RhD antigen: a systematic review and economic evaluation. Health Technol Assess. 2018 Mar;22(13):1-172.

Scott F, Bonifacio M, Sandow R, et al. Rare autosomal trisomies: important and not so rare. Prenat Diagn. 2018 Sep;38(10):765-771.

Soster E, Mossfield T, Menezes M, et al.; Global Expanded NIPT Consortium. Clinical outcomes of screen-positive genome-wide cfDNA cases for trisomy 20: results from the global expanded NIPT Consortium. Mol Cytogenet. 2024 Apr 16;17(1):9.

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 Jun 10;16(1):10.

South ST, Lee C, Lamb AN, et al.; Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. Genet Med. 2013 Nov;15(11):901-9.

van der Meij KRM, Sistermans EA, Macville MVE, et al. TRIDENT-2: national implementation of genome-wide non-invasive prenatal testing as a first-tier screening test in the Netherlands. Am J Hum Genet. 2019;105(6):1091-1101.

Van Opstal D, van Maarle MC, Lichtenbelt K, et al. Origin and clinical relevance of chromosomal aberrations other than the common trisomies detected by genome-wide NIPS: results of the TRIDENT study. Genet Med. 2018 Apr;20(5):480-485.

Wan J, Li R, Zhang Y, et al. Pregnancy outcome of autosomal aneuploidies other than common trisomies detected by noninvasive prenatal testing in routine clinical practice. Prenat Diagn. 2018 Oct;38(11):849-857.

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 Sep;43(10):1344-1354.

Xiong L, Barrett AN, Hua R, et al. Noninvasive prenatal testing for fetal inheritance of maternal β-thalassaemia mutations using targeted sequencing and relative mutation dosage: a feasibility study. BJOG. 2018 Mar;125(4):461-468.

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 Nov 17;12(1):19750.

Xue Y, Zhao G, Li H, et al. Non-invasive prenatal testing to detect chromosome aneuploidies in 57,204 pregnancies. Mol Cytogenet. 2019 Jun 20;12:29.

Yan X, Ding K, Zhang X, et al. Analysis of prenatal diagnosis and pregnancy outcomes for rare autosomal trisomies detected by non-invasive prenatal testing in 33,079 cases. BMC Med Genomics. 2025 Feb 7;18(1):29.

Yang H, Llewellyen A, Walker R, et al. High-throughput, non-invasive prenatal testing for fetal rhesus D status in RhD-negative women: A systematic review and meta-analysis. BMC Med. 2019; 17;37. PMID: 30760268.

Yang L, Bu G, Ma Y, et al. Comparison of noninvasive prenatal screening for defined pathogenic microdeletion/microduplication syndromes and nonsyndromic copy number variations: a large multicenter study. J Comp Eff Res. 2022 Dec;11(17):1277-1291.

Yang S, Zhuang Y, Li J, et al. Expanded non-invasive prenatal testing offers better detection of fetal copy number variations but not chromosomal aneuploidies. PLoS One. 2025 Jan 24;20(1):e0312184.

Young E, Bowns B, Gerrish A, et al. Clinical service delivery of noninvasive prenatal diagnosis by relative haplotype dosage for single-gene disorders. J Molec Diagn. 2020; 22(9): 1151-1161.

Zhang J, Li J, Saucier JB, et al. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free DNA. Nat Med. 2019; 25:439-447.

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 May;284:5-11.

Zou Y, Feng C, Qin J, et al. Performance of expanded non-invasive prenatal testing for fetal aneuploidies and copy number variations: A prospective study from a single center in Jiangxi province, China. Front Genet. 2023 Jan 13;13:1073851.

Policy History/Revision Information

Date	Summary of Changes

06/01/2025	**Coverage Rationale**
	• Added language to indicate Cell-Free Fetal DNA testing using maternal plasma to determine fetal genotype is proven and medically necessary when the individual undergoing testing is alloimmunized or at risk for alloimmunization due to maternal RhD status or the presence of red cell antigen antibodies, and all of the following:
	  ○ Paternal genotyping shows heterozygosity for RhD or paternal RhD status is unknown
	  ○ Indicated invasive diagnostic testing [e.g., amniocentesis, chorionic villus sampling (CVS)] for fetal genotyping has been offered and declined
	• Removed language indicating:
	  ○ DNA-based noninvasive prenatal tests of fetal Aneuploidy are proven and medically necessary as screening tools for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) or trisomy 13 (Patau syndrome), with or without fetal sex chromosomes, for individuals with a singleton or twin pregnancy in any one of the following circumstances:
	  ▪ Birthing person aged 35 years or older at delivery and/or donor oocyte aged 35 years or older
	  ▪ Fetal ultrasound findings indicating an increased risk of Aneuploidy
	  ▪ History of a prior pregnancy with a trisomy due to translocation
	  ▪ Positive first- or second-trimester screening test results for Aneuploidy
	  ▪ Parental balanced Robertsonian translocation with an increased risk of fetal trisomy 13 or trisomy 21
	  ▪ Screening after pre-test counseling from a board-certified genetic counselor or from the prenatal care physician or healthcare professional using shared decision-making (SDM)
	  ○ Vanadis® is unproven and not medically necessary due to insufficient evidence of efficacy
	• Replaced language indicating:
	  ○ "*DNA-based noninvasive prenatal tests are* unproven and not medically necessary for *all other* indications *[not listed in the policy as proven and medically necessary]*; including [the listed indications] due to insufficient evidence of efficacy" with "*Cell-Free Fetal DNA testing using maternal plasma is considered* unproven and not medically necessary for indications *beyond screening for trisomies 21, 18, 13, and sex chromosome aneuploidy; this includes but is not limited to* the [listed indications] due to insufficient evidence of efficacy"
	• Revised list of unproven and not medically necessary indications for Cell-Free Fetal DNA testing using maternal plasma:
	  ○ Added "Cell-Free Fetal DNA expanded panel testing (panels that include testing beyond trisomies 21, 18, 13, and sex chromosome aneuploidy)"
	  ○ Removed:
	  ▪ Screening for:
	  – Aneuploidy other than trisomies 21, 18, 13, or sex chromosomes
	  – Fetal RhD
	  ▪ For the sole purpose of determining the sex of the fetus unless the determination of fetal sex is essential to the diagnosis of a condition
	  ▪ Repeat testing due to low fetal fraction
	  ▪ Pregnancies involving one or more of the following:
	  – Three or more fetuses
	  – Missed abortion or fetal demise in a single or multiple gestation pregnancy
	  – Vanishing twin syndrome

Date	Summary of Changes
	○ Replaced “screening for fetal antigen status” with “screening for fetal antigen status *other than RhD*”
	Medical Records Documentation Used for Review
	• Updated list of Medical Records Documentation Used for Reviews: ○ Added: ▪ Individual undergoing testing is alloimmunized or at risk for alloimmunization due to maternal RhD status, including presence or absence of red cell antigen antibodies ▪ Paternal genotyping shows heterozygosity for RhD or paternal RhD status is unknown ▪ Member has been offered and declined invasive diagnostic testing [e.g., amniocentesis, chorionic villus sampling (CVS)] for fetal genotype ○ Removed: ▪ Maternal age ▪ History of prior pregnancy with a trisomy, if applicable ▪ History of parental balanced Robertsonian translocation ▪ Abnormal first- or second-trimester screening test result ▪ Counseling provided by genetic counselor or prenatal provider on the risks and benefits of testing using shared decision making
	Definitions
	• Removed definition of: ○ Massively Parallel Sequencing (MPS) ○ Mosaicism ○ Next Generation Sequencing (NGS) ○ Shared Decision-Making (SDM) ○ Single Nucleotide Polymorphisms (SNPs) ○ Trisomy 13 (Patau Syndrome) ○ Trisomy 18 (Edwards Syndrome) ○ Trisomy 21 (Down Syndrome)
	Applicable Codes
	• Removed CPT codes 0327U, 81420, and 81507 • Removed list of applicable ICD-10 diagnosis codes: O09.00, O09.01, O09.02, O09.03, O09.10, O09.11, O09.12, O09.13, O09.211, O09.212, O09.213, O09.219, O09.291, O09.292, O09.293, O09.299, O09.30, O09.31, O09.32, O09.33, O09.40, O09.41, O09.42, O09.43, O09.511, O09.512, O09.513, O09.519, O09.521, O09.522, O09.523, O09.529, O09.611, O09.612, O09.613, O09.619, O09.621, O09.622, O09.623, O09.629, O09.70, O09.71, O09.72, O09.73, O09.811, O09.812, O09.813, O09.819, O09.821, O09.822, O09.823, O09.829, O09.891, O09.892, O09.893, O09.899, O09.90, O09.91, O09.92, O09.93, O09.A0, O09.A1, O09.A2, O09.A3, O26.20, O26.21, O26.22, O26.23, O26.841, O26.842, O26.843, O26.849, O26.851, O26.852, O26.853, O26.859, O26.891, O26.892, O26.893, O26.899, O26.90, O26.91, O26.92, O26.93, O28.0, O28.1, O28.2, O28.3, O28.4, O28.5, O28.8, O28.9, O30.001, O30.002, O30.003, O30.009, O30.011, O30.012, O30.013, O30.019, O30.021, O30.022, O30.023, O30.029, O30.031, O30.032, O30.033, O30.039, O30.041, O30.042, O30.043, O30.049, O30.091, O30.092, O30.093, O30.099, O35.00X0, O35.01X0, O35.02X0, O35.03X0, O35.04X0, O35.05X0, O35.06X0, O35.07X0, O35.08X0, O35.09X0, O35.10X0, O35.11X0, O35.12X0, O35.13X0, O35.14X0, O35.15X0, O35.19X0, O35.AXX0, O35.BXX0, O35.CXX0, O35.DXX0, O35.EXX0, O35.FXX0, O35.GXX0, O35.HXX0, O35.2XX0, O99.210, O99.211, O99.212, O99.213, O99.280, O99.281, O99.282, O99.283, O99.284, O99.285, O99.310, O99.311, O99.312, O99.313, O99.320, O99.321, O99.322, O99.323, O99.330, O99.331, O99.332, O99.333, O99.340, O99.341, O99.342, O99.343, O99.810, O99.814, Q95.0, Q95.1, Q95.2, Q95.3, Q95.5, Q95.8, Q95.9, Z34.00, Z34.01, Z34.02, Z34.03, Z34.80, Z34.81, Z34.82, Z34.83, Z34.90, Z34.91, Z34.92, Z34.93, Z36.0, Z36.1, Z36.2, Z36.3, Z36.4, Z36.5, Z36.81, Z36.82, Z36.83, Z36.89, Z36.8A, Z36.9, Z3A.09, Z3A.10, Z3A.11, Z3A.12, Z3A.13, Z3A.14, Z3A.15, Z3A.16, Z3A.17, Z3A.18, Z3A.19, Z3A.20, Z3A.21, Z3A.22, Z3A.23, Z3A.24, Z3A.25, Z3A.26, Z3A.27, Z3A.28, Z3A.29, Z3A.30, Z3A.31, Z3A.32, Z3A.33, Z3A.34, Z3A.35, Z3A.36, Z3A.37, Z3A.38, Z3A.39, Z3A.40, Z3A.41, Z3A.42, and Z3A.49
	Supporting Information
	• Updated *Description of Services*, *Clinical Evidence*, *FDA*, and *References* sections to reflect the most current information • Archived previous policy version MATERNITY 025.34

Instructions for Use

This Clinical Policy provides assistance in interpreting UnitedHealthcare Oxford standard benefit plans. When deciding coverage, the member specific benefit plan document must be referenced as the terms of the member specific benefit plan may differ from the standard plan. In the event of a conflict, the member specific benefit plan document governs. Before using this policy, please check the member specific benefit plan document and any applicable federal or state mandates. UnitedHealthcare Oxford reserves the right to modify its Policies as necessary. This Clinical Policy is provided for informational purposes. It does not constitute medical advice.

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