Prenatal Screening; Cell-Free Fetal DNA Testing

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DESCRIPTION

National guidelines recommend that all pregnant individuals will be offered screening for fetal chromosomal abnormalities, most of which are aneuploidies, referring specifically to T21, T18 and T13 before 20 weeks of gestation, regardless of age. There are numerous limitations to standard screening for these disorders using maternal serum and fetal ultrasound. Noninvasive prenatal screening (NIPS) analyzing cell-free fetal DNA in maternal serum is a potential complement or alternative to conventional serum screening. Noninvasive prenatal screening (NIPS) using cell-free fetal DNA has also been proposed to screen for microdeletions and sex chromosome aneuploidies. Prenatal testing for twin zygosity using cell-free fetal DNA has been proposed to inform decisions about early surveillance for twin-twin transfusion syndrome and other monochorionic twin-related abnormalities.

Fetal Aneuploidy
Fetal chromosomal abnormalities occur in approximately 1 in 160 live births. Most fetal chromosomal abnormalities are aneuploidies, defined as an abnormal number of chromosomes, which are the structures that contain genetic information. The trisomy syndromes are aneuploidies involving 3 copies of 1 chromosome. The most important risk factor for trisomy syndromes is maternal age. The approximate risk of trisomy 21 (T21 Down syndrome) affected birth is 1 in 1100 at age 25 to 29. The risk of fetus with T21 (at 16 weeks gestation) is about 1 in 250 at age 35 and 1 in 75 at age 40.

Trisomy 21 (T21) Down syndrome is the most common chromosomal aneuploidy and is the driving force for current maternal serum screening programs. Other trisomy syndromes include T18 (Edwards syndrome) and T13 (Patau syndrome), which are the next most common forms of fetal aneuploidy, although the percentage of cases surviving to birth is low and survival beyond birth is limited. The prevalence of these other aneuploidies is much lower than the prevalence of T21 and identifying them is not currently the main intent of prenatal screening programs. Also, the clinical implications of identifying T18 and T13 are unclear because survival beyond birth is limited for both conditions.

Fetal Aneuploidy Screening
Standard aneuploidy screening involves combinations of maternal serum markers and fetal ultrasound done at various stages of pregnancy. The detection rate for various combinations of noninvasive testing ranges from 60% to 96% when the false-positive rate is set at 5%. When tests indicate a high risk of trisomy syndrome, direct karyotyping of fetal tissue obtained by amniocentesis or chronic villous sampling (CVS) is required to confirm that T21 or another trisomy is present. Both amniocentesis and CVS are invasive procedures and have procedure-associated risk of fetal injury, fetal loss (miscarriage) and infection. A new screening strategy that reduces unnecessary amniocentesis and CVS procedures and increases detection of T21, T18 and T13 could improve outcomes. Confirmation of positive noninvasive screening tests with amniocentesis or CVS is recommended; with more accurate tests, this may reduce the need for invasive testing and associated morbidities.

Commercial, noninvasive, sequencing based testing of maternal serum for fetal trisomy syndromes is now available. The test technology involves detection of cell-free fetal DNA fragments present in the plasma of pregnant women. As early as 8 to 10 weeks of gestation, these fetal DNA fragments comprise 6% to 10% or more of the total cell-free fetal DNA in a maternal plasma sample. The test is unable to provide a result if fetal fraction is too low, that is, below 4%. Fetal fraction can be affected by maternal and fetal characteristics. For example, fetal fraction was found to be lower at higher maternal weights and higher with increasing fetal crown-rump length.

Twin Zygosity Testing
Twin gestations occur in approximately 1 in 30 live births in the United States and have a 4 to10 fold increased risk of perinatal complications. Dizygotic or "fraternal" twins occur

from ovulation and fertilization of 2 oocytes, which results in dichorionic (DC) placentation and 2 separate placentas. In contrast to DC twins, MC twin pregnancies share their blood supply. Monochorionic (MC) twins account for about 20% of twin gestations and are at higher risk of structural defects, miscarriage, preterm delivery, and selective fetal growth restriction compared to DC twins. Up to 15% of MC twin pregnancies are affected by twin-to-twin transfusion syndrome (TTTS), a condition characterized by relative hypovolemia of 1 twin and hypervolemia of the other. According to estimates from live births, TTTS occurs in up to 15% of MC twin pregnancies. In these twin pregnancies, serial fetal ultrasound examinations are necessary to monitor for development of TTTS as well as selective intrauterine growth restriction because these disorders have high morbidity and mortality and are amenable to interventions that can improve outcomes. Noninvasive prenatal testing (NIPT) using cell-free fetal DNA to determine zygosity in twin pregnancies could potentially inform decisions about early surveillance for TTTS and other MC twin-related abnormalities. In particular, determining zygosity with NIPT could potentially assist in the assessment of chorionicity when ultrasound findings are not clear.

Cell-Free Fetal DNA Analysis Methods

Sequencing-based tests use one of two general approaches to analyzing cell-free fetal DNA. The first category of tests uses quantitative or counting methods. The most widely used technique to date uses massively parallel sequencing (MPS; also known as next-generation or "next gen" sequencing). DNA fragments are amplified by polymerase chain reaction; during the sequencing process, the amplified fragments are spatially segregated and sequenced simultaneously in a massively parallel fashion. Sequenced fragments can be mapped to the reference human genome to obtain numbers of fragment counts per chromosome. The sequencing-derived percent of fragments from the chromosome of interest reflects the chromosomal representation of the maternal and fetal DNA fragments in the original maternal plasma sample. Another technique is direct DNA analysis, which analyzes specific cell-free DNA fragments across samples and requires approximately a tenth the number of cell-free DNA fragments as MPS. The digital analysis of selected regions (DANSR™) is an assay that uses direct DNA analysis.

The second general approach is single nucleotide variant-based methods. This uses targeted amplification and analysis of approximately 20,000 single nucleotide variants on selected chromosomes (e.g., 21, 18, 13) in a single reaction. A statistical algorithm is used to determine the number of each type of chromosome. Some of the commercially available cell-free fetal DNA prenatal tests also test for other abnormalities including sex chromosome abnormalities and selected microdeletions.

A newer approach to cell free DNA testing called the Vanadis NIPT does not involve amplification or sequencing. The assay uses maternal serum and applies a series of enzymes to create labelled rolling circle replication products (RCPs) from chromosomal cell-free DNA targets, which are then converted to fluorescent DNA molecules and labeled with chromosome-specific fluorophores. The labeled fluorescent DNA molecules are deposited to a microfilter plate and counted with an automated imaging device. The

ratio between the number of each chromosome-specific fluorescent DNA molecules is transferred for risk calculation to proprietary software to calculate the likelihood of a trisomy. Currently, Vanadis NIPT provides results for trisomy 21, trisomy 18 and trisomy 13; although, additional aneuploidies and microdeletions might be added in the future.

Copy Number Variants and Clinical Disorders

Microdeletions (also known as submicroscopic deletions) are chromosomal deletions that are too small to be detected by microscopy or conventional cytogenetic methods. They can be as small as 1 and 3 megabases long. Along with microduplications, microdeletions are collectively known as copy number variants. Copy number variants can lead to disease when the change in copy number of a dose-sensitive gene or genes disrupts the ability of the gene(s) to function and affects the amount of protein produced. A number of genomic disorders associated with microdeletion have been identified, which may be associated with serious clinical features, such as cardiac anomalies, immune deficiency, palatal defects, and developmental delay as in DiGeorge syndrome. Some of the syndromes (e.g., DiGeorge) have complete penetrance yet marked variability in clinical expressivity. A contributing factor is that the breakpoints of the microdeletions may vary, and there may be a correlation between the number of haploinsufficient genes and phenotypic severity.

A proportion of microdeletions are inherited, and some are de novo. Accurate estimates of the prevalence of microdeletion syndromes during pregnancy or at birth are not available. The risk of a fetus with a microdeletion syndrome is independent of maternal age. There are few population-based data and most studies published to date have based estimates on phenotypic presentation. The 22q11.2 (DiGeorge) microdeletion is the most common associated with a clinical syndrome. Table 1 provides prevalence estimates for the most common microdeletion syndromes. These numbers likely underestimate the prevalence of these syndromes in the prenatal population because the population of variant carriers includes phenotypically normal or very mildly affected individuals.

Table 1. Recurrent Microdeletion Syndromes

Syndrome	Location	Estimated Prevalence
DiGeorge	22q11.2	1/2000
1p36 deletion	1p36-	1/5000
Prader-Willi and Angelman	Del 15q11.2	1/20,000
Wolf-Hirschhorn	4p-	1/50,000 to 1/20,000
Cri du chat	5p-	1/50,000
Miller-Dieker	Del 17p13.3	1 /100,000

Routine prenatal screening for microdeletion syndromes is not recommended by national organizations. Current practice is to offer invasive prenatal diagnostic testing in select cases to women when a prenatal ultrasound indicates anomalies (eg, heart defects, cleft palate) that could be associated with a particular microdeletion syndrome. Samples are

analyzed using fluorescence in situ hybridization, chromosomal microarray analysis, or karyotyping. Additionally, families at risk (e.g., those known to have the deletion or with a previously affected child) generally receive genetic counseling and those who conceive naturally may choose prenatal diagnostic testing. Most affected individuals, though, are identified postnatally based on clinical presentation and may be confirmed by genetic testing. Using 22q11.2 deletion syndrome as an example, although clinical characteristics vary, palatal abnormalities (e.g., cleft palate) occur in approximately 69% of individuals, congenital heart disease in 74%, and characteristic facial features are present in a majority of individuals of northern European heritage.

Noninvasive Prenatal Screening (NIPS) Using Cell-Free Fetal DNA for Chromosomal Trisomies in Singleton Pregnancies

Clinical Context and Test Purpose

The purpose of noninvasive prenatal screening (NIPS) using cell-free fetal DNA is to screen for fetal chromosomal abnormalities (e.g., trisomies 21 [T21], 18 [T18], 13 [T13]). It can be used as a complement or as an alternative to conventional serum screening. National guidelines have recommended that all pregnant women be offered screening for aneuploidies. Positive cell-free fetal DNA tests need to be confirmed using invasive testing and with more accurate screening tests, this may reduce the need for invasive testing and associated morbidities.

The purpose of NIPS using analysis of cell-free fetal DNA in individuls who have a singleton pregnancy is to inform a decision whether to proceed with diagnostic testing.

Populations

The relevant population of interest is individuals with a singleton pregnancy in their first trimester or early in their second trimester.

Interventions

The intervention of interest is NIPS using analysis of cell-free fetal DNA for detection of chromosomal trisomies.

Comparators

The following tests are currently being used to make decisions about identifying fetal chromosomal abnormalities: conventional serum and ultrasound screening followed by invasive diagnostic testing as well as standard of care without screening.

Outcomes

The primary outcomes of interest are test accuracy and validity, reductions in miscarriages associated with invasive confirmatory testing, and reduction in use of other noninvasive and invasive tests received by pregnant individuals.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Several systemic reviews and meta-analyses of studies on the diagnostic accuracy of sequencing-based tests for the detection of fetal aneuploidies have been published.

In 2017, a systemic review and meta-analysis was published by Iwarsson et. al., the aim of this study was to review the performance of noninvasive prenatal testing (NIPT) for detection of trisomy 21, 18 and 13 (T21, T18, and T13) in a general pregnant population as well as on high-risk pregnancies. In a general pregnant population, there is moderate evidence that the pooled sensitivity is 0.993 (95% CI, 0.955-0.999) and specificity was 0.999 (95% CI, 0.998-0.999) for the analysis of T21. Pooled sensitivity and specificity for T13 and T18 was not calculated in this population due to the low number of studies. In a high-risk pregnant population, there is moderate evidence that the pooled sensitivities for T21 and T18 are 0.998 (95% CI, 0.981-0.999) and 0.977 (95% CI, 0.958-0.987) respectively, and low evidence that the pooled sensitivity for T13 is 0.975 (95% CI, 0.819-0.997). The pooled sensitivity for all three trisomies is 0.999 (95% CI, 0.998-0.999). The authors concluded this is the first meta-analysis using GRADE that shows that NIPT performs well as a screen for trisomy 21 in general pregnant population. Although the false positive rate is low compared with first trimester combined screening, women should still be advised to confirm a positive result by invasive testing if termination of pregnancy is under consideration.

Several studies have evaluated noninvasive prenatal screening for fetal aneuploidies (T21, and when available T18 and T13) in high-risk singleton pregnancies. The sensitivity and specificity of the tests were uniformly high. Sensitivity ranged from 99.1% to 100% and the specificity from 99.7% to 100%. Several companies market this testing, studies available are mostly prospective and are industry funded. Studies generally included women at a wide range of gestational ages (e.g., 8-36 weeks or 11-20 weeks) spanning first and second trimesters. The approach to analysis varied. Some studies analyzed samples from enrolled women, and other analyzed samples from all women with pregnancies known to have a trisomy syndrome and selected controls. The studies compared the results of cell-free DNA testing with the criterion standards of karyotyping for specific trisomies.

In 2016, Taylor-Phillips et. al. published a comprehensive systematic review and meta-analysis on the accuracy of noninvasive prenatal testing using cell-free DNA for detection of Down syndrome (T21), Edwards (T18) and Patau (T13) syndromes. To be included, studies had to confirm trisomy status using an invasive test, fetal pathologic examination, or postnatal phenotype assessment. Most studies were limited to samples of high-risk women and singleton pregnancies. Quality appraisal identified high risk of bias in included studies, funnel plots showed evidence of publication bias. Pooled sensitivity was 99.3% (95% confidence interval (CI), 98.9 to 99.6%) for Down syndrome, 97.4% (95% CI, 95.8% to 98.4%) for Edwards, and 97.4% (955 CI, 86.1% to 99.6%) for Patau

syndrome. The pooled specificity was 99.9% (99.9% to 100%) for all three trisomies. The authors concluded NIPT using cell-free fetal DNA was very high sensitivity and specificity for Down syndrome, with slightly lower sensitivity for Edwards and Patau syndrome. However, it is not 100% accurate and should not be used as a final diagnosis for positive cases.

A 2016 study by Norton et. al. reported on the performance of sequential DNA screening in a large cohort of women who participated in the California Prenatal Screening Program and compared findings with an estimations of cell-free fetal DNA findings. A total of 452,901 women underwent sequential screening, 2575 (0.57%) had a fetal chromosomal abnormality; 2101 were detected for a detection rate (DR) of 81.6%, and 19,929 euploid fetuses had positive sequential screening for a false positive rate (FPR) of 4.5%. If no results, cases were presumed normal, cell-free DNA (cfDNA) screening would have detected 1820 chromosome abnormalities (70.7%) with an FPR of 0.7%. If no results, cases were considered screen positive, 1985 (77.1%) cases would be detected at a total screen positive rate of 3.7%. in either case, the detection rate of sequential screening for all aneuploidies in the cohort was greater than cfDNA ($P<0.001$). A limitation of this study was that results of cell-free fetal DNA tests were estimated using statistical modeling and were not observed. The authors concluded for primary population screening, cfDNA providers lower DR (detection rate) than sequential screening if considering detection of all chromosomal abnormalities. Assuming that no results of cfDNA are high risk improves cfDNA detection but with a greater FPR (false positive rate). cfDNA should not be adopted as primary screening without further evaluation of the implications for detection of all chromosomal abnormalities and how to best evaluate no results cases.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

The 2013 and 2014 BlueCross BlueShield Association TEC Assessments each constructed decision models to predict health outcomes of sequencing-based testing compared with standard testing. The model in the 2013 TEC assessment focused on T21. In this model, the primary health outcomes of interest included the number of cases of aneuploidy correctly identified, number of cases missed, number of invasive procedures potentially avoided (i.e., with a more sensitive test), and the number of miscarriages

potentially avoided as a result of fewer invasive procedures. The results were calculated for a high-risk population of women aged 35 years or older (estimated antenatal prevalence of T21, 0.95%), and an average-risk population including women of all ages electing an initial screen (estimated antenatal prevalence of T21, 0.25%). For women testing positive on initial screen and offered an invasive, confirmatory procedure, it was assumed that 60% would accept amniocentesis or CVS. Sensitivities and specificities for both standard and sequencing-based screening tests were varied to represent the range of possible values; estimates were taken from published studies whenever possible.

According to the model results, sequencing-based testing improved outcomes for both high-risk and average-risk women. As an example, assuming there are 4.25 million births in the United States per year and two-thirds of the population of average-risk pregnant women (2.8 million) accepted screening, the following outcomes would occur for the 3 screening strategies under consideration:

- Standard screening. Of the 2.8 million screened with the stepwise sequential screen, 87,780 would have an invasive procedure (assuming 60% uptake after a positive screening test and a recommendation for confirmation), 448 would have a miscarriage, and 3976 of 4200 (94.7%) T21 (Down syndrome) cases would be detected.
- Sequencing as an alternative to standard screening. If sequencing-based testing were used instead of standard screening, the number of invasive procedures would be reduced to 7504 and the number of miscarriages reduced to 28, while the cases of Down syndrome detected would increase to 4144 of 4200 (97.6% of total), using conservative estimates.
- Sequencing following standard screening. Another testing strategy would be to add sequencing-based testing only after a positive standard screen. In this scenario, invasive procedures would be further decreased to 4116, miscarriages would remain at 28, but fewer Down syndrome cases would be detected (3948/4200 [94.0% of total]). Thus, while this strategy has the lowest rate of miscarriages and invasive procedures, it detects fewer cases than sequencing-based testing alone.

The model in the 2014 TEC Assessment included T13 and T18 (but not sex chromosome aneuploidies, due to the difficulty of defining relevant health outcomes). The model was similar but not identical to that previously used to evaluate T21. As in earlier model, outcomes of interest included the number of cases of aneuploidy correctly detected and the number of cases missed, and findings were calculated separately for a high-risk population of women aged 35 or older and a low-risk population. The model assumed that 75% of high-risk and 50% of low-risk women who tested positive on the initial screen would proceed to an invasive test. (The T21 model assumed a 60% uptake rate of invasive confirmatory testing.) A distinctive feature of the 2014 modelling study was that it assumed screening for T21 was done concurrently to screening for T13 and T18 and that women who choose invasive testing do so because of a desire to detect T21. Consequently, miscarriages associated with invasive testing were not considered an adverse effect of T13 or T18 screening.

The model compared 2 approaches to screening: (1) a positive sequencing-based screen followed by diagnostic invasive testing; and (2) a positive standard noninvasive screen followed by diagnostic invasive testing. As in the T21 modelling study, sensitivities, and specificities for both standard and sequencing-based screening tests were varied to represent the range of possible values; estimates were taken from published studies whenever possible. Assuming that a hypothetical population of 100,000 pregnant women was screened, the model had the following findings:

- High-risk women: Assuming 75% uptake after a positive screen, the maximum cases detectable in the hypothetical population of 100,000 pregnancies is 127 trisomy 18 cases and 45 trisomy 13 cases. Standard noninvasive screening would identify 123 of the 127 trisomy 18 cases and sequencing-based screening would identify 121 of 127 cases. In addition, standard noninvasive screening would identify 37 of 45 trisomy 13 cases and sequencing-based screening would identify 39 of 45 trisomy 13 cases.
- Low-risk women: Assuming 50% uptake after a positive screen, the maximum cases detectable in the hypothetical population of 100,000 pregnancies is 20 trisomy 18 cases and 6 trisomy 13 cases. Each initial screening test would identify 19 of the 20 trisomy 18 cases and 5 of the 6 trisomy 13 cases.

Results of the modeling suggest that sequencing-based tests detect a similar number of T13 and T18 cases and miss fewer cases than standard noninvasive screening. Even in a hypothetical population of 100,000 women, however, the potential number of detectable cases is low, especially for T13 and for low-risk women.

In 2012, Palomaki et. al. modeled the use of the Sequenom sequencing based test offered to women after a positive screening test, with invasive testing offered only in the case of a positive sequencing-based test. The model included cases positive for T21 or T18 (but not T13 due to its lower prevalence). As in the 2013 TEC Assessment, Palomaki assumed 4.25 million births in the United States per year, with two-thirds of those receiving standard screening. The models assumed a 99% detection rate, 0.5% false positive rate, and 0.9% failure rate for sequencing-based testing. Compared with the highest performing standard screening test, the addition of sequencing-based screening would increase the Down syndrome detection rate from 4450 to 4702 and decrease the number of miscarriages associated with invasive testing from 350 to 34.

In 2013, Ohno and Caughey published a decision model comparing the use of sequencing-based tests in high-risk women with confirmatory testing (i.e., as screening test) and without confirmatory testing (i.e. as diagnostic test). Results of the model concluded that using sequencing-based tests with confirmatory test results in fewer losses of normal pregnancies compared with sequencing-based tests used without a confirmatory test. The model assumed estimates using the total population of 520,000 high risk women presenting for first trimester care each year in the United States. Sequencing based tests used with confirmatory testing resulted in 1441 elective terminations (all with Down syndrome). Without confirmatory testing, sequencing based

testing resulted in 3873 elective terminations, 1449 with Down syndrome and 2424 without Down syndrome. There were 29 procedure-related pregnancy losses when confirmatory tests were used. The decision model did not address T18 or T13.

It is important to note sequencing-based testing without confirmatory testing carries the risk of misidentifying normal pregnancies as positive for trisomy. Due to the small but finite false positive rate, together with the low baseline prevalence of trisomy in all populations, a substantial percentage of positive results on sequencing test could be false positive results.

Section Summary
A meta-analysis of data available from published studies reported sensitivities of 98.8% to 98.9% and specificities of 99.9% for NIPS for detecting T21, T18, and T13 in high-risk women with singleton pregnancies. Calculations indicated that 64 to 70 affected cases would be missed out of 100,000 pregnancies. The available studies providing data separately for an unselected population found sensitivities ranging from 94.9% (MPS) to 100% (TMPS), and specificities of 99.9% for detection of T21, T18, and T13. The specificity of 99.9% is similar to that seen in high-risk women, with an estimated 0 (MPS) to 32 (TMPS) affected cases missed out of 100,000 pregnancies. Modeling studies using published estimates of diagnostic accuracy and other parameters predict that sequencing-based testing as an alternative to standard screening would increase the number of T21 (i.e., Down syndrome) cases detected and, when included in the model, a large decrease in the number of invasive tests and associated miscarriages.

Noninvasive Prenatal Screening (NIPS) Using Cell-Free Fetal DNA for Chromosomal Trisomies in Twin and Multiple Pregnancies

Clinical Context and Test Purpose
The purpose of noninvasive prenatal screening (NIPS) using analysis of cell-free fetal DNA in patients who have a twin or other multiple pregnancy is to inform decision whether to proceed with diagnostic testing.

Populations
The relevant population of interest is individuals with first and second trimester twin or other multiple pregnancy.

Interventions
The intervention of interest is NIPS using analysis of cell-free fetal DNA for detection of chromosomal trisomies.

Genetic counseling may also be necessary. The timing for testing is generally in the first trimester of pregnancy and can be early in the second trimester.

Comparators
The following tests are currently being used to make decisions about identifying fetal chromosomal abnormalities in twin or other multiple pregnancy: conventional serum and ultrasound screening following by invasive diagnostic testing as well as standard of care without screening.

Outcomes
The primary outcomes of interest are test accuracy and validity, reductions in miscarriages associated with invasive confirmatory testing, and reduction in use of other noninvasive and invasive tests received by pregnant individuals.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In 2019, a meta-analysis by Gil et. al. completed an analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies (fetal trisomies 21, 18, 13) and sex chromosome aneuploidies (SCA) in both single and twin pregnancies. The inclusion criteria were peer reviewed study reporting on clinical validation for implementation of maternal cfDNA testing in screening for aneuploidies in which data on pregnancy outcome were provided for more than 85% of the study population. In total 35 relevant studies were identified, and these were used for the meta-analysis on the performance of cfDNA testing in screening for aneuploidies. These studies reported cfDNA results in relation to fetal karyotype from invasive testing for clinical outcome. In the combined total of 1963 cases of trisomy 21 and 223 932 non-trisomy 21 singleton pregnancies, the weighted pooled DR and FPR were 99.7% (95% CI, 99.1-99.9%) and 0.04% (95% CI, 0.02-0.07%), respectively. In a total of 563 cases of trisomy 18 and 222 013 non-trisomy 18 singleton pregnancies, the weighted pooled DR and FPR were 97.9% (95% CI, 94.9-99.1%) and 0.04% (95% CI, 0.03-0.07%), respectively. In a total of 119 cases of trisomy 13 and 212 883 non-trisomy 13 singleton pregnancies, the weighted pooled DR and FPR were 99.0% (95% CI, 65.8-100%) and 0.04% (95% CI, 0.02-0.07%), respectively. In a total of 36 cases of monosomy X and 7676 unaffected singleton pregnancies, the weighted pooled DR and FPR were 95.8% (95% CI, 70.3-99.5%) and 0.14% (95% CI, 0.05-0.38%), respectively. In a combined total of 17 cases of SCA other than monosomy X and 5400 unaffected singleton pregnancies, the weighted pooled DR and FPR were 100% (95% CI, 83.6-100%) and 0.004% (95% CI, 0.0-0.08%), respectively. For twin pregnancies, in a total of 24 cases of trisomy 21 and 1111 non-trisomy 21 cases, the DR was 100% (95% CI, 95.2-100%) and FPR was 0.0% (95% CI, 0.0-0.003%), respectively. The authors concluded screening by analysis of cfDNA in maternal blood in singleton pregnancies could detect >99% of fetuses with trisomy 21, 98% of trisomy 18 and 99% of trisomy 13 at a combined FPR of 0.13%. The number of reported cases of SCA is too small for accurate assessment of performance of screening. In twin pregnancies, performance of screening for trisomy 21 is encouraging but the number of cases reported is small.

In 2017, Du et. al. assessed the performance of massively parallel sequencing (MPS) testing of cell-free fetal DNA (cfDNA) from maternal plasma for trisomies 21, 18, and 13 in twin pregnancies. MPS technology has been widely used to screen for trisomies 21, 18 and 13 in singleton pregnancies. Ninety-two women with twin pregnancies were recruited. The results were identified through karyotypes of amniocentesis or clinical examination and follow-up of the neonates. Cell-free fetal DNA testing correctly identified two T21 pregnancies, and there was 1 false positive T13 test. No cases of T18 were identified.

Foster et. al. in 2017, evaluated two sets of maternal blood samples from twin pregnancies using noninvasive prenatal testing (NIPT) for fetal aneuploidy. Clinical study A, 115 stored samples from pregnancies with known outcome and Clinical Study B 487 prospectively collected samples for which outcomes were requested from providers. NIPT was used to screen for the presence of fetal aneuploidy on chromosomes 13, 18, 21, X and Y in all cases, and results were compared with outcomes when known. In Clinical Study A, all 115 samples were classified correctly by NIPT: three cases of trisomy 21 (one fetus affected), one of monochorionic trisomy 18 (both fetuses affected) and 111 euploid (normal number of chromosomes). In Clinical Study B, a NIPT result was reported for 479 (98.4%) of the 487 samples. Aneuploidy was detected or suspected in nine (1.9%) cases: seven cases of trisomy 21 detected, one case of trisomy 21 Suspected, and one case with trisomy 21 detected and trisomy 18 suspected. Information on aneuploidy outcome was available for 171 (35.75) cases in Clinical Study B. Of the nine cases with aneuploidy detected or suspected, six were confirmed to be a true positive in at least one twin based on karyotype or birth outcome and two were suspected to be concordant based on ultrasound findings; the one known discordant result was for aneuploidy suspected case. No false negatives were reported. Limitations of this study include the number of affected pregnancies was small and the majority were trisomy 21. This precluded determination of detection rates of trisomies 13 and 18. Another limitation was incomplete clinical outcomes with aneuploidy outcome information available for only 35.7% of cases in Clinical Study B. Authors concluded, although there is considerable evidence for robust NIPT performance in singleton pregnancies, there is still relatively little published data about its performance in twins. In this study, the detection rate for trisomy 21 in twin pregnancies appears to be in line with that in singletons. The limited number of affected cases for other trisomies precluded conclusive determination of those detection rates. In summary, the findings reported support the view that cfDNA NIPT performs well in twin pregnancies, with overall very low false-positive frequencies.

Observational Studies

Four observational studies published after the systematic reviews were conducted for NIPS in twin or multiple pregnancies.

Observational Studies of NIPS in Twin or Multiple Pregnancies- Study Results

Study	Initial N	Final N	Excluded Samples	Prevalence of	Sensitivity	Specificity

				Condition		
Dyr et. al. 2019	30,826	28,992	1,834 (5.95%) non-reportable (low fetal fraction)	(Confirmed cases) T21:16; T18: 8; T13:3	T21: 98.4%; T18: 99.99%; T13: 99.99%	T21: 99.9%; T18: >99.99%; T13: 99.28%
Kypri et. al. 2019	306 twin pregnancies	300	6 with insufficient fetal fraction	T21: 3; T18: 1; T13: 1	100%	100%
Norwitz et. al. 2019	126 twin pregnancies	117	10 confirmed euploid samples did not receive a cfDNA result. Of 87 euploid samples with gestational age ≥10 weeks, 0/21 MZ and 10/66 DZ samples did not receive a result: Overall no-result rate 10.6% (95% CI 5.3% - 19.7%)	11 aneuploid; 106 euploid T21: 5 (1 MZ, 4 DZ); T18: 5 (all DZ); T13: 1 (DZ)	DZ gestations:100% (95% CI 69.2% - 100%)	Overall aneuploidy (96/96):100% (95% CI 94.8%-100%)

Motevasselian et. al. 2020	500 twin pregnancies	356	144 excluded: 94 did not come for follow-up, 22 no karyotype; 7 intrauterine death of both fetuses; 2 twin pregnancies had selective embryonic reduction, 19 terminated due to preterm labor (n = 11), premature rupture of membranes (n = 7) and severe preeclampsia (n = 1)	T21: 3; T18: 1; T13: 1	100%	99.7% (1 false positive)
Chibuk et.al. (2021)	422	371	Non-reportable cfDNA test (48) or failed amnioanalysis (3)	T21: 61	T21: 98.0% (87.8 to 99.9)	T21: 96.0% (93.0 to 97.7)
Khalil et.al. (2021)	1003	958	5 withdrew, 37 lost to follow-up, 3 sample	T21: 13; T18: 1; T13: 1	T21: 100 (75 to 100) T18: 0 (0 to 97) T13: 100 (3 to 100)	T21:100 (61 to 100) T18: 90 (42 to 100) T13: 100 (61 to 100)

			failed (1 T21, 2 euploid)			
Judah et. al. (2021)	1442	1272	No result or no confirmatory testing	T21: 20	T21: 95.0	T21: 99.6
Cheng et.al. (2021)	1048 twin pregnancies	1029	All 13 pregnancies with a positive NIPS had karyotype, 19/1035 with NIPS-negative result lost to follow-up	T21: 1; T18: 0; T13: 0	T21: 100%	
Xu et. al. (2021)	2399 twin pregnancies	2399	49 twin pregnancies had no pregnancy outcomes or karyotypes for one of the fetuses	T21: 7; T18: 1; T13: 0	T21: 100 (59.0 to 100) T18: 100 (2.5 to 100) T13: Could not be calculated	T21: 100 (99.8 to 100) T18: 99.9 (99.7 to 100) T13: 99.8 (99.5 to 99.9)

Observational Studies of NIPS in Twin or Multiple Pregnancies - Study Limitations

Study	Limitations
Dyr et.al. 2019	Study population not representative of intended use – Indications for NIPT varied

Motevasselian et. al. (2020)	Study population not representative of intended use – Indications for NIPT varied

Observational Studies of NIPS in Twin or Multiple Pregnancies - Study Design and Conduct Limitations

Study	Selection	Data Completeness	Statistical
Dyr et.al. 2019	Selection not random or consecutive (i.e., convenience)	High loss to follow-up or missing data: incomplete follow-up	Confidence interval and/or p values not reported
Kypri et. al. 2019			Confidence interval and/or p values not reported
Norwitz et. al. 2019	Selection not described		
Motevasselian et. al. (2020)		High number of samples excluded: 28% of pregnancies excluded	Confidence interval and/or p values not reported
Chibuk et. al. (2021)	Selection not described: unclear if convenience or consecutive samples	High loss to follow-up or missing data: incomplete follow-up	
Khalili et. al. (2021)	Selection not random or consecutive (i.e., convenience).	High loss to follow-up or missing data: incomplete follow-up	
Judah et. al. (2021)	Selection not random or consecutive (i.e., convenience)	High loss to follow-up or missing data: incomplete follow-up	Confidence intervals and/or p values not reported

Cheng et. al. (2021)	Selection not random or consecutive (i.e., convenience)	High loss to follow-up or missing data: incomplete follow-up	Confidence intervals and/or p values not reported
Xu et. al. (2021	Selection not described: Unclear if convenience or consecutive samples	Inadequate description of indeterminate and missing samples; High number of samples excluded excluded no-call cases and those with fetal demise or selective termination	

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Direct evidence is not available for the evaluation of noninvasive prenatal testing to detect fetal aneuploidies in women pregnant with twins or multiples. Additionally, it is not possible to construct a chain of evidence for clinical utility due to the lack of sufficient evidence on clinical validity.

Section Summary
Nonrandomized studies and meta-analyses have assessed the clinical validity of NIPS for detecting aneuploidies in twin pregnancies. Studies reported high sensitivity and specificity of NIPS to identify trisomies compared to standard methods. However, the total number of cases of aneuploidy identified in these studies is fewer than 300, resulting in wide confidence intervals and estimates that are too imprecise to allow conclusions about clinical validity. The quantity and quality of evidence remains insufficient to draw

conclusions about clinical validity. There is lack of direct evidence of clinical utility and a chain of evidence cannot be constructed due to insufficient evidence on clinical validity.

Noninvasive Prenatal Screening for Fetal Microdeletions Using Cell-Free DNA

Clinical Context and Test Purpose
The purpose of NIPS using analysis of cell-free fetal DNA in individuals who are pregnant is to inform a decision whether to proceed with diagnostic testing.

NIPS using cell-free DNA is being researched as a tool to screen for microdeletions. Microdeletions (also referred to as submicroscopic deletions) are chromosomal deletions that are too small to be detected by microscopy or conventional cytogenetic methods or microscopy. Microdeletions, along with microduplications, are collectively known as copy number variations (CNVs). CNVs can lead to disease development when the change in copy number of a dose-sensitive gene or genes disrupts the ability of the gene(s) to function and effects the amount of protein produced.

Several genomic disorders associated with microdeletions have been identified. Microdeletion syndromes have distinctive and, in many cases, serious clinical features, such as cardiac anomalies, immune deficiency, palatal defects and cognitive delay. While some are inherited, other occur de nevo. Examples of microdeletion syndromes include but are not limited to the following:

- Angelman syndrome
- DiGeorge syndrome or velocardiofacial syndrome (most common),
- Miller-Dieker syndrome
- Neurofibromatosis type I, Neurofibromatosis type II
- Prader-Willi syndrome
- Rubinstein-Taybi syndrome
- Smith-Magenis syndrome
- Williams syndrome
- Wolf-Hirschhorn syndrome.

Populations
The relevant population of interest are individuals who are pregnant.

Interventions
The intervention of interest is NIPS for fetal microdeletions using analysis of cell-free fetal DNA.

Genetic counseling may also be necessary.

The timing for testing is generally in the first trimester of pregnancy and can be early in the second trimester.

Comparators
Routine prenatal screening for microdeletion and microduplication syndromes is not recommended by national organizations. Current practice is to offer invasive prenatal diagnostic testing in select cases to women when a prenatal ultrasound indicates anomalies (e.g., heart defects, cleft palate) that could be associated with a particular microdeletion syndrome.

Outcomes
The primary outcomes of interest are test accuracy and validity, reductions in miscarriages associated with invasive confirmatory testing, and reduction in the use of other noninvasive and invasive tests received by the pregnant individuals.

Clinically Valid
A test must detect the presence of absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial and adverse).

Review of Evidence
In 2022, Madankumar et. al., assessed the performance of SNP-based cell-free DNA (cfDNA) prenatal screening in the detection of 22q11.2DS or DiGeorge syndrome. Patients who had SNP-based cfDNA prenatal screening for 22q11.2DS were prospectively enrolled at 21 centers in 6 countries. Prenatal or newborn DNA samples were requested in all cases for genetic confirmation with chromosomal microarray. The primary outcome was sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of cfDNA for detection of all deletions, including the classical deletion and nested deletions that are ≥500kb, in the 22q11.2 low copy repeat A-D region. Secondary outcomes included the prevalence of 22q11.2DS and performance of an updated cfDNA algorithm that was evaluated blinded to pregnancy outcome. Of 20,887 women enrolled, genetic outcome was available in 18,289 (87.6%). Twelve 22q11.2DS cases were confirmed in the cohort, including five (41.7%) nested deletions, yielding a prevalence of 1:1524. In the total cohort, cfDNA reported 17,976 (98.3%) as low risk for 22q11.2DS and 38 (0.2%) as high-risk; 275 (1.5%) were non-reportable. Overall, 9 of 12 cases of 22q11.2 were detected, yielding a sensitivity of 75.0% (95% CI: 42.8, 94.5); specificity of 99.84% (95% CI: 99.77, 99.89); PPV of 23.7% (95% CI: 11.44, 40.24) and NPV of 99.98% (95% CI: 99.95, 100). None of the cases with a non-reportable result was diagnosed with 22q11.2DS. The updated algorithm detected 10/12 cases (83.3%; 95% CI: 51.6-97.9) with a lower false positive rate (0.05% vs. 0.16%, $p<0.001$) and a PPV of 52.6% (10/19; 95% CI 28.9-75.6).

In 2021 Familiari et.al. completed a systematic review of the current available literature on the performance of cell-free DNA as a screening for microdeletion and microduplication syndromes (MMs). We identified 42 papers, seven included, for a total of 474,189 pregnancies and 210 cases of MMs. Diagnostic verification of positive cases was available overall in 486 (71.68 %) of 678 cases. The weighted pooled SPR, FPR and PPV were 0.19% (95% CI = 0.09-0.33), 0.07 (95% CI = 0.02-0.15) and 44.1 (95% CI = 31.49-63.07). In conclusion, the pooled PPV of cfDNA testing in screening for MMs was

about 40%, ranging from 29% to 91%, for an overall FPR <0.1%. The authors concluded no confirmatory analysis was available in cases that did not undergo invasive testing, which were the vast majority of cases with a negative test, and therefore, the DR and the negative predictive value cannot be determined.

Cui et al. (2019) evaluated the clinical utility of non-invasive prenatal testing (NIPT) for the detection of copy number variants (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.

Hu et al. (2019) studied 8,141 single pregnancies with a genome wide SSP-NIPT in order to establish positive predictive value for sub-chromosomal microdeletions and microduplications at 3 Mb resolution (4.89 million reads). Maternal age ranged from 15-46 with 13.79% over age 35 and 40.88% between ages 25-29. 13.79% presented for NIPT because of advanced maternal age; 26 cases had abnormal ultrasound findings. Other indications included: poor fertility and abnormal serum screening. Fifty-one (0.63%) were positive for chromosome microdeletions or microduplications, however 13 (36.11%) were true positives. Twenty-three (63.89%) were false-positives, and 15 were unverified cases. Of the 13 true positives, nine were de novo mutations and four were inherited mutations. Seven cases were identified as disease-causing and six were of unknown clinical significance. The authors state that microdeletion NIPT has a low PPV and has not demonstrated an acceptable low false-positive rate to be considered practical for prenatal screening. Diagnostic testing is recommended.

Ravi et al. (2018) reported on the clinical validity of using a SNP based NIPT assay to detect fetal 22q11.2 deletions during pregnancy. Women from six prenatal centers were enrolled in the study and were undergoing 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 patients that were undergoing 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

patients that were < 9 weeks gestation, had a fetal demise, had atypical 22q distal deletions on invasive testing, or equivocal invasive test results were excluded. Patients with inconclusive or no call NIPT results were excluded and no redraws were requested. The study was internally blinded, but ultimately included ten patients 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 1,351 SNPs spanning a 2.91 Mb section of the 22q11.2 region that constitutes ~87% of all deletions detected in individuals with the 22q11.2 deletion syndrome. Risk status for the 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 a fetal fraction 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 1,442 in pregnancy, the estimated positive predictive value (PPV) is 19.6%.

Schwartz et al. (2018) reported on a cytogenetic laboratory's experience with the follow up of NIPT results positive for microdeletions. Three hundred and forty-nine patients were positive by NIPT for microdeletions 1p, 4p, 5p, 15q, or 22q and had an invasive diagnostic test with chromosome microarray analysis as a follow up. Thirty-two (9.2%) patients had a microdeletion confirmed. Of those, 39% of the cases had additional abnormal microarray findings. Unrelated abnormal microarray findings were detected in 11.8% of the patients tested. This cohort had stretches of homozygosity in the microdeletion region which could be an explanation for false positive NIPT results. In this study the NIPT positive predictive value of the NIPT microdeletion test was 9.2%.

Petersen et al (2017) compared test results from amniotic or chorionic samples of unselected women referred for diagnostic testing due to a positive NIPS result. The PPV of NIPS to identify a microdeletion syndrome was 13% in Petersen et al (2017) and 18% in Gross et al (2017). Gross et al (2017) reported that 8 (73%) of the 11 true-positive cases in their study could have been identified without NIPS (i.e., by ultrasound followed by invasive testing). Soster et al (2021) conducted a retrospective analysis of 55,517 samples submitted for genome-wide cfDNA screening at a commercial laboratory between 2015 and 2018. Diagnostic testing results were available in 42.5% (n = 1,142) of screen-positive samples, and 0.82% of screen-negative samples, with overall 2.98% of samples with diagnostic outcomes. Test characteristics for microdeletions are shown in the below tables. A limitation of these studies is the lack of reporting on false negatives because follow-up after negative screening results was voluntary and/or not available from the retrospective review of de-identified data.

Studies from 2 companies offering microdeletion testing have evaluated data from clinical samples submitted for screening. In 2016, Gross et. al. published a study

evaluating the performance of the Natera cell-free DNA test to identify 22q11.2 deletion syndrome. The study retrospectively analyzed 21,949 samples submitted for screening. After 1172 cases were excluded (919 failed quality control, 46 were twins/triploidy, 207 were out of specification), 20,776 cases were evaluated for the microdeletion. A total of 97 (0.46%) of the 20,776 cases were considered at high risk for the 22q11.2 deletion. One of these was confirmed to be a 22q11.2 microdeletion in the mother, not in the fetus, and another was suspected to be a maternal deletion. Diagnostic testing results were available for 61 (64.2%) cases, which confirmed 11 (18.0%) true positives and identified 50 (82.0%) false positives, resulting in a positive predictive value (PPV) of 18.0%. Information regarding invasive testing was available for 84 (88.4%) high risk cases: 57.1% (48/84) had invasive testing and 42.9% (36/84) did not. Ultrasound anomalies were present in 81.8% of true positive and 18.0% of false-positive cases. Limitations of the analysis included lack of follow up data on both high risk and low risk cases. Although attempts were made to follow-up all high-risk cases, confirmatory diagnostic information was unavailable in 34 cases (36%). This included cases for which patients chose not to have confirmatory testing results or were reluctant to share confirmatory testing results, as well as cases for which patients was lost to follow-up. Providers were encouraged to report false-negative cases, but no such cases were reported. However, because follow-up on low-risk cases was not carried out, calculation of negative predictive value was not carried out, calculation of the negative predictive value was not possible. Authors concluded the decision to add 22q11.2 deletion screening as an adjunct to existing NPIT needs to balance the medical benefits of early diagnosis of 22q11.2 deletions against efficacy of the test, the prevalence in the referral group (which would be expected to be higher when NIPT referrals include patients with positive combined test results and abnormal ultrasound findings), additional clinical service consideration and cost. The data on clinical experience presented in this study may be helpful in this assessment.

Nonrandomized Studies of Noninvasive Screening for Copy Number Variants-Characteristics

Study	Test	Copy Number Variant, Syndrome	Population	Reference Test
Gross et. al. (2016)	Natera	22q11.2, DiGeorge	20,776 samples from high-risk pregnant women submitted for screening	Diagnostic testing in 61
Petersen et. al. (2017)	Various	1p36,1p36; 5p-, Cri du chat; 15q-, Prader-Willi; 22q11.2, DiGeorge	52 cases referred for diagnostic testing following	Diagnostic CMA, FISH, or karyotyping

			positive NIPS for MDS	
Soster et. al. (2021)	Genome-wide cfDNA test	1p36 deletion, Wolf–Hirschhorn, Cri-du-chat, Langer–Giedion, Jacobsen, Prader–Willi, Angelman, and DiGeorge syndrome	55,517 samples submitted for genome-wide cfDNA screening at a commercial laboratory	Karotype (58.5%); microarray (10.8%), FISH (1.6%), other or unspecified (16.7%), multiple tests (12.5%)

cfDNA: cell-free DNA; CMA: chromosomal microarray; FISH: fluorescence in-situ hybridization; MDS: microdeletion syndromes; MPS: massively sequencing; NIPS: noninvasive prenatal screening.

Nonrandomized Studies of Noninvasive Screening for Copy Number Variants-Results

Study	Initial N	Final N	Excluded Samples	Positive Tests, n (%)	Clinical Validity
Gross et. al. (2016)	21,949	20,776	1172	97 (0.46)	TP, n% 11 (0.05) PPV, % 18 FP 86 FN Unknown
Petersen et. al. (2017)	52	52	NR	52	TP, n% 7 PPV, % 13 FP 45 FN Unknown
Soster et. al. (2021)	55,517	1569	Samples without diagnostic	2687 (5.06%)	TP, n(%)

			results for microdeletions		• 22q: 38 • 1p36: 7 • 15q: 8 • 4p: 9 • 5p: 6 • 11q: 5 • 8q: 2 PPV, % 72.6 FP • 22q: 1 • 1p36: 0 • 15q: 0 • 4p: 0 • 5p: 2 • 11q: 0 • 8q: 0 FN • 22q: 5 • 1p36: 0 • 15q: 0

					• 4p: 0 • 5p: 0 • 11q: 0 • 8q: 0

FN: false-negatives; FP: false-positives; NR: not reported; PPV: positive predictive value; TP: true-positives.

In 2015, a study by Helgeson et. al. used the Sequenom MPS-based test and the investigators analyzed 175,393 blood samples from high-risk pregnant women. Between October 2013 and July 2014, 123,096 samples were tested for 4 microdeletions: 1p36, 5p-, 15q-, and 22q11.2. From August 2014 to October 2014, 52,297 samples were tested for those 4 microdeletions plus an additional 3: 4p-, 8q- and 11q-. The preferred reference standard was diagnostic testing (chromosomal microarray analysis, fluorescence in situ hybridization or karyotype analysis). Cases were considered "confirmed" if the deletion was detected in the pregnant woman and/or fetus and considered "false-positive" if diagnostic testing was negative for the deletion in either the fetus or pregnant woman (maternal plasma samples contain DNA fragments from both the pregnant woman and the fetus; microdeletions detected could be either or both of them). In the absence of diagnostic testing, cases were considered "suspected" if diagnostic testing was not performed and phenotypic data were consistent with the clinical presentation common to the deletion. Fifty-five (0.03%) of the samples had one of the testing microdeletions. Nearly half (48%) of the positive tests were in pregnancies referred for testing due to ultrasound findings. Two patients were lost to follow-up, and diagnostic testing and/or clinical phenotype information was available for the remaining 53 patients. Microdeletions were confirmed (in the pregnant woman and/or fetus) 41 (77.4%) of 53 cases, and an additional 9 cases did not have confirmatory testing but had clinical features consistent with one of the microdeletions. There were 3 false positive cases, 1 case of 1p36 deletion and 2 cases of 5p deletion. The PPV ranged from 60% to 100% for cases with diagnostic and/or clinical follow-up information. The false-positive rate was 0.0017% for confirmed cases; if cases lost to follow-up were all false positives, the rate would be 0.0029%. In the 25 of 55 microdeletions identified by NIPS, a maternal component was identified. Twenty of these cases were associated with a 22q11.2 deletion, four with a 15q deletion, and one with an 8q deletion. In at least 5 cases, deletions were confirmed in the pregnant woman but not in the fetus. Clinical outcomes were unavailable for most pregnancies in which a deletion was not detected. There false negatives were reported, all for 22q11.2 based on phenotypic presentation, but data on false negatives were incomplete. Not all patients had confirmatory testing, so it is not possible to identify all false negatives or false positives accurately.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

There are no direct data on whether sequencing-based testing for microdeletions improves outcomes compared with standard care.

The clinical utility of testing for any particular microdeletion or any panel of microdeletions is uncertain.

There is a potential that prenatal identification of individuals with microdeletion syndromes could improve health outcomes due to the ability to allow for informed reproductive decision making and/or initiate earlier treatment; however, data demonstrating improvement are unavailable. Given the variability of expressivity of microdeletion syndromes and the lack of experience with routine genetic screening for microdeletions, clinical decision making based on genetic test results is not well defined. It is not clear what follow-up testing or treatments might be indicated for screen-detected individuals.

Most treatment decisions would be made after birth, and it is unclear whether testing in utero would lead to earlier detection and treatment of clinical disease after birth. Moreover, clinical decision making when a maternal microdeletion is detected in pregnant women without previous knowledge of a genetic variant is unclear.

Section Summary
Several studies on the clinical validity of microdeletion testing have been published; they are based on large numbers of samples submitted to the testing companies. In a recent systematic review, 7 nonrandomized studies met inclusion criteria, representing 210 cases of microdeletions or microduplications. The overall pooled PPV was 44.1% (95% CI 31.49 to 63.07: range 28.9% to 90.6%). In additional nonrandomized studies, PPV ranged from 13% to 73%. These studies have limitations (e.g., missing data on confirmatory testing, lack of complete data on false negatives).

Maternal plasma DNA sequencing-based tests for fetal microdeletions have been proposed for use in a similar setting as noninvasive screening for fetal aneuploidies. However, there is currently no widely accepted clinical use for screening for microdeletions and microduplications in early pregnancy. Other potential uses are for diagnosis of suspected genetic disorders.

The clinical utility of NIPS for microdeletions is not well-established. Although there is potential for clinical utility in screening for some syndromes associated with microdeletions early in pregnancy, the clinical management changes that would be associated with early diagnosis of these syndromes are not well-established, and the potential for outcome improvements associated with early diagnosis (i.e., before the diagnosis would be suspected on the basis of physical exam findings or findings on routine imaging) is not well-established. The incidence of microdeletions syndromes is low, and not all individuals with a microdeletion will have clinical symptoms.

Noninvasive Screening for Sex Chromosome Aneuploidies Using Cell-Free Fetal DNA

Clinical Context and Test Purpose
The purpose of noninvasive prenatal screening (NIPS) using analysis of cell-free fetal DNA for sex chromosome aneuploidies in pregnancy is to inform a decision whether to proceed with diagnostic testing.

Sex chromosome aneuploidies belong to a group of genetic conditions that are caused or affected by the loss or damage of sex chromosomes (genosomes). This may refer to: 47, XXX; 48, XXXX; 49 XXXXY syndrome; 49, XXXXX; Klinefelter's syndrome, XXY; Turner syndrome, X; XXX gonadal dysgenesis; XX male syndrome; XXYY syndrome; XYY syndrome and occur in approximately 1 in 400 births. These aneuploidies are typically diagnosed postnatally, sometimes not until adulthood, such as during an evaluation of diminished fertility. Alternatively, sex chromosome aneuploidies may be diagnosed incidentally during invasive karyotype testing of pregnant women at high risk for Down syndrome. Potential benefits of early identification (e.g., the opportunity for early management of the manifestations of the condition), must be balanced against potential harms that can include stigmatization and distortion of a family's view of the child.

Populations
The relevant population of interest are individuals with first and second trimester singleton pregnancy.

Interventions
The intervention of interest is non-invasive prenatal screening (NIPS) using analysis of cell free fetal DNA.

Comparators
The following tests are currently being used to make decisions about identifying fetal chromosomal abnormalities: conventional serum and ultrasound screening followed by invasive diagnostic testing as well as standard of care without screening.

Outcomes
The primary outcomes of interest are test accuracy and validity, reductions in miscarriages associated with invasive confirmatory testing, and reduction in use of other noninvasive and invasive tests received by pregnant individuals.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

The Cochrane review by Badeau et. al. (2017) evaluated and compared the diagnostic accuracy of noninvasive prenatal screening (NIPS) for sex chromosome anomalies using massively parallel shotgun sequencing (MPSS) and targeted massively parallel sequencing (TMPS). Common fetal aneuploidies include Down syndrome (trisomy 21 or T21), Edward syndrome (trisomy 18 or T18), Patau syndrome (trisomy 13 or T13), Turner syndrome (45,X), Klinefelter syndrome (47,XXY), Triple X syndrome (47,XXX) and 47,XYY syndrome (47,XYY). Prenatal screening for fetal aneuploidies is standard care in many countries, but current biochemical and ultrasound tests have high false negative and false positive rates. The discovery of fetal circulating cell-free DNA (ccfDNA) in maternal blood offers the potential for genomics-based non-invasive prenatal testing (gNIPT) as a more accurate screening method. Two approaches used for gNIPT are massively parallel shotgun sequencing (MPSS) and targeted massively parallel sequencing (TMPS). The gNIPT results were confirmed by a reference standard such as fetal karyotype or neonatal clinical examination. The database search was from January 2007 to July 2016, the studies could include pregnant women of any age, ethnicity and gestational age with singleton or multifetal pregnancy. The women must have had a screening test for fetal aneuploidy by MPSS or TMPS and a reference standard such as fetal karyotype or medical records from birth. Sixty-five studies of 86,139 pregnant women (3141 aneuploids and 82,998 euploids) were included. No study was judged to be at low risk of bias across the four domains of the QUADAS-2 tool, but applicability concerns were generally low. Of the 65 studies, 42 enrolled pregnant women at high risk, five recruited an unselected population and 18 recruited cohorts with a mix of prior risk of fetal aneuploidy. Among the 65 studies, 44 evaluated MPSS and 21 evaluated TMPS; of these, five studies also compared gNIPT with a traditional screening test (biochemical, ultrasound or both). Forty-six out of 65 studies (71%) reported gNIPT assay failure rate, which ranged between 0% and 25% for MPSS, and between 0.8% and 7.5% for TMPS.In the population of unselected pregnant women, MPSS was evaluated by only one study; the study assessed T21, T18 and T13. TMPS was assessed for T21 in four studies involving unselected cohorts; three of the studies also assessed T18 and 13. In pooled analyses (88 T21 cases, 22 T18 cases, eight T13 cases and 20,649 unaffected pregnancies (non T21, T18 and T13)), the clinical sensitivity (95% confidence interval (CI)) of TMPS was 99.2% (78.2% to 100%), 90.9% (70.0% to 97.7%) and 65.1% (9.16% to 97.2%) for T21, T18 and T13, respectively. The corresponding clinical specificity was above 99.9% for T21, T18 and T13.In high-risk populations, MPSS was assessed for T21, T18, T13 and 45,X in 30, 28, 20 and 12 studies, respectively. In pooled analyses (1048 T21 cases, 332 T18 cases, 128 T13 cases and 15,797 unaffected pregnancies), the clinical sensitivity

(95% confidence interval (CI)) of MPSS was 99.7% (98.0% to 100%), 97.8% (92.5% to 99.4%), 95.8% (86.1% to 98.9%) and 91.7% (78.3% to 97.1%) for T21, T18, T13 and 45,X, respectively. The corresponding clinical specificities (95% CI) were 99.9% (99.8% to 100%), 99.9% (99.8% to 100%), 99.8% (99.8% to 99.9%) and 99.6% (98.9% to 99.8%). In this risk group, TMPS was assessed for T21, T18, T13 and 45,X in six, five, two and four studies. In pooled analyses (246 T21 cases, 112 T18 cases, 20 T13 cases and 4282 unaffected pregnancies), the clinical sensitivity (95% CI) of TMPS was 99.2% (96.8% to 99.8%), 98.2% (93.1% to 99.6%), 100% (83.9% to 100%) and 92.4% (84.1% to 96.5%) for T21, T18, T13 and 45,X respectively. The clinical specificities were above 100% for T21, T18 and T13 and 99.8% (98.3% to 100%) for 45,X. Indirect comparisons of MPSS and TMPS for T21, T18 and 45,X showed no statistical difference in clinical sensitivity, clinical specificity or both. Due to limited data, comparative meta-analysis of MPSS and TMPS was not possible for T13.We were unable to perform meta-analyses of gNIPT for 47,XXX, 47,XXY and 47,XYY because there were very few or no studies in one or more risk groups. The authors concluded, these results show that MPSS and TMPS perform similarly in terms of clinical sensitivity and specificity for the detection of fetal T31, T18, T13 and sex chromosome aneuploidy (SCA). However, no study compared the two approaches head-to-head in the same cohort of patients. The accuracy of gNIPT as a prenatal screening test has been mainly evaluated as a second-tier screening test to identify pregnancies at very low risk of fetal aneuploidies (T21, T18 and T13), thus avoiding invasive procedures. Genomics-based non-invasive prenatal testing methods appear to be sensitive and highly specific for detection of fetal trisomies 21, 18 and 13 in high-risk populations. There is paucity of data on the accuracy of gNIPT as a first-tier aneuploidy screening test in a population of unselected pregnant women. With respect to the replacement of invasive tests, the performance of gNIPT observed in this review is not sufficient to replace current invasive diagnostic tests. We conclude that given the current data on the performance of gNIPT, invasive fetal karyotyping is still the required diagnostic approach to confirm the presence of a chromosomal abnormality prior to making irreversible decisions relative to the pregnancy outcome. However, most of the gNIPT studies were prone to bias, especially in terms of the selection of participants.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No studies identified provided direct evidence of clinical utility that noninvasive prenatal screening (NIPS) using analysis of cell-free fetal DNA change the management of pregnant individuals.

Sex chromosome aneuploidies (e.g., 45 X [Turner syndrome]; 47, XXY, 47, XYY) occur in approximately 1 in 400 live births. These aneuploidies are typically diagnosed postnatally, sometimes not until adulthood, such as during an evaluation of diminished fertility. Alternatively, sex chromosome aneuploidies may be diagnosed incidentally during invasive karyotype testing of pregnant women at high risk for Down syndrome. It is not possible to construct a chain of evidence for clinical utility due to the lack of sufficient evidence on clinical validity and diagnostic challenges noted.

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Section Summary
There is less data on the diagnostic performance of sequencing-based tests for detecting sex chromosome aneuploidies. The available data have suggested that diagnostic performance for detecting these other fetal aneuploidies is not as high as it is for detection of T21, T18, and T13 and there is a higher rate of false-positive tests. The clinical utility of prenatal diagnosis of sex chromosome aneuploidies is uncertain. Potential benefits of early identification (e.g., the opportunity for early management of the manifestations of the condition) must be balanced against potential harms that can include stigmatization and distortion of a family's view of the child.

Noninvasive Prenatal Testing with Cell-Free DNA for Zygosity in Twin Pregnancies

Clinical Context and Test Purpose
The purpose of noninvasive prenatal testing (NIPT) using analysis of cell-free fetal DNA (cfDNA) in individuals who have a twin pregnancy is to inform decisions about early surveillance for twin- to-twin transfusion syndrome (TTTS) and other monochorioinc (MC) twin-related abnormalities.

Populations
The relevant population of interest is individuals with twin pregnancies.

Twin gestations occur in approximately 1 in 30 live births in the United States and have a 4- to 10-fold increased risk of perinatal complications. Monochorionic (MC) twins account for about 20% of twin gestations and are at higher risk of structural defects, miscarriage, preterm delivery, and selective fetal growth restriction compared to dichorionic (DC) twins. Up to 15% of MC twin pregnancies are affected by twin-to-twin transfusion syndrome (TTTS), a condition characterized by relative hypovolemia of 1 twin and hypervolemia of the other. In these twin pregnancies, serial fetal ultrasound examinations are necessary to monitor for development of TTTS as well as selective intrauterine growth restriction because these disorders have high morbidity and mortality and are amenable to interventions that can improve outcomes.

Interventions
The intervention of interest is NIPT to determine zygosity using analysis of cell-free fetal DNA.

NIPT to determine zygosity in twin pregnancies could potentially inform decisions about early surveillance for TTTS and other monochorioinc twin-related abnormalities.

The timing for testing is generally in the first trimester of pregnancy and can be early in the second trimester.

Genetic counseling may also be necessary.

Comparators
Ultrasound examination performed in the first trimester or early second trimester is used to distinguish between monochorionic (MC) and dichorionic (DC) twins.

Outcomes
The primary outcomes of interest are test accuracy and validity, reduction in the use of other noninvasive and invasive tests received by the pregnant individuals, and reduction in morbidity and mortality associated with twin-twin transfusion syndrome and other monochorionic twin-related abnormalities.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Validation Study of Cell-Free Fetal DNA Testing for Twin Zygosity
In 2019, Norwitz et. al. analyzed maternal plasma cell-free DNA samples from twin pregnancies in a prospective blinded study to validate a single-nucleotide polymorphism (SNP)-based non-invasive prenatal test (NIPT) for zygosity, fetal sex, and aneuploidy. Zygosity was evaluated by looking for either one or two fetal genome complements, fetal sex was evaluated by evaluating Y-chromosome loci, and aneuploidy was assessed through SNP ratios. Pregnant women (≥18 years of age) with sonographically confirmed twin pregnancies were enrolled at 21 locations in compliance with local laws and institutional review board-approved protocols. Following informed consent, 20 mL of maternal blood was collected; samples were analyzed at a Clinical Laboratory Improvement Act (CLIA)-certified and College of American Pathologists (CAP)-accredited laboratory (Natera, Inc.; San Carlos, CA, USA) using an SNP-based NIPT methodology. Samples were accumulated from April 2013 to February 2017 and held frozen prior to this prospective trial. All cases and the corresponding independently determined confirmatory data for zygosity, fetal sex, and aneuploidy status of the pregnancies were de-identified prior to NIPT analysis such that the testing was carried out in a blinded manner. The total number of twin pregnancies in the study was 126. Of 126 total twin pregnancies, 95 samples with confirmed zygosity were available. Two of the 95 samples did not receive results due to low fetal fraction. Among the 93

pregnancies that yielded results, monozygotic sensitivity was 100% (29/29) and monozygotic specificity was 100% (64/64). Study limitations include techniques to confirm zygosity varied, unclear if random or consecutive samples and unclear when index testing occurred. These results need to be confirmed in additional, well-conducted studies to draw conclusions about clinical validity.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

There are no direct data on whether cell-free fetal DNA testing for twin zygosity improves outcomes compared with standard care.

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Section Summary
One validation study conducted in 95 twin pregnancies found 100% sensitivity (95% CI 88.1% to 100%) and 100% specificity (95% CI 94.4% to 100%) for determining zygosity. These results need to be confirmed in additional, well-conducted studies to draw conclusions about clinical validity. There are no studies of the clinical utility of NIPT using cell-free fetal DNA to determine zygosity, and the evidence on clinical validity is limited to 1 validation study of fewer than 100 twin pregnancies.

Noninvasive Prenatal Screening using Vanadis NIPT for Chromosomal Trisomies in Singleton Pregnancies

Clinical Context and Test Purpose
The purpose of Vanadis NIPT using cell-free fetal DNA is to screen for fetal chromosomal abnormalities (eg, trisomies 21, 18, 13 [T21, T18, T13]). It can be used as a complement or alternative to conventional serum screening. National guidelines have recommended that all pregnant individuals be offered screening for aneuploidies. Positive cell-free fetal DNA tests need to be confirmed using invasive testing and, if more accurate than standard screening may reduce the need for invasive testing and associated morbidities.

The purpose of Vanadis NIPT using analysis of cell-free fetal DNA in individuals who have singleton pregnancy is to inform a decision whether to proceed with diagnostic testing.

Populations
The relevant population of interest are individuals with first- and second-trimester singleton pregnancy.

Interventions
The intervention of interest is Vanadis NIPT using analysis of cell-free fetal DNA for detection of chromosomal trisomies 21, 18, and 13.

Comparators
The following tests are currently being used to make decisions about identifying fetal chromosomal abnormalities: conventional serum and ultrasound screening followed by invasive diagnostic testing as well as standard of care without screening.

Outcomes
The primary outcomes of interest are test accuracy and validity, reductions in miscarriages associated with invasive confirmatory testing, and reduction in the use of other noninvasive and invasive tests received by the pregnant individuals. The timing for testing is generally in the first trimester of pregnancy and can be early in the second trimester.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

In a proof-of-concept study, Vanadis NIPT analyzed chromosome 21. For the case-control study 2 sample sets were collected; confirmed trisomy 21 pregnancies samples were collected from pregnant women carrying 1 affected fetus, with samples collected in association with termination, and as controls women with euploid singleton pregnancies were collected in association with first trimester screening after gestational week 9. In total 17 samples from pregnancies affected with trisomy 21 were collected and 165 samples from normal pregnancies. Using an age adjusted risk cut-off higher than 1%, all affected and normal samples were classified correctly. Additionally, a prospective high risk sample cohort consisted of plasma samples collected prospectively before invasive testing from singleton pregnancies at week 11-22 classified as high risk for trisomy 21. In total there were 13 positive trisomy 21 pregnancies which all were classified correctly using an age adjusted risk cut-off of 1%. No false positives were recorded. Additional and larger studies are required to demonstrate the application and performance of the Vanadis NIPT assay in a prospectively collected population cohort for screening trisomy 21 and additional chromosomes.

In 2019 the clinical performance of Vanadis NIPT was reported. Maternal plasma samples from 1200 singleton pregnancies from prospectively and retrospectively collected high-risk cohorts were analyzed by Vanadis NIPT with reference outcomes determined by either cytogenetic testing, of amniotic fluid or chorionic villi, or clinical

examination of neonates. Of these samples,158 fetal aneuploidies were identified. Sensitivity was 100% (112/112) for trisomy 21 (95% CI, 96.8%-100%), 89% (32/36) for trisomy 18 (95% CI, 73.9%-96.9%), and 100% (10/10) for trisomy 13 (95% CI, 69.2%-100%); with respective specificities of 100% (95% CI, 99.6%-100%), 99.5% (95% CI, 98.9%-99.8%), and 99.9% (95% CI, 99.5%-100%). There were 5 first pass failures (0.4%), all in unaffected pregnancies. Sex classification was performed on 979 of the samples and 99.6% (975/979) provided a concordant result.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

There are no direct data on whether cell-free fetal DNA testing with Vanadis NIPT for singleton pregnancy improves outcomes compared with standard care.

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Section Summary
One proof of concept study and 1 clinical validation study of Vanadis NIPT have been published. Among 1200 singleton pregnancies, Vanadis NIPT had a sensitivity of 100% (95% CI, 96.8% to 100%) and specificity of 100% (95% CI, 99.6% to 100%) for trisomy 21; the respective values for trisomy 18 were 89% (95% CI, 73.9% to 96.9%) and 99.5% (95% CI, 98.9% to 99.8%), and for trisomy 13 were 100% (95% CI, 69.2% to 100%) and 99.9% (95% CI, 99.5% to 100%). These results need to be confirmed in additional, well-conducted studies to draw conclusions about clinical validity. There are no studies of the clinical utility of Vanadis NIPT using cell-free fetal DNA to determine aneuploidy in singleton pregnancy, and the current evidence is limited to 1 proof of concept study and 1 clinical validation study.

Summary of Evidence
For individuals who have a singleton pregnancy who receive NIPS for T21, T18, and T13 using cell-free fetal DNA, the evidence includes observational studies and systematic reviews. Published studies on available tests and meta-analyses of these studies have consistently demonstrated very high sensitivity and specificity for detecting Down syndrome (T21) in singleton pregnancies. Most studies included only women at high-risk of T21, but several studies have reported similar levels of diagnostic accuracy in average-risk women. Compared with standard serum screening, both the sensitivity and specificity of cell-free fetal DNA screening are considerably higher. As a result,

screening with cell-free fetal DNA for T21 will result in fewer missed cases of Down syndrome, fewer invasive procedures, and fewer cases of pregnancy loss following invasive procedures. Screening for T18 and T13 along with T21 may allow for preparation for fetal demise or termination of the pregnancy prior to fetal loss. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have a twin or multiple pregnancy who receive NIPS for aneuploidies using cell-free fetal DNA, the evidence includes observational studies, nonrandomized studies, and systematic reviews. Studies reported high sensitivity and specificity of NIPS to identify trisomies compared to standard methods. However, the total number of cases of aneuploidy identified in these studies is small resulting in a wide confidence intervals and estimates that are to imprecise to allow conclusions about clinical validity. There is a lack of direct evidence of clinical utility, and a chain of evidence cannot be conducted due to the paucity of evidence on clinical validity. In 2020, the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine updated their practice bulletin regarding screening for fetal chromosomal abnormalities which includes the following: "Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13. Twin fetuses in a single pregnancy each contribute different amounts of cell-free DNA into the maternal circulation. It is possible that an aneuploid fetus would contribute less fetal DNA, therefore masking the aneuploid test result. Recent studies have suggested that sensitivity for trisomy 21 with cell-free DNA in twin pregnancies may be similar to singletons when a test result is returned; however, there is a higher rate of test failure. 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 cell-free DNA is used." The evidence is insufficient to determine the effects of the technology on health outcomes.

For pregnant individuals who receive NIPS for microdeletions using cell-free fetal DNA, there are several studies on the clinical validity of microdeletion testing that have been published; they are based on large numbers of samples submitted to the testing companies. In a recent systematic review, 7 nonrandomized studies met inclusion criteria, representing 210 cases of microdeletions or microduplications. The overall pooled PPV was 44.1% (95% CI 31.49 to 63.07: range 28.9% to 90.6%). In additional nonrandomized studies, PPV ranged from 13% to 73%. These studies have limitations (e.g., missing data on confirmatory testing, lack of complete data on false negatives). The clinical utility of NIPS for microdeletions is not well-established. Although there is potential for clinical utility in screening for some syndromes associated with microdeletions early in pregnancy, the clinical management changes that would be associated with early diagnosis of these syndromes are not well-established, and the potential for outcome improvements associated with early diagnosis (i.e., before the diagnosis would be suspected on the basis of physical exam findings or findings on routine imaging) is not

well-established. The incidence of microdeletions syndromes is low, and not all individuals with a microdeletion will have clinical symptoms. In 2020, the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine updated their practice bulletin regarding screening for fetal chromosomal abnormalities which includes the following: "screening for a limited number of microdeletions with cell-free DNA is available; however, this testing has not been validated clinically and is not recommended." The evidence is insufficient to determine the effects of the technology on health outcomes.

For pregnant individuals who receive NIPS for sex chromosome aneuploidies (SCA) using cell-free fetal DNA, the evidence includes observational studies, mainly in high-risk pregnancies, and systematic reviews. Meta-analyses of available data have suggested high sensitivities and specificities, but the small number of cases makes definitive conclusions difficult. In addition, the clinical utility of identifying sex chromosome aneuploidies during pregnancy is uncertain. No direct comparative evidence was identified that demonstrated cell-free fetal DNA–based screening for SCA resulted in a change in clinical management. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have twin pregnancy who receive noninvasive prenatal testing (NIPT) for twin zygosity using cell-free fetal DNA, the evidence includes one validation study conducted in 95 twin pregnancies found 100% sensitivity (95% CI 88.1% to 100%) and 100% specificity (95% CI 94.4% to 100%) for determining zygosity. This evidence is too limited to draw conclusions about performance characteristics and would need to be confirmed in additional, well-conducted studies. Moreover, the clinical utility of NIPT for twin zygosity compared to standard methods such as ultrasound is unclear and has not been evaluated in published studies. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have a singleton pregnancy who receive NIPS for T21, T18, and T13 using Vanadis NIPT, the evidence includes 2 industry sponsored studies. The available studies on clinical validity have limitations, and the added benefit of Vanadis NIPT compared with current approaches is unclear. Moreover, the clinical utility of Vanadis NIPT remains unclear and has not been evaluated in published studies. Additional, well-conducted studies to draw conclusions about clinical validity and clinical utility are needed. The evidence is insufficient to determine the effects of the technology on health outcomes.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories that offer LDTs must be licensed by CLIA for high complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of noninvasive

prenatal screening tests using cell-free fetal DNA. Commercially available tests include but are not limited to the following:

- **ClariTest Core Non-Invasive Prenatal Screening** (GenPath Diagnostics)**: I**s a non-invasive prenatal screen (NIPS) that identifies the risk for fetal chromosomal abnormalities. ClariTest Core can be performed as early as 10 weeks gestation from a simple blood draw (circulating free DNA). Results are available within five to seven days. This NIPS can be used to screen singleton pregnancies for common trisomies 21, 18 and 13, sex chromosome aneuploidies and microdeletions. Twin gestations can be screened for common trisomies 21, 18 and 13 and for presence of the Y chromosome. Results will be reports as high risk or low risk.
- ****Harmony Prenatal test** (Ariosa Diagnostics, now Roche): Tests for trisomies 21, 18, and 13. The tests uses directed DNA analysis and results are reported as risk score.
- ****InformaSeq prenatal test** (Integrated Genetics, now LabCorp): Tests for trisomies 21, 18, and 13, with optional testing for select sex chromosome abnormalities. Uses Illumina platform and reports results in similar manner.
- **Innatal Prenatal Screen** (Progenity)**:** Tests for trisomies 21, 18, and 13 and sex chromosome disorders using cell free DNA technology. The results are summarized on a single-page report to include fetal fraction, clear result banner (easily triage patients who need additional follow-up), easy to find fetal sex and for patients with positive results, extra care is given to provide risk assessment and next steps i.e., individualized PPV combining test performance with maternal and gestational age and result navigators to guide what the results mean and what happens next.
- ****Invitae Non-invasive Prenatal Screening (NIPS)** (Invitae)**:** Tests for trisomies 21, 18, and 13 in singleton or twin pregnancies as early as 10 weeks using cell-free DNA (cfDNA). Optional add-ons for singleton pregnancy only include microdeletions (1p36 deletion syndrome; DiGeorge syndrome; Angleman/Prader-Willi syndrome; Cri du Chat syndrome; Wolf-Hirschhorn syndrome) and sex chromosome aneuploidies (Turner syndrome; Triple X syndrome; Klinefelter syndrome; Jacob's syndrome).
- ****MaterniT21 PLUS test** (Sequenom Laboratories, now LabCorp)**:** Tests for trisomy 21, 18, and 13 and fetal sex aneuploidies. Their enhanced sequencing series includes testing for trisomies 16 and 22 and 7 microdeletions: 22q deletion syndrome (DiGeorge syndrome), 5p (cri du chat syndrome), 15q (Prader-Willi and Angelman syndromes), 1p36 deletion syndrome, 4p (Wolf-Hirschhorn syndrome), 8q (Langer-Giedion syndrome), and 11q (Jacobsen syndrome). The test uses massive parallel sequencing (MPS) and reports results as positive or negative. The enhanced sequencing series is offered on an opt-out basis.
- ****Nifty Test** (BGI Diagnosis Co.)**:** Tests for trisomies 21,18, and 13 with optional testing for other genetic conditions such as deletion syndromes and sex chromosome aneuploidies as early as 10 weeks of pregnancy.
- ****Panorama prenatal test** (Natera)**:** Tests for detecting trisomies 21, 18, and 13, as well as select sex chromosome abnormalities. Uses single-nucleotide variant

technology; results reported as risk score. An extended panel tests for 5 microdeletions: 22q deletion syndrome (DiGeorge syndrome), 5p (cri du chat syndrome), 15q11-13 (Prader-Willi and Angelman syndromes), and 1p36 deletion syndrome. Screening for 22q11.2 will be included in the panel unless the opt-out option is selected; screening for the remaining 4 microdeletions is offered on an opt-in basis.

- ****Prequel Prenatal Screen** (Myriad)**:** Tests for trisomies 21, 18, and 13 using cell free DNA as early as 10 weeks into pregnancy. This test can also screen for sex chromosome aneuploidies and microdeletions which are optional.
- ****QNatal Advanced** (Quest Diagnostics)**:** Tests for trisomies 21, 18, and 13. Also, when a clear result is seen, fetal sex aneuploidies and select microdeletions will be reported as additional findings.
- **Vanadis NIPT** (PerkinElmer, Inc.; PerkinElmer Genomics): Fetal aneuploidy (trisomy 21, 18 and 13) DNA sequence analysis of selected regions using maternal plasma without fetal fraction cutoff, algorithm reported as a risk score for each trisomy.
- **The VisbiliT test** (Sequenom Laboratories, now LabCorp)**:** tests for trisomy 21 (T21) associated with Down syndrome, trisomy 18 (T18) associated with Edwards syndrome and fetal gender in a single gestation pregnancy. The results are provided as a personalized risk score.
- ****Veracity (NIPD Genetics) tests** for T21, T18, and T13, sex chromosome aneuploidies, and microdeletions.
- ****Verifi prenatal test (Verinata Health, now Illumina):** Tests for trisomies 21, 18, and 13 and fetal sex chromosome aneuploidies. The test uses massive parallel sequencing (MPS) and calculates a normalized chromosomal value [NPS]; reports result as 1 of 3 categories: No Aneuploidy Detected, Aneuploidy Detected, or Aneuploidy Suspected.
- ****Verifi Prenatal Plus (Verinata Health, now Illumina):** Tests for trisomies 21, 18 and 13 with the following options: sex chromosome aneuploidy; all chromosome aneuploidies (including sex chromosome aneuploidies) and select microdeletion syndromes. Results are reported as "Positive: Aneuploidy Detected" or Negative: No Aneuploidy Detected." Results for chromosome 21, 18, 13, X and Y will continue to be reported individually. Results for the remaining chromosomes are reported collectively. Results for positive microdeletion syndrome will be reported as "Results consistent with microdeletion in a certain genomic region."

** These tests offer add-on screening options for microdeletions and sex aneuploidies.

Practice Guidelines and Position Statements

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

In 2020, the American College of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM) updated their joint practice bulletin (No.

226, replaces practice bulletin 163) regarding prenatal screening for fetal chromosomal abnormalities that included the following recommendations:

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to all pregnant women regardless of maternal age or risk of chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.
- If screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously.
- Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing.
- All patients should be offered a second-trimester ultrasound for fetal structural defects since these may occur with or without fetal aneuploidy; ideally this is performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein).
- Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results.
- Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected. The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings become evident such as fetal anomalies identified on ultrasound examination.
- Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.
- If an enlarged nuchal translucency or an anomaly is identified on ultrasound examination, the patient should be offered genetic counseling and diagnostic testing for genetic conditions as well as a comprehensive ultrasound evaluation including detailed ultrasonography at 18–22 weeks of gestation to assess for structural abnormalities.

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test. However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.
- In clinical situations of an isolated soft ultrasonographic marker (such as echogenic cardiac focus, choroid plexus cyst, pyelectasis, short humerus or femur length) where aneuploidy screening has not been performed, the patient should be counseled regarding the risk of aneuploidy associated with the finding and cell-free DNA, quad screen testing, or amniocentesis should be offered. If aneuploidy testing is performed and is low risk, then no further risk assessment is needed. If more than one marker is identified, then genetic counseling, maternal–fetal medicine consultation, or both are recommended.
- 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 incorporated into pretest counseling for patients with multiple gestations.
- Cell-free DNA screening can be performed in twin pregnancies. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13.
- Because preimplantation genetic testing is not uniformly accurate, prenatal screening and prenatal diagnosis should be offered to all patients regardless of previous preimplantation genetic testing.

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- The use of multiple serum screening approaches performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver contradictory risk estimates.
- 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 cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.
- Patients with unusual or multiple aneuploidies detected by cell-free DNA should be referred for genetic counseling and maternal–fetal medicine consultation.

European Society of Human Genetics and American Society of Human Genetics

In 2015, the public and professional policy committee of the European Society of Human Genetics and the social issues committee of the American Society of Human Genetics issued a joint statement on NIPS (also called noninvasive prenatal testing [NIPT]). Relevant recommendations are as follows:

- "NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as cTFS (*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 (including that the DNA sequenced is both maternal and fetal in origin, and that the fetal fraction derives from the placenta as well as the developing fetus). Thus, women should be advised to have a positive result confirmed through diagnostic testing, preferably by amniocentesis, if they are considering a possible termination of pregnancy.

- The better test performance, including lower invasive testing rate of NIPT-based screening should not lead to lower standards for pretest information and counseling. This is especially important in the light of the aim of providing pregnant women with meaningful options for reproductive choice. There should be specific attention paid to the information needs of women from other linguistic and cultural backgrounds or who are less health literate.
- If NIPT is offered for a specific set of conditions (e.g., trisomies 21, 18 and 13), it may not be reasonably possible to avoid additional findings, such as other chromosomal anomalies or large-scale insertions or deletions. As part of pretest information, women and couples should be made aware of the possibility of such additional findings and the range of their implications. There should be a clear policy for dealing with such findings, as much as possible also taking account of pregnant women's wishes with regard to receiving or not receiving specific information.
- Expanding NIPT-based prenatal screening to also report on sex chromosomal abnormalities and microdeletions not only raises ethical concerns related to information and counseling challenges but also risks reversing the important reduction in invasive testing achieved with implementation of NIPT for aneuploidy and is therefore currently not recommended."

National Society of Genetic Counselors

In 2018, the National Society of Genetic Counselors (NSGC) updated their position statement on prenatal cell-free DNA screening, which states: The National Society of Genetic Counselors supports prenatal cell-free DNA (cfDNA) screening, also known as NIPT (noninvasive prenatal testing) or NIPS (noninvasive prenatal screening), as an option for pregnant patients. Because cfDNA screening cannot definitively diagnose or rule out genetic conditions**,** qualified providers should communicate the benefits and limitations of cfDNA screening to patients prior to testing. Many factors influence cfDNA screening performance, therefore it may not be the most appropriate option for every pregnancy.

Prior to undergoing cfDNA screening, patients should have the opportunity to meet with qualified prenatal care providers who can facilitate an individualized discussion of patients' values and needs, including the option to decline all screening or proceed directly to diagnostic testing. Clinicians with expertise in prenatal screening, such as genetic counselors, should provide post-test genetic counseling to patients with increased-risk screening results. Diagnostic testing should be offered to patients with increased-risk results to facilitate informed decision making

American College of Medical Genetics and Genomics
In 2016, the American College of Medical Genetics and Genomics (ACMG) updated their position statement on noninvasive prenatal screening (NIPS) for fetal aneuploidy that includes the following recommendations:

ACMG recommends:

- Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes).
- Referring patients to trained genetics professional when an increased risk of aneuploidy is reported after NIPS.
- Offering diagnostic testing when a positive screening test result is reported after NIPS.
- Providing accurate, balanced up-to-date information, at an appropriate literacy level when a fetus is diagnosed with a chromosomal or genomic variation in an effort to educate prospective parents about the condition of concern. These materials should reflect the medical and psychosocial implications of the diagnosis.

ACMG does not recommend:

- NIPS to screen for autosomal aneuploidies other than those involving chromosomes 13, 18, 21.

ACMG recommends:

- Informing all pregnant women, as part of pretest counseling for NIPS, of the availability of the expanded use of screening for sex chromosome aneuploidies.
- Providers should make efforts to deter patients from selecting sex chromosome aneuploidy screening for the sole purpose of biologic sex identification in the absence of clinical indication for this information.
- Informing patients about the causes and increased possibilities of false-positive results for sex chromosome aneuploidies as part of pretest counseling and screening for these conditions. Patients should also be informed of the potential for results of conditions that, once confirmed, may have a variable prognosis (e.g., Turner syndrome) before consenting to screening for sex chromosomes aneuploidies.
- Referring patients to trained genetics professional when an increased risk of sex chromosome aneuploidy is reported after NIPS.
- Offering diagnostic testing when a positive screening test result is reported after screening for sex chromosome aneuploidies.

ACMG recommends:

- Informing all pregnant women of the availability of the expanded use of NIPS to screen for clinically relevant:

- Obstetric care providers should discuss with their patients the desire for prenatal screening as opposed to diagnostic testing (i.e., CVS or amniocentesis)
- Obstetric care providers should discuss with their patients the desire for maximum fetal genomic information through prenatal screening.
- Obstetric care providers should inform their patients of the higher likelihood of false-positive and false-negative results for these conditions as compared to results obtained when NIPS is limited to common aneuploidy screening.
- Obstetric care providers should inform their patients of the potential for results of conditions that once confirmed may have an uncertain prognosis.
- Referring patients to trained genetics professional when NIPS identifies a CNV.
- Offering diagnostic testing (CVS or amniocentesis) with CMA when NIPS identifies CNV.

ACMG does not recommend:

- NIPS to screen for genome wide CNVs. If this level of information is desired, then diagnostic testing (e.g., chorionic villous sampling or amniocentesis) followed by CMA is recommended.

ACMG recommends:

- In pregnancies with multiple gestations and/or donor oocytes, testing laboratories should be contacted regarding the validity of NIPS before it is offered to the patient as a screening option.

International Society for Prenatal Diagnosis

In 2015, the International Society for Prenatal Diagnosis published a position statement on prenatal diagnosis of chromosomal abnormalities, an update of their 2013 statement. (Note: that a number of the authors of the 2015 report had financial links to industry.) The following is the summary of screening protocol recommendations:

The following protocol options are currently considered appropriate:

1. cfDNA (cell-free DNA) screening as a primary test offered to all pregnant women.
2. cfDNA secondary to a high-risk assessment based on serum and ultrasound screening protocols (options 4-9 below).
3. cfDNA contingently offered to a broader group of women ascertained as having high or intermediate risks by conventional screening. Contingent provision of cfDNA could also include a protocol in which women with very high risks are offered invasive prenatal diagnosis while those with intermediate risk are offered cfDNA.
4. Ultrasound nuchal translucency at 11-13 completed weeks combined with serum markers at 9-13 weeks gestation.

5. Extending option (4) to include other first trimester serum or sonographic markers. Ultrasound performance needs to be prospectively validated by the center where the screening is performed.
6. A contingent test whereby women with borderline risks from option (4) have option (5) at a specialist center and risk is subsequently modified.
7. Four maternal serum markers (quadruple test) at 15-19 weeks, for women who first attend after 13 weeks 6 days gestation.
8. Combining options (4) and (7) in either a stepwise or contingent protocol – provided that all screening test data are included in the final risk assessment. Integrated screening can be offered when CVS is not available. A serum integrated test when NT measurement is unavailable.
9. Contingent second trimester ultrasound to modify risks for aneuploidy for women having options (4), (7), or (8). Ultrasound performance must be prospectively validated by the center where the screening is performed.

Except in exceptional circumstances, the following are not recommended:

1. The use of maternal age as a sole criterion for aneuploidy risk assessment.
2. First trimester measurement of NT with no additional tests.
3. Conventional screening tests for chromosome abnormalities following successful and unambiguous cfDNA screening.

Summary

I. "High sensitivities and specificities are potentially achievable with cfDNA [cell-free DNA] screening for some fetal aneuploidies, notably trisomy 21.
II. Definitive diagnosis of Down syndrome and other fetal chromosome abnormalities can only be achieved through testing on cells obtained by amniocentesis or CVS.
III. The use of maternal age alone to assess fetal Down syndrome risk in pregnant women is not recommended.
IV. A combination of ultrasound NT measurement and maternal serum markers in the first trimester should be available to women who want an early risk assessment and for whom cfDNA screening cannot be provided.
V. A four-marker serum test should be available to women who first attend for their prenatal care after 13 weeks 6 days of pregnancy and where cfDNA screening cannot be provided.
VI. Protocols that combine first trimester and second trimester conventional markers are valid.
VII. Second trimester ultrasound can be a useful adjunct to conventional aneuploidy screening protocols.
VIII. When cfDNA screening is extended to microdeletion and microduplication syndromes or rare trisomies the testing should be limited to clinically significant disorders or well-defined severe conditions. There should be defined estimates for the detection rates, false-positive rates, and information about the clinical significance of a positive test for each disorder being screened."

PRIOR APPROVAL

Not applicable.

POLICY

See Related Medical Policy

- 02.04.50 Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders
- 02.04.82 Noninvasive Fetal RhD Genotyping Using Cell-Free Fetal DNA

Cell-Free Fetal DNA for Fetal Aneuploidy Screening in a Singleton Pregnancy (81420, 81507, 0009M)
Noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for fetal aneuploidy (trisomies 21, 18 and 13) may be considered **medically necessary** in individuals with a singleton pregnancy undergoing screening for fetal aneuploidy.

Cell-Free Fetal DNA for Fetal Aneuploidy Screening in Twin and Multiple Gestation Pregnancies (81420, 81507, 0009M)
Noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for fetal aneuploidy (trisomies 21, 18 and 13) in individuals with a twin or multiple gestation pregnancies undergoing screening for fetal aneuploidy is considered **investigational,** because the evidence is insufficient to determine the effects of this testing on net health outcomes.

Cell-Free Fetal DNA Screening for Fetal Sex Chromosome Aneuploidies (81479, 81599)
Noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for fetal sex chromosome aneuploidies is considered **investigational,** because the evidence is insufficient to determine the effects of this testing on net health outcomes.

Cell Free Fetal DNA Screening for Microdeletions (81422)
Noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for microdeletions is considered **investigational.**

Overall, the evidence base is insufficient to permit definitive conclusions about the performance of NIPS to assess the risk of microdeletion syndromes. Larger, well-designed clinical validity studies assessing test performance and clinical utility studies assessing pregnancy outcomes are needed before this testing can be adopted for routine use in general or average-risk obstetric populations. An updated joint practice bulletin in 2020 by the American College of Obstetricians and Gynecologists (ACOG) and the Society of Maternal-Fetal Medicine (SMFM) states cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time.

The evidence is insufficient to determine the effects of this testing on net health outcomes.

Twin Zigosity (0060U)
Noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for twin zygosity is considered **investigational,** because the evidence is insufficient to determine the effects of this testing on net health outcomes**.**

Vanadis NIPT (0168U)
Noninvasive prenatal screening (NIPS) using Vanadis NIPT of maternal plasma to screen for trisomy 21, 18 and 13 is considered **investigational** in all situations.

The available studies on clinical validity have limitations, and the added benefit of Vanadis NIPT noninvasive prenatal screening compared with current approaches is unclear. Moreover, the clinical utility of Vanadis NIPT noninvasive prenatal screening remains unclear and has not been evaluated in published studies. The evidence is insufficient to determine the effects of the technology on health outcomes.

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81420 Fetal chromosomal aneuploidy (eg, trisomy 21, monosomy X) genomic sequencing analysis panel, circulating cell free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18, and 21
- 81422 Fetal chromosomal microdeletion(s) genomic sequence analysis (e.g., DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free DNA in maternal blood
- 81507 Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma algorithm reported as a risk score for each trisomy
- 81479 Unlisted molecular pathology procedures (may be utilized for noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for sex chromosome aneuploidies)
- 81599 Unlisted multi-analyte assay with algorithmic analysis (may be utilized for noninvasive prenatal screening (NIPS) using cell-free fetal DNA of maternal plasma for sex chromosome aneuploidies)
- 0009M Fetal aneuploidy (trisomy 21 and 18) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as risk score for each trisomy
- 0060U Twin zygosity, genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood (Natera, Inc. Panorama Prenatal Test)

SELECTED REFERENCES

- Hook EB, Cross PK, Schreinemachers DM. Chromosomal abnormality rates at amniocentesis and in live-born infants. JAMA. Apr 15 1983;249(15):2034-2038. PMID 6220164
- American College of Obstetricians and Gynecologists (ACOG). Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol. Jan 2007;109(1):217-227. PMID 17197615
- Ashoor G, Syngelaki A, Poon LC, et al. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound Obstet Gynecol. Jan 2013;41(1):26-32. PMID 23108725
- Blue Cross Blue Shield Association Technology Evaluation Center (BCBSA TEC). Sequencing-based tests to determine fetal down syndrome (trisomy 21) from maternal plasma DNA. TEC Assessment Program. 2013;27(10).
- Blue Cross Blue Shield Association Technology Evaluation Center (BCBSA TEC). Noninvasive maternal plasma sequencing-based screening for fetal aneuploides other than trisomy 21. 2014;In Press.
- Gil MM, Akolekar R, Quezada MS, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. Fetal Diagn Ther. Feb 8 2014;35(3):156-173. PMID 24513694
- Palomaki GE, Deciu C, Kloza EM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med. Mar 2012;14(3):296-305. PMID 22281937
- Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med. Nov 2011;13(11):913-920. PMID 22005709
- Ehrich M, Deciu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol. Mar 2011;204(3):205 e201-211. PMID 21310373
- Bianchi DW, Platt LD, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. May 2012;119(5):890-901. PMID 22362253
- Sehnert AJ, Rhees B, Comstock D, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem. Jul 2011;57(7):1042-1049. PMID 21519036
- Norton ME, Brar H, Weiss J, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol. Aug 2012;207(2):137 e131-138. PMID 22742782
- Ashoor G, Syngelaki A, Wagner M, et al. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol. Apr 2012;206(4):322 e321-325. PMID 22464073

- Sparks AB, Struble CA, Wang ET, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. Apr 2012;206(4):319 e311-319. PMID 22464072
- Nicolaides KH, Syngelaki A, Gil M, et al. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn. Jun 2013;33(6):575-579. PMID 23613152
- Porreco RP, Garite TJ, Maurel K, et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. Am J Obstet Gynecol. Mar 19 2014. PMID 24657131
- Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. Apr 23 2015;372(17):1589-1597. PMID 25830321
- Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol. May 2015;45(5):530-538. PMID 25598039
- Pergament E, Cuckle H, Zimmermann B, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. Obstet Gynecol. Aug 2014;124(2 Pt 1):210-218. PMID 25004354
- Canick JA, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenat Diagn. May 14 2012:1-5. PMID 22585317
- Ohno M, Caughey A. The role of noninvasive prenatal testing as a diagnostic versus a screening tool--a cost-effectiveness analysis. Prenat Diagn. Jul 2013;33(7):630-635. PMID 23674316
- Wapner RJ, Babiarz JE, Levy B, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. Am J Obstet Gynecol. Mar 2015;212(3):332 e331-339. PMID 25479548
- Gross SJ, Stosic M, McDonald-McGinn DM, et al. Clinical Experience with Single-Nucleotide Polymorphism-Based Noninvasive Prenatal Screening for 22q11.2 Deletion Syndrome. Ultrasound Obstet Gynecol. Sep 23 2015. PMID 26396068
- Helgeson J, Wardrop J, Boomer T, et al. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. Prenat Diagn. Oct 2015;35(10):999-1004. PMID 26088833
- Zhao C, Tynan J, Ehrich M, et al. Detection of fetal subchromosomal abnormalities by sequencing circulating cell-free DNA from maternal plasma. Clin Chem. Apr 2015;61(4):608-616. PMID 25710461
- Committee Opinion No. 640: Cell-free DNA Screening for Fetal Aneuploidy. Obstet Gynecol. Jun 29 2015. PMID 26114726
- American College of Obstetricians and Gynecologists (ACOG). Committee Opinion: Noninvasive Prenatal Testing for Fetal Aneuploidy. 2012;

http://www.acog.org/Resources_And_Publications/Committee_Opinions/Committee_on_Genetics/Noninvasive_Prenatal_Testing_for_Fetal_Aneuploidy.

- Dondorp W, de Wert G, Bombard Y, et al. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Summary and recommendations. Eur J Hum Genet. Apr 1 2015.
- PMID 25828867
- Devers PL, Cronister A, Ormond KE, et al. Noninvasive prenatal testing/noninvasive prenatal diagnosis: the position of the National Society of Genetic Counselors. J Genet Couns. Jun 2013;22(3):291-295. PMID 23334531
- Gregg AR, Gross SJ, Best RG, et al. ACMG statement on noninvasive prenatal screening for fetal aneuploidy. Genet Med. May 2013;15(5):395-398. PMID 23558255
- Benn P, Borrell A, Chiu RW, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. Aug 2015;35(8):725-734. PMID 25970088
- Benn P, Borell A, Chiu R, et al. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. Jul 2013;33(7):622-629. PMID 23616385
- UpToDate. Prenatal Screening for Down Syndrome Using Cell-Free DNA. Glenn E. Palomaki, PhD, Geralyn M Messerlian, PhD, Jacquelyn V Halliday, MS, Topic last updated January 4, 2016.
- UpToDate. Prenatal Screening for Common Aneuploidies Using Cell Free DNA. Gleen E. Palomaki PhD, Geralyn M. Messerlian PhD, Jacquelyn V. Halliday M.S. Topic last updated January 7, 2019.
- UpToDate. Down Syndrome Overview of Prenatal Screening. Geralyn M. Messerlian PhD, Gleen E. Palomaki PhD. Topic last updated April 28, 2016.
- Gregg A, Skotko B, Benkendorf J, et. al. Noninvasive prenatal screening for fetal aneuploidy 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genetics in Medicine online publication July 28, 2016 doi:10.1038/gim.2016.97
- Ashoor G, Syngelaki A, Poon LC, et al. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. Ultrasound Obstet Gynecol. Jan 2013;41(1):26-32. PMID 23108725
- Gil MM, Akolekar R, Quezada MS, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. Fetal Diagn Ther. Feb 8 2014;35(3):156-173. PMID 24513694
- Sehnert AJ, Rhees B, Comstock D, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem. Jul 2011;57(7):1042-1049. PMID 21519036
- Sparks AB, Struble CA, Wang ET, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol. Apr 2012;206(4):319 e311-319. PMID 22464072

- Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. Apr 23 2015;372(17):1589-1597. PMID 25830321
- Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. Ultrasound Obstet Gynecol. May 2015;45(5):530-538. PMID 25598039
- Wapner RJ, Babiarz JE, Levy B, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. Am J Obstet Gynecol. Mar 2015;212(3):332 e331-339. PMID 25479548
- Gross SJ, Stosic M, McDonald-McGinn DM, et al. Clinical Experience with Single-Nucleotide Polymorphism-Based Noninvasive Prenatal Screening for 22q11.2 Deletion Syndrome. Ultrasound Obstet Gynecol. Sep 23 2015. PMID 26396068
- Helgeson J, Wardrop J, Boomer T, et al. Clinical outcome of subchromosomal events detected by whole-genome noninvasive prenatal testing. Prenat Diagn. Oct 2015;35(10):999-1004. PMID 26088833
- Zhao C, Tynan J, Ehrich M, et al. Detection of fetal subchromosomal abnormalities by sequencing circulating cell-free DNA from maternal plasma. Clin Chem. Apr 2015;61(4):608-616. PMID 25710461
- Nicolaides KH, Syngelaki A, Ashoor G, et. al. Noninvasive prenatal testing for fetal trisomies in a routinely screening first-trimester population. Am J Obstet Gynecol Nov 2012;207(5):374 e371-376. PMID 23107079
- Mackie FL, Hemming K, Allen S, et. al. The accuracy of cell-free fetal DNA based on non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. BJOG Jan 2017;124(1):32-46. PMID 27245374
- Taylor-Phillips S, Freeman K, Geppert J, et. al. Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards, and Patau syndromes: a systematic review and meta-analysis. BMJ Open Jan 18 2016;6(1):e010002. PMID 26781507
- Iwarsson E, Jacobsson B, Dagerhamn J, et. al. Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18, and 13 in general pregnant population and in a high risk population – a systematic review and meta-analysis. Acta Obstet Gynecol Scan. Jan 2017;96(1):7-18. PMID 27779757
- Gil MM, Accurti V, Santacruz B, et. al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies updated meta-analysis. Ultrasound Obstet Gynecol Apr 11 2017. PMID 28397325
- Du E, Feng C, Cao Y, et. al. Massively parallel sequencing (MPS) of cell-free fetal DNA (cfDNA) for trisomies 21, 18, and 13 in twin pregnancies. Twin Res Hum Genet. Jun 2017;20(3):242-249. PMID 28485265
- Fosler L, Winters P, Jones KW, et. la. Aneuploidy screening by noninvasive prenatal testing in twin pregnancy. Ultrasound Obstet Gynecol. Apr 2017;49(4):470-477. PMID 27194226
- Society for Maternal-Fetal Medicine Publications Committee. SMFM Statement: Clarification of recommendations regarding cell-free DNA aneuploidy screening. Am J Obstet Gynecol. Dec 2015;213(6):753-754. PMID 26458766

- The American College of Obstetrician and Gynecologists and Society for Maternal-Fetal Medicine, Practice Bulletin 163 Screening for Fetal Aneuploidy. Obstet Gynecol. May 2016;127(5):979-981. PMID 27101120
- Natera Panorama.
- Sequenom MaterniT21 Plus.
- Harmony Prenatal Test.
- Illumina Verifi Prenatal and Verifi Plus Prenatal Testing. Also available at https:.www.illumina.com
- LabCorp InformaSeq non-invasive prenatal test.
- Quest Diagnostics QNatal Advanced.
- Sequenom VisibiliT
- Chitty LS, Hudgins L, Norton ME. Current controversies in prenatal diagnosis 2: Cell-free DNA prenatal screening should be used to identify all chromosome abnormalities. Prenat Diagn. Feb 2018;38(3):160-165
- Badeau M, Lindsay C, Blais J, et. al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. Cochrane Database Syst Rev. Nov 10 2017;11:CD011767. PMID 29125628
- Liao H, Liu S, Wang H. Performance of non-invasive prenatal screening for fetal aneuploidy in twin pregnancies: a meta-analysis. Prenat Diagn Sep 2017;37(9):874-882. PMID 28728213
- Petersen AK, Cheung SW, Smith JL, et. al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. Am J Obstet Gynecol. Dec 2017;217(6):691. PMID 29032050
- Invitae Non-Invasive Prenatal Screening.
- NIFTY Non-Invasive Prenatal Screening.
- Myriad Prequel Prenatal Screen.
- Innatal Prenatal Screen.
- ClariTest Non-Invasive Prenatal Screening.
- Liao H, Liu S, Wang H. Performance of non-invasive prenatal screening for fetal aneuploidy in twin pregnancies: a meta-analysis. Prenat Diagn 2017 Sep;37(9):874-882. PMID 28728213
- Gil MM, Galeva S, Jani J, et. al. Screening for trisomies by cfDNA testing of maternal blood in twin pregnancy: update of the Fetal Medicine Foundation results and meta-analysis. Ultrasound Obstet Gynecol 2019 Jun;53(6):734-742. PMID 31165549
- 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 Gyncol 2016 Feb 47(2):177-83. PMID 26396068
- Rose N, Kaimal A, Dugoff L, et. al. Screening for Fetal Chromosomal Abnormalities ACOG Practice Bulletin Number 226. October 2020 Volume 136 and Issue 4

- Norwitz ER, McNeill G, Kalyan A, et al. Validation of a Single-Nucleotide Polymorphism-Based Non-Invasive Prenatal Test in Twin Gestations: Determination of Zygosity, Individual Fetal Sex, and Fetal Aneuploidy. J Clin Med. Jun 28 2019; 8(7). PMID 31261782
- Chitty LS, Hudgins L, Norton ME. Current controversies in prenatal diagnosis 2: Cell-free DNA prenatal screening should be used to identify all chromosome abnormalities. Prenat Diagn. Feb 2018; 38(3): 160-165. PMID 29417608
- Gil MM, Galeva S, Jani J, et al. Screening for trisomies by cfDNA testing of maternal blood in twin pregnancy: update of The Fetal Medicine Foundation results and meta-analysis. Ultrasound Obstet Gynecol. Jun 2019; 53(6): 734-742. PMID 31165549
- Dyr B, Boomer T, Almasri EA, et al. A new era in aneuploidy screening: cfDNA testing in 30,000 multifetal gestations: Experience at one clinical laboratory. PLoS ONE. 2019; 14(8): e0220979. PMID 31393959
- Kypri E, Ioannides M, Touvana E, et al. Non-invasive prenatal testing of fetal chromosomal aneuploidies: validation and clinical performance of the veracity test. Mol Cytogenet. 2019; 12: 34. PMID 31338126
- Motevasselian M, Saleh Gargari S, Younesi S, et al. Non-invasive prenatal test to screen common trisomies in twin pregnancies. Mol Cytogenet. 2020; 13: 5. PMID 32042312
- Ericsson O, Ahola T, Dahl F, et al. Clinical validation of a novel automated cell-free DNA screening assay for trisomies 21, 13, and 18 in maternal plasma. Prenat Diagn. 2019;39(11):1011-1015. doi:10.1002/pd.5528
- Benn P, Valenti E, Shah S, et al. Factors associated with informative redraw after an initial no result in noninvasive- prenatal testing. Obstet Gynecol. 2018 Aug;132(2):428-435
- Benn P, Martin K, McKanna T, et al. Combining the use of a fetal fraction-based algorithm and probability of an informative redraw in noninvasive prenatal testing for fetal aneuploidy. J Genet Couns. 2019; 00:1-7
- 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
- Chen YP, He ZQ, Shi Y, et al. Not all chromosome aberrations can be detected by NIPT in women at advanced maternal age: a multicenter retrospective study. Clin Chim Acta. 2018 Nov;486:232-236
- Chibuk J, Rafalko J, Boomer T, et al. Cell-free DNA screening in twin pregnancies: A more accurate and reliable screening tool. Prenatal diagnosis. 2020; 1-9
- 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
- Dahl F, Ericsson O, Karlberg O, et al. Imaging single DNA molecules for high precision NIPT. Sci Rep. 2018 Mar 14;8(1):4549
- Guy C, Haji-Sheikhi F, Rowland CM, et al. Prenatal cell-free DNA screening for fetal aneuploidy in pregnant women at average or high risk: results from a large US clinical laboratory. Mol Genet Genomic Med. 2019 Mar;7(3):e545

- Hong SY, Shim SH, Park HJ, et al. Experiences and efficacy of noninvasive prenatal test using maternal plasma in a single center: 1,591 cases. J Genet Med 2020; 17(1):11-1
- Hu H, Wang L, Wu J, et al. Noninvasive prenatal testing for chromosome aneuploidies and subchromosomal microdeletions/microduplications in a cohort of 8141 single pregnancies. Hum Genomics. 2019 Mar 12;13(1):1
- Jelsema R, Blake S, Prins S, et al. Collective utility of NIPT zygosity determination and chorionicity assignment via ultrasound. Am J Obstet Gynecol. 2019; 220(1):S592
- Jones KJ, Wang E, Bogard P, et al. Targeted cell-free DNA analysis with microarray quantitation for assessment of fetal sex and sex chromosome aneuploidy risk. Ultrasound Obstet Gynecol. 2018 Feb;51(2):275-276
- Ontario Health Technology Assessment Series. Noninvasive prenatal testing for trisomies 21, 18, and 13, sex chromosome aneuploidies, and microdeletions: a Health Technology Assessment. 2019;19(4):1-166
- 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
- Schwartz S, Kohan M, Pasion R, et al. Clinical experience of laboratory follow-up with noninvasive prenatal testing using cellfree DNA and positive microdeletion results in 349 cases. Prenat Diagn. 2018 Feb;38(3):210-218
- Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin Summary, Number 226. Obstet Gynecol. Oct 2020; 136(4): 859-867. PMID 32976375
- Soster E, Boomer T, Hicks S, et al. Three years of clinical experience with a genome-wide cfDNA screening test for aneuploidies and copy-number variants. Genet Med. Jul 2021; 23(7): 1349-1355. PMID 33731879
- Familiari A, Boito S, Rembouskos G, et al. Cell-free DNA analysis of maternal blood in prenatal screening for chromosomal microdeletions and microduplications: a systematic review. Prenat Diagn. Mar 12 2021. PMID 33710639
- La Verde M, De Falco L, Torella A, et al. Performance of cell-free DNA sequencing-based non-invasive prenatal testing: experience on 36,456 singleton and multiple pregnancies. BMC Med Genomics. Mar 30 2021; 14(1): 93. PMID 33785045
- Van Den Bogaert K, Lannoo L, Brison N, et al. Outcome of publicly funded nationwide first-tier noninvasive prenatal screening. Genet Med. Jun 2021; 23(6): 1137-1142. PMID 33564150
- Khalil A, Archer R, Hutchinson V, et al. Noninvasive prenatal screening in twin pregnancies with cell-free DNA using the IONA test: a prospective multicenter study. Am J Obstet Gynecol. Jul 2021; 225(1): 79.e1-79.e13. PMID 33460583
- Cheng Y, Lu X, Tang J, et al. Performance of non-invasive prenatal testing for foetal chromosomal abnormalities in 1048 twin pregnancies. Mol Cytogenet. Jun 30 2021; 14(1): 32. PMID 34193223

- Xu Y, Jin P, Lei Y, et al. Clinical Efficiency of Non-invasive Prenatal Screening for Common Trisomies in Low-Risk and Twin Pregnancies. Front Genet. 2021; 12: 661884. PMID 3404063
- Judah H, Gil MM, Syngelaki A, et al. Cell-free DNA testing of maternal blood in screening for trisomies in twin pregnancy: updated cohort study at 10-14 weeks and meta-analysis. Ultrasound Obstet Gynecol. Aug 2021; 58(2): 178-189. PMID 33838069
- UpToDate. Chromosomal translocations, deletions and inversions. Iris Schrijver M.D., James L. Zehnder M.D., Topic last updated May 21, 2021. Also available at https://www.uptodate.com
- Madankumar R, Edwards L, Strong N, et. al., Cell-free DNA screening for prenatal detection of 22q11.2 deletion syndrome. American Journal of Obstetrics and Gynecology (2022), doi: https://doi.org/10.1016/ j.ajog.2022.01.002

POLICY HISTORY

Date	Reason	Action
February 2022	Annual Review	Policy Revised
February 2021	Annual Review	Policy Revised
February 2020	Annual Review	Policy Renewed
February 2019	Annual Review	Policy Revised
February 2018	Annual Review	Policy Revised
February 2017	Annual Review	Policy Revised
February 2016		New Policy

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

Wellmark Blue Cross and Blue Shield
Medical Policy Analyst
PO Box 9232
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