***Current Policy Effective Date 1/1/25**
(See policy history boxes for previous effective dates)

Title: Genetic Testing-Noninvasive Prenatal Screening For Fetal Aneuploidies, Microdeletions, Single-Gene Disorders, and Twin Zygosity Using Cell-Free Fetal DNA

Description/Background

National guidelines recommend that all pregnant individuals be offered screening for fetal chromosomal abnormalities, most of which are aneuploidies, an abnormal number of chromosomes. Trisomy syndromes are aneuploidies involving 3 copies of 1 chromosome. Trisomies 21, 18, and 13 are the most common forms of fetal aneuploidy that survive to birth. There are numerous limitations to standard screening for these disorders using the maternal serum and fetal ultrasound. Noninvasive prenatal screening analyzing fetal cell-free DNA (cfDNA) 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. Prenatal testing for twin zygosity using fetal cfDNA 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. The trisomy syndromes are aneuploidies involving three copies of one chromosome. The most important risk factor for trisomy syndromes is maternal age. The approximate risk of a trisomy 21 (T21; Down syndrome)-affected birth is 1 in 1100 at age 25 to 29. The risk of a fetus with T21 (at 16 weeks of gestation) is about 1 in 250 at age 35 and 1 in 75 at age 40.[1]

T21 is the most common chromosomal aneuploidy. 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. Detection of T18 and T13 early in pregnancy can facilitate preparation for fetal loss or early intervention.

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 a trisomy syndrome, direct karyotyping of fetal tissue obtained by amniocentesis or chorionic 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 risks of fetal injury, fetal loss and infection. A new screening strategy that reduces unnecessary amniocentesis and CVS procedures or increases detection of T21, T18, and T13 could improve outcomes. Confirmation of positive noninvasive screening tests with amniocentesis or CVS is recommended. Amniocentesis might be preferred over CVS for confirming cell-free DNA positive results due to the potential for placental mosaicism leading to false positive results.[2,3] With more accurate screening tests, fewer individuals would receive positive screening results.

Commercial, noninvasive, sequencing-based testing of maternal serum for fetal trisomy syndromes is now available. The testing technology involves the detection of cell-free fetal DNA fragments present in the plasma of pregnant women. As early as eight to ten 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 tests are unable to provide a result if the fetal fraction is too low (i.e., <4%). The fetal fraction can be affected by maternal and fetal characteristics. For example, the 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- to 10-fold increased risk of perinatal complications.[4] Dizygotic or "fraternal" twins occur from ovulation and fertilization of two oocytes, which results in dichorionic (DC) placentation and two 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.[4] Up to 15% of MC twin pregnancies are affected by twin to twin transfusion syndrome (TTTS), a condition characterized by relative hypovolemia of one twin and hypervolemia of the other.[5] 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.[5] 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.[5]

Single-Gene Disorders

Single-gene disorders (also known as monogenic disorders) are caused by a variation in a single gene. Individually, single-gene disorders are rare, but collectively are present in approximately 1% of births. The Vistara Single-Gene Disorder Test panel screens for 25 conditions that result from variants across 30 genes, which have a combined incidence of 1 in 600 (0.17%).[6] These include Noonan syndrome and other Noonan spectrum disorders, skeletal disorders (e.g., osteogenesis imperfecta, achondroplasia), craniosynostosis syndromes, Cornelia de Lange syndrome, Alagille syndrome, tuberous sclerosis, epileptic encephalopathy, SYNGAP1-related intellectual disability, CHARGE syndrome, Sotos syndrome, and Rett syndrome. The UNITY Fetal Risk Screen™ provides maternal carrier testing for several autosomal recessive conditions (alpha and beta-thalassemia, cystic fibrosis, sickle cell disease, and spinal muscular atrophy) followed by reflex single-gene NIPT of the fetus when a maternal carrier is identified. The clinical presentation and severity of these disorders can vary widely. Some, but not all, can be detected by prenatal ultrasound examination.

Cell-Free 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 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 fetal 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 UNITY Fetal Risk Screen™ employs a proprietary molecular counting method called the Quantitative Counting Template to determine the number of input DNA molecules when sequencing. Quantitative counting templates are inserted into the maternal cfDNA specimen, which is designed to co-amplify at the same rate as the corresponding gene of interest and can be used to calculate the number of genes of interest.

The second general approach is single nucleotide variant-based methods. They use 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. At least some of the commercially available cell-free DNA prenatal tests also test for other abnormalities including sex chromosome abnormalities and selected microdeletions.

A newer approach to cfDNA testing called the Vanadis NIPT does not involve polymerase chain reaction (PCR) amplification or sequencing. The procedure consists of the digestion of cfDNA using a restriction enzyme. The digested cfDNA is then hybridized and ligated to chromosome-specific DNA probes forming a circular DNA. All non-circular DNA is removed by exonuclease treatment. Finally, the circular DNA containing the cfDNA is amplified with rolling circle amplification to form rolling circle products that are labeled with chromosome-specific fluorescently labeled DNA probes. The fluorescently labeled rolling circle products are imaged and counted with an automated microscopy scanner. The microscope takes multiple images from each well with different spectral filters, i.e. each wavelength range presents a specific chromosome. With image analysis algorithms, the fluorescently labeled rolling circle products are counted for each sample. The ratio between the number of chromosome-specific rolling circle products is then transferred to risk calculation software to calculate the likelihood of a trisomy. Currently, Vanadis NIPT provides results for trisomy 21, trisomy 18 and trisomy 13, and fetal sex determination.

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 one and three megabases long. Along with microduplications, microdeletions are collectively known as copy number variants. Copy number variants can lead to disease when the change in the 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 (eg, 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 haplo-insufficient 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) deletion is the most common microdeletion 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

Adapted from Chitty et al (2018).[7]

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. For those who do have prenatal screening for microdeletion syndromes, diagnostic testing is necessary to confirm positive results. Diagnostic testing is generally done by chorionic villus sampling (CVS) or amniocentesis. CVS uses placental cells collected for genetic evaluation under ultrasound guidance without entering the amniotic sac. Diagnostic amniocentesis uses a small sample of the fluid that surrounds the fetus, which contains cells that are shed primarily from the fetal skin, bladder, gastrointestinal tract, and amnion. Confined placental mosaicism can cause false-positive cell-free DNA results, and as such, amniocentesis might be preferred over CVS for diagnostic testing in cases of positive cell-free DNA. Both CVS and amniocentesis procedures increase the risk for miscarriage.[3,2]

Samples are analyzed using fluorescence in situ hybridization, chromosomal microarray analysis, or karyotyping. Additionally, families at risk (eg, 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 (eg, 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.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Act 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:

- **Myriad Prequel™ Prenatal Screen** (Myriad Women's Health, Counsyl) utilizes whole genome sequencing for detecting aneuploidy including T21, T18, T13
- **MaterniT21™** PLUS (Sequenom Laboratories) core test includes T21, T18, and T13 and fetal sex aneuploidies. The enhanced sequencing series includes testing for T16 and T22 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 massively parallel sequencing (MPS) and reports results as positive or negative. The enhanced sequencing series is offered on an opt-out basis.
- **Harmony™** (Ariosa Diagnostics, now Roche) tests for T21, T18, and T13. The test uses directed DNA analysis and results reported as risk score.
- **Panorama™** (Natera) is a prenatal test for detecting T21, T18, and T13, as well as select sex chromosome abnormalities. It uses single-nucleotide variant technology; results reported as risk score. An extended panel tests for five 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 four microdeletions is offered on an opt-in basis.
- **Verifi®** (Verinata Health, now Illumina) is a prenatal test for T21, T18, and T13. The test uses MPS and calculates a normalized chromosomal value, reporting results as one of three categories: no aneuploidy detected, aneuploidy detected, or aneuploidy suspected.
- **InformaSeq℠** (Integrated Genetics, now LabCorp) is a prenatal test for detecting T21, T18, and T13, with optional additional testing for select sex chromosome abnormalities. It uses the Illumina platform and reports results in a similar manner.

- **QNatal™ Advanced** (Quest Diagnostics) tests for T21, T18, and T13.
- **Prenatal cell-free DNA screening** (or cfDNA screening, often referred to as NIPT or noninvasive prenatal testing) (Mayo Medical Laboratories) tests for aneuploidy including Down syndrome (trisomy 21), Patau syndrome (trisomy 13), and Edward syndrome (trisomy 18). Fetal sex will be reported.
- **UNITY Fetal Risk Screen™ (BillionToOne)** Obstetrics (single-gene noninvasive prenatal test), cellfree DNA sequence analysis of 1 or more targets (eg, CFTR, SMN1, HBB, HBA1, HBA2) to identify paternally inherited pathogenic variants, and relative mutation-dosage analysis based on molecular counts to determine fetal inheritance of maternal mutation, algorithm reported as a fetal risk score for the condition (eg, cystic fibrosis, spinal muscular atrophy, beta hemoglobinopathies [including sickle cell disease], alpha thalassemia).
- **UNITY Aneuploidy™ Screen (BillionToOne)** tests for T21, T18, T13, sex chromosome aneuploidy, fetal sex (optional), zygosity (included for twins), and 22q11.2 microdeletion (optional).
- **Vanadis NIPT Solution** (PerkinElmer) tests for T21, T18, T13, and fetal sex determination, using digestion of cell-free DNA using a restriction enzyme to calculate a likelihood of a trisomy.
- **Veracity** (NIPD Genetics) tests for T21, T18, and T13, sex chromosome aneuploidies, and microdeletions.
- **VisibiliT** (Sequenom Laboratories, now LabCorp) tests for T21 and T18, and tests for sex.
- **Vistara™** Single-Gene NIPT tests 25 autosomal dominant and X-linked conditions across 30 genes.
- **Vasistera™** NIPT is a blood-based genetic, prenatal screening test of the pregnant person that screens for common chromosomal conditions that affect a baby's health. Vasistera screens for trisomy 21, trisomy 18, and trisomy 13. Fetal sex reporting is optional.

Medical Policy Statement

The safety and effectiveness of noninvasive prenatal screening for fetal aneuploidies using cell-free fetal DNA have been established. It may be considered a useful diagnostic option when indicated.

The peer reviewed medical literature has not demonstrated the clinical utility of noninvasive prenatal screening for microdeletions and single gene disorders using cell-free fetal DNA. Therefore, this service is considered experimental/investigational.

Inclusionary and Exclusionary Guidelines

Inclusions:

- Nucleic acid sequencing-based testing of maternal plasma to screen for trisomy 21, 18, and 13 in individuals or any combination of the three with singleton and twin pregnancies.

(Karyotyping would be necessary to exclude the possibility of a false positive nucleic acid sequencing-based test.)

- Nucleic acid sequencing-based testing of maternal plasma for fetal sex or fetal sex chromosome aneuploidy only when certain fetal abnormalities are noted on ultrasound such as cases of ambiguous genitalia or cystic hygroma when the determination of fetal sex is necessary to help guide medical management.

Exclusions:

- Nucleic acid sequencing-based testing of maternal plasma for trisomy 21, 18, and 13 in individuals with pregnancies of multiple gestations of 3 or more fetuses.
- Nucleic acid sequencing-based testing of maternal plasma for fetal sex determination and/or fetal sex chromosome aneuploidies other than the situation specified above.
- Nucleic acid sequencing-based testing of maternal plasma for microdeletions.
- Nucleic acid sequencing-based testing of maternal plasma for twin zygosity.
- Vanadis® NIPT of maternal plasma to screen for trisomy 21, 18 and 13.
- NIPT of maternal plasma to screen for single-gene disorders (e.g. Vistara or UNITY Fetal Risk Screen™).
- Nucleic acid sequencing-based testing of maternal plasma, other than in the situations specified above.

Policy Guidelines:

Karyotyping would be necessary to exclude the possibility of a false-positive, nucleic acid sequencing–based test. Before testing, individuals should be counseled about the risk of a false-positive test. In Committee Opinion No. 640, the American College of Obstetricians and Gynecologists (2015) recommended that all patients receive information on the risks and benefits of various methods of prenatal screening and diagnostic testing for fetal aneuploidies, including the option of no testing.

Studies published to date on noninvasive prenatal screening for fetal aneuploidies have reported rare but occasional false-positives. False-positive findings have been found to be associated with factors including placental mosaicism, vanishing twins, and maternal malignancies. Diagnostic testing is necessary to confirm positive cell-free fetal DNA tests, and management decisions should not be based solely on the results of cell-free fetal DNA testing. The American College of Obstetricians and Gynecologists further recommended that patients with indeterminate or uninterpretable (i.e., “no call”) cell-free fetal DNA test results be referred for genetic counseling and offered ultrasound evaluation and diagnostic testing because “no call” findings have been associated with an increased risk of aneuploidy.

Cell-free DNA screening does not assess risk of neural tube defects. Individuals should continue to be offered ultrasound or maternal serum alpha-fetoprotein screening.

GENETIC COUNSELING

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81420	81479[a]	81507	81599[b]	0327U

[a] If the codes above do not apply and the test does not involve an algorithmic analysis [when specified as cell-free fetal DNA-based prenatal testing involving multianalyte assays and not involving an algorithmic analysis for fetal aneuploidy]

[b] If the codes above do not apply and the test involves multianalyte assays and an algorithmic analysis [when specified as cell-free fetal DNA-based prenatal testing involving multianalyte assays and an algorithmic analysis for fetal aneuploidy]

Other codes (investigational, not medically necessary, etc.):

81422	81479[c]	0060U	0489U

[c] When code 81479 represents Vistara™ Single-Gene NIPT test.

Rationale

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

NONINVASIVE PRENATAL SCREENING FOR CHROMOSOMAL TRISOMIES IN SINGLETON PREGNANCIES

Clinical Context and Test Purpose

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

The following PICO was used to select literature to inform this review.

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

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 screening with diagnostic testing as needed or standard 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).

REVIEW OF EVIDENCE
A Cochrane review by Badeau et al (2017) included 65 studies on the screening of women with a singleton pregnancy (Table 2).[8] None of the studies was rated at low-risk of bias, although they were considered to have a low bias in the domains of the index test and reference standard. Results were assessed separately for massively parallel sequencing (MPS) and targeted MPS (TMPS), for unselected pregnant women and high-risk women, and for T21, T18, and T13 (Tables 3 and 4). For both unselected and high-risk pregnant women, sensitivity for T21 was 99.2% or higher and specificity was 99.9% or higher.

Adding screening for T18 and T13 resulted in an overall sensitivity of 94.9% in unselected pregnant women and 98.8% in high-risk women. Specificity was 99.9% for both groups. Reviewers calculated that out of 100,000 high-risk pregnancies, 5851 would be affected by T21, T18, or T13. Of these 5781 (MPS) and 5787 (TMPS) would be detected and 70 (MPS) and 64 (TMPS) cases would be missed (Table 4). Of the 94,149 unaffected women, 94 would undergo an unnecessary invasive test. Reviewers concluded that the performance of the nucleic acid sequencing–based test was sensitive and highly specific to detect fetal trisomies T21, T18, and T13 in high-risk women but was not sufficient to replace current invasive diagnostic tests. Available data were considered insufficient to evaluate diagnostic performance in an unselected population.

Table 2. Characteristics of Systematic Reviews

					No. of Studies Rated as "High" or "Unclear" Risk of Bias		
Study	**No. of Studies**	**Study Populations**	**Designs of Studies**	**Reference Standard of Studies**	**No Domains**	**1-2 Domains**	**>2 Domains**

Badeau et al (2017)[8]	65	Women with singleton pregnancy	RCTs, cohort studies, case-control	Fetal karyotyping or neonatal clinical examination	0	41	24

Table 3. Systematic Review Results for Unselected Pregnant Women

Test	Affected Pregnancies (Unaffected Pregnancies)	Sensitivity (95% CI), %	Specificity (95% CI), %	FN per 100,000 Cases	FP per 100,000 Cases	Disease Prevalence (95% CI)
T21 MPS	8 (1733)	100 (67.6 to 100)	100 (99.8 to 100)	0	0	0.46 (0.24 to 5.21)
T21 TMPS	88 (20,679)	99.2 (78.2 to 100)	100 (>99.9 to 100)	4	0	
T18 MPS	2 (1739)	100 (34.3 to 100)	99.9 (99.7 to 100)	0	100	0.11 (0.06 to 0.36)
T18 TMPS	22 (20,553)	90.9 (70.0 to 97.7)	100 (99.9 to 100)	10	0	
T13 MPS	1 (1740)	100 (20.7 to 100)	100 (99.8 to 100)	0	0	0.12 (0.01 to 0.52)
T13 TMPS	8 (14,154)	65.1 (9.16 to 97.2)	100 (99.9 to 100)	41	0	
T21, T18, T13 MPS	11 (1730)	100 (74.1 to 100)	99.9 (99.8 to 99.9)	0	99	0.63 (0.32 to 5.73)
T21, T18, T13 TMPS	118 (20,649)	94.9 (89.1 to 97.7)	99.9 (99.8 to 99.9)	32	99	

CI: confidence interval; FN: false-negative (missed cases); FP: false-positive; MPS: massively parallel sequencing; TMPS: targeted massively parallel sequencing; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

Table 4. Systematic Review Results for High-Risk Pregnant Women

Test	Affected Pregnancies (Unaffected Pregnancies)	Sensitivity (95% CI), %	Specificity (95% CI), %	FN per 100,000 Cases	FP per 100,000 Cases	Disease Prevalence (95% CI)
T21 MPS	1048 (15,937)	99.7 (98 to 100)	99.9 (99.8 to 100)	15	95	4.95 (0.44 to 27.66)
T21 TMPS	246 (4380)	99.2 (96.8 to 99.8)	100 (99.8 to 100)	40	0	
T18 MPS	332 (16,180)	97.8 (92.5 to 99.4)	99.9 (99.8 to 100)	32	99	1.46 (0.22 to 17.02)
T18 TMPS	112 (4010)	98.2 (93.1 to 99.6)	100 (99.8 to 100)	26	0	
T13 MPS	128 (13,810)	95.6 (86.1 to 98.9)	99.8 (99.8 to 99.9)	46	198	1.09 (0.04 to 3.54)
T13 TMPS	20 (293)	100 (83.9 to 100)	100 (98.7 to 100)	0	0	
T21, T18, T13 MPS	1508 (15,797)	98.8 (97.2 to 99.5)	99.9 (99.7 to 100)	70	94	5.85 (0.67 to 46.81)
T21, T18, T13 TMPS	378 (4282)	98.9 (97.2 to 99.6)	99.9 (99.8 to 100)	64	94	

CI: confidence interval; FN: false-negative (missed cases); FP: false-positive; MPS: massively parallel sequencing; TMPS: targeted massively parallel sequencing; T13: trisomy 13; T18: trisomy 18; T21: trisomy 21.

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 individuals receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
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 (RCTs).

No studies identified provided direct evidence of the clinical utility that NIPS using analysis of cell-free fetal DNA changed the management of patients having singleton pregnancies.

Chain of Evidence
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.

Two TEC Assessments (2013, 2014) constructed decision models to predict health outcomes of sequencing-based testing compared with standard testing.[9,10] The model in the 2013 TEC Assessment focused on T21. In this model, the primary health outcomes of interest included the of number of: cases of aneuploidy correctly identified, cases missed, invasive procedures potentially avoided (i.e., with a more sensitive test), and miscarriages potentially avoided as a result of fewer invasive procedures. The results were calculated for a high-risk population of women ages 35 years or older (estimated antenatal prevalence of T21, 0.95%) and for 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 the initial screen and offered an invasive, confirmatory procedure, it was assumed that 60% would accept amniocentesis or chorionic villous sampling. 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 were 4.25 million births in the U.S. 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 (94.7%) of 4200 Down syndrome (T21) 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 (97.6% of total) of 4200, 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 the 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 ages 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 modeling study was that it assumed screening for T21 was done concurrently with screening for T13 and T18 and that women who choose invasive testing would do so because of a desire to detect T21. Consequently, miscarriages associated with invasive testing were not considered an adverse event of T13 or T18 screening.

The model compared two approaches with 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 modeling 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 would be 127 T18 cases and 45 T13 cases. Standard noninvasive screening would identify 123 of the 127 T18 cases, and sequencing-based screening would identify 121 of 127 cases. Additionally, standard noninvasive screening would identify 37 of 45 T13 cases, and sequencing-based screening would identify 39 of 45 T13 cases.
- Low-risk women: Assuming 50% uptake after a positive screen, the maximum cases detectable in the hypothetical population of 100,000 pregnancies would be 20 T18 cases and 6 T13 cases. Each initial screening test would identify 19 of the 20 T18 cases and 5 of the 6 T13 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 addition to the TEC Assessments, several other decision models have been published. For example, Ohno and Caughey (2013) published a decision model comparing the use of sequencing-based tests in high-risk women with confirmatory testing (i.e., as a screening test) and without confirmatory testing (i.e., as a diagnostic test).[11] 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 U.S. Sequencing-based tests used with confirmatory testing resulted in 1441 elective terminations (all with Down syndrome). Without confirmatory testing, sequencing-based tests resulted in 3873 elective terminations, 1449 with Down syndrome and 2424 without Down syndrome. There were 29 procedure-related pregnancies losses when confirmatory tests were used. The decision model did not address T18 or T13.

Section Summary: Noninvasive Prenatal Screening for Chromosomal Trisomies in Singleton Pregnancies

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 FOR SEX CHROMOSOME ANEUPLOIDIES IN SINGLETON PREGNANCIES

Clinical Context and Test Purpose

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

The following PICO was used to select literature to inform this review.

Populations

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

Interventions

The intervention of interest is 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 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).

REVIEW OF EVIDENCE

The Cochrane review by Badeau et al (2017) evaluated the diagnostic accuracy of NIPS for sex chromosome anomalies.[8] Twelve studies were identified on the 45, X chromosome with sensitivities of 91.7% to 92.4% and specificities of 99.6% to 99.8% (Table 5). Reviewers calculated that of 100000 pregnancies, 1039 would be affected by 45, X chromosome. Of these, 953 (MPS) and 960 (TMPS) would be detected and 86 and 79 cases, respectively, would be missed. Of the 98,961 unaffected women, 396 and 198 pregnant women would undergo an unnecessary invasive test. Badeau et al (2017) were unable to perform meta-analyses of NIPS for chromosomes 47, XXX, 47, XXY, and 47, XYY due to insufficient evidence.

Table 5. Systematic Review Testing Results for Sex Chromosome Aneuploidies in High-Risk Pregnancies

Test	Affected Pregnancies (Unaffected Pregnancies)	Sensitivity (95% CI), %	Specificity (95% CI), %	FN per 100,00 Cases	FP per 100,00 Cases	Disease Prevalence (95% CI)
45, X MPS	119 (7440)	91.7 (78.3 to 97.1)	99.6 (98.9 to 99.8)	86	396	1.04 (0.27 to 18.58)
45, X TMPS	79 (985)	92.4 (84.1 to 96.5)	99.8 (98.3 to 100)	79	198	
Sex chromosomes MPS[a]	151 (7452)	91.9 (73.8 to 97.9)	99.5 (98.8 to 99.8)	124	492	1.53 (0.45 to 18.58)
Sex chromosomes TMPS[a]	96 (968)	93.8 (86.8 to 97.2)	99.6 (98.1 to 99.9)	95	394	

CI: confidence interval; FN: false-negative; FP: false-positive; MPS: massively parallel sequencing; TMPS: targeted massively parallel sequencing
[a] Chromosomes 45, X, 47, XXX, 47 XXY and 47, XYY combined

A systematic review published after the Cochrane review had similar results, showing high sensitivity (94.1%; 95% CI 90.8% to 96.3%) and specificity (94.1%; 95% CI 90.8% to 96.3%), but more false positives (235 per 100,000) than tests for the common trisomies.[12] Subgroup analyses showed variation in PPV by type of SCA, from 32% (95% CI 27.0% to 37.4%) for Monosomy X to 70% (95% CI 63.9% to 77.1%) for XYY syndrome, explained by higher sensitivity and specificity for the Y chromosome and high risk of false-positive results for SCAs involving the X chromosome only.

The body of evidence is limited by imprecision of estimates due to small sample sizes, lack of confirmatory testing, and inability to generalize findings to pregnancies in average risk populations.

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.

REVIEW OF EVIDENCE

Direct Evidence

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.

No studies identified provided direct evidence of the clinical utility that NIPS using analysis of cell-free fetal DNA changed the management of patients having singleton pregnancies.

Sex chromosome aneuploidies (eg, 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 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 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.

Chain of Evidence
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: Noninvasive Prenatal Screening for Sex Chromosome Aneuploidies in Singleton Pregnancies
There is less data on the diagnostic performance of sequencing-based tests for detecting sex chromosome aneuploidies than for detecting Trisomy 21, Trisomy 18, and Trisomy 13. The available data suggests the tests have high sensitivity and specificity, but a higher rate of false positives than tests to detect the common trisomies. The body of evidence is limited by imprecision of estimates due to small sample sizes, lack of confirmatory testing, and inability to generalize findings to pregnancies in average risk populations. The clinical utility of prenatal diagnosis of sex chromosome aneuploidies is uncertain. Potential benefits of early identification (eg, 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 SCREENING FOR FETAL ANEUPLOIDIES IN TWIN PREGNANCIES

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

The following PICO was used to select literature to inform this review.
Populations
The relevant population of interest are individuals with first- and second-trimester twin pregnancy.

Interventions
The intervention of interest is NIPS 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
The following tests are currently being used to make decisions about identifying sex chromosome aneuploidies: 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.

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).

REVIEW OF EVIDENCE

Systematic Reviews

Two recent, good methodological quality systematic reviews with meta-analyses have examined the evidence for NIPS for aneuploidies in twin pregnancies (Tables 6 to 8).[12,13]

Judah et al (2021) report on cell-free fetal DNA (cfDNA) testing in 1442 twin pregnancies.[13] Study populations included a mix of pregnancies at high and average risk for aneuploidies. The cfDNA test classified correctly 19 (95.0%) of the 20 cases of T21, 9 (90.0%) of 10 cases of T18, 1 (50.0%) of 2 cases of T13, and 1235 (99.6%) of 1240 cases without any of the 3 trisomies. The pooled weighted detection rate and false positive rate (FPR) were 99.0% (95% CI 92.0, 99.9%) and 0.02% (95% CI 0.001, 0.43%), respectively. In the combined total of 50 cases of T18 and 6840 non-trisomy 18 pregnancies the pooled weighted detection rate and FPR were 92.8% (95% CI 77.6, 98.0%) and 0.01% (95% CI 0.00, 0.44%), respectively. In the combined total of 11 cases of T13 and 6290 non-trisomy 13 pregnancies the pooled weighted detection rate and FPR were 94.7% (95% CI 9.14, 99.97%) and 0.10% (95% CI 0.03., 0.39%). The body of evidence was limited by the small number of cases and individual study limitations included high risk of selection bias (e.g., screening performed in populations that had previously been screened using methods including maternal age, first-trimester combined test, or second-trimester serum biochemistry.) The study authors concluded that the detection rate of T21 was high, but lower than that in singleton pregnancies. The number of cases of T18 and T13 was too small for an accurate assessment of the predictive performance of the test.

In a systematic review of NIPS with cfDNA testing in average-risk pregnancies, Rose et al (2022) included 11 studies that reported at least 1 performance characteristic of NIPS to detect trisomies in multifetal gestations.[14] Of these, 7 studies (N = 4271 twin pregnancies) were included in meta-analyses. The study authors concluded that performance characteristics were generally comparable to NIPS performance in singleton pregnancies but that few studies have comprehensively evaluated NIPS performance in twin gestations. In addition to the small number of cases overall, individual study limitations included a lack of complete follow-up data to be able to ascertain true negative and true positive cases, and an inability to distinguish low- and high-risk cohorts in some studies.

Table 6. Comparison of Studies Included in Systematic Reviews of Noninvasive Prenatal Screening in Twin Pregnancies

Study (year)	Judah et al (2021)	Rose et al (2022)
Chen (2019)		● (not included in meta-analysis)
Chibuk (2020)	●	

Du (2017)	●	
Dyr (2019)		● (not included in meta-analysis)
Gil (2019)		●
He (2020)	●	●
Huang (2014)	●	
Judah (2021)	●	
Khalil (2021)	●	●
Kypri (2019)		●
Lau (2013)	●	
Le Conte (2018)	●	●
Montevasselian (2020)		●
Norwitz (2019)	●	● (not included in meta-analysis)
Oneda (2020		● (not included in meta-analysis)
Tan (2016)	●	
Yang (2018)	●	
Yin (2019)	●	
Yu (2019)	●	●

Table 7. Systematic Reviews of Noninvasive Prenatal Screening for Fetal Aneuploidies in Twin Pregnancies-Characteristics

					Risk of Bias Assessment		
Study	**N Studies**	**Study Populations**	**N Pregnancies**	**Reference Standard of Studies**	**No Domains**	**1-2 Domains**	**>2 Domains**
Judah et al (2021)[13]	12	Twin gestations, mix of high and low risk for aneuploidies	1442 (75)	Karyotyping	All were high risk of selection bias, most high risk of flow/timing bias		
Rose et al (2022)[14]	11 (7 included in meta-analyses)	Twin gestations in individuals at average risk	4271 in studies included in meta-analyses	Karyotyping	1 serious risk of bias, 6 moderate risk		

NR: not reported.

Table 8. Systematic Reviews of Noninvasive Prenatal Screening for Fetal Aneuploidies in Twin Pregnancies- Results

	Trisomy Affected Pregnancies	Sensitivity (95% CI), %	Specificity (95% CI), %	PPV	NPV	FP	FN	Other Performance Characteristics
Judah et al (2021)[13]								

T21	137	99.0 (92.0 to 99.9)	98 (57 to 99)			16 (13 from 1 study)	2	LR positive: 4224 (230 to 77525) LR negative:0.010 (0.001 to 0.085)
T18	50	92.8 (77.6 to 98.0)	99 (43 to 100)			5	0	LR positive: 6198 (253 to 151,590) LR negative:0.072 (0.021 to 0.240)
T13	11	94.7 (9.14 - 99.97)	90 (61 to 97)			9	0	LR positive: 916 (226 to 3714) LR negative:0.053 (0.000 to 7.173)
Rose et al (2022)[14]						**FP rate**		**Diagnostic Odds Ratio**
T21	54 total (not reported separately by trisomy)	98.2 (88.2 to 99.7)	99.9 (99.8 to 99.9)	94.7 (84.9 to 98.3)	100 (99.8 to 100)	0.07 (0.02 to 0.22)		6586.60 (1696.39 to 25573.83)
T18		90.0 (67.6 to 97.5)	100 (99.8 to 100)	90.0 (67.6 to 97.5)	100.(99.8 to 100)	0.05 (0.01 to 0.20)		3606.40 (710.38 to 18,308.67
T13		80.0 (30.9 to 97.3)	99.9 (99.4 to 100)	81.8 (1.8 to 99.9)	100.0 (99.8 to 100)	0.07 (0.01 to 0.59)		1350.78 (206.12 to 8852.31)

cfDNA: cell-free DNA; CI: confidence interval; DZ: dizygotic; MZ: monozygotic; N: sample size; T: trisomy.

Nonrandomized Studies

Observational studies not included in the systematic reviews discussed above are summarized in Table 9.[15,16, 17, 33, 34] These studies reported a total of 96 trisomies (78 of T21, 11 of T18, 7 of T13). Study limitations were similar to those identified in the systematic reviews (Tables 10 and 11), including small numbers of cases resulting in the imprecision of estimates, and lack of complete follow-up data.

Table 9. Observational Studies of Noninvasive Prenatal Screening for Fetal Aneuploidies in Twin Pregnancies

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity	
					Sensitivity	Specificity
Xu et al (2021)[18,]	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)
Cheng et al (2021)[19,]	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%	
La Verde et al (2021)[20,]	800	800	NA	T21: 8	T21: 100% (59.7,100.0)	T21: 100% (99.39, 100.0)
Van den Bogaert et al (2021)[21,]	2770	2040	No follow-up data available	T21: 11	T21: 100%	T21: 100%
Dugoff et al (2023)[17]	1764	1447	78 cases with a vanishing twin and 239 with inadequate follow-up were excluded	T21: 41 T18: 10 T13: 5	T21: 97.6% (83.8 to 99.7) T18: 100% (72.3 to 100) T13: 80% (11.1 to 99.2)	T21: 100% (99.7 to 100) T18: 99.9% (99.5 to 100) T13: 100% (99.7 to 100)
Claudel et al (2023)	2577	1865	692 cases with vanishing twin, miscarried pregnancies, in utero death, stillbirth, neonate death or inadequate follow-up were excluded	T21:10 T18: 0 T13: 2	T21: 100% (61 to 100)	T21: 99.8% (99.4 to 99.9)
Eiben et al (2023)	1658	1656	2 samples failed NIPS and were excluded from analysis; 1625 fetuses were determined to have a low-risk and 31 were determined to have a high-risk	T21:20 T18: 8 T13: 3 12 T21, 6 T18, and 1 T13 had sufficient follow-up for assessment	T21: >99.9% T18: >99.9% T13: NA High-risk cases only (n=31)	T21: >99.9% T18: 99.7% T13: 99.7% High-risk cases only (n=31)

CI: confidence interval; NA: not available; NIPS: noninvasive prenatal screening; T: trisomy.

Table 10. Observational Studies of Noninvasive Prenatal Screening for Fetal Aneuploides in Twin or Multiple Pregnancies-Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Xu et al (2021)[18,]					
Cheng et al (2021)[19,]					
La Verde et al (2021)[20,]					

Van den Bogaert et al (2021)[21]					
Dugoff et al (2023)[17]					
Claudel et al (2023)					
Eiben et al (2023)					

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 11. Observational Studies of Noninvasive Prenatal Screening for Fetal Aneuploidies in Twin Pregnancies- Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Xu et al (2021)[18]	1. Unclear if convenience or consecutive samples				1, 2, excluded no-call cases and those with fetal demise or selective termination	
Cheng et al (2021)[19]	2. Convenience sample				3. Incomplete follow-up	1. Confidence intervals not reported
La Verde et al[20]	1. Unclear if convenience or consecutive samples				3. Incomplete follow-up	
Van den Bogaert et al[21]					3. Incomplete follow-up	1. Confidence intervals not reported
Dugoff et al (2023)[17]	2. Convenience sample				3. Incomplete follow-up	
Claudel et al (2023)						
Eiben et al (2023)						

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not

same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Clinical Utility

Direct Evidence
Direct evidence is not available for the evaluation of noninvasive prenatal testing (NIPT) to detect fetal aneuploidies in individuals pregnant with twins or multiples.

Chain of Evidence
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: Noninvasive Prenatal Screening for Fetal Aneuploidies in Twin Pregnancies
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. According to the American Congress of Obstetrics and Gynecologists (ACOG, 2020), cell free DNA screening can be performed in twin gestations. Sensitivity for trisomy 21 using cell free DNA for twin pregnancy is similar to singleton pregnancy although test failure may be higher. Because each fetus in a single pregnancy contribute different amounts of cell free DNA into the maternal circulation it is possible that an aneuploidy fetus would contribute less fetal DNA, masking the aneuploid test result. Nonetheless, noninvasive prenatal testing using cell-free DNA is considered an appropriate noninvasive prenatal screening option.

NONINVASIVE 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 patients who are pregnant is to inform a decision whether to proceed with diagnostic testing.

The following PICO was used to select literature to inform this review.

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

Interventions
The intervention of interest is NIPS 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

(eg, 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.

REVIEW OF EVIDENCE

Clinical Validity

Systematic Review
Three recent, good methodological quality systematic reviews have evaluated NIPS for microdeletion syndromes (Table 12).

Familiari et al (2021) conducted a systematic review of the literature on screening for fetal microdeletions and microduplications using cell-free fetal DNA (Table 11).[22] A total of 7 studies met inclusion criteria, representing 210 cases of microdeletions or microduplications. The overall pooled positive predictive value (PPV) was 44.1% (95% CI 31.49 to 63.07; range 28.9% to 90.6%). Limitations in the individual studies included retrospective design, low number of cases for each condition, lack of a standardized confirmation of the disease, low detail regarding the presence of absence of ultrasound anomalies and sonographic protocol used, different gestational ages at the time of the test, and variation in background risk. The authors noted that confirmatory testing was seldom reported in studies, under the assumption that all anomalies would have been identified in the newborn by physical exam. However, because many newborns with microdeletion and microduplication syndromes will not demonstrate phenotypical anomalies, standard neonatal examination cannot be considered a reliable ascertainment method and the detection rate and negative predictive value could not be determined from this body of evidence.

In a systematic review of NIPS using cfDNA in general risk pregnancies conducted for ACMG, Rose et al (2022) included 17 studies of screening for copy number variants (microdeletions and microduplications).[14] Meta-analyses were not conducted due to study heterogeneity. Although screening identified a small number of CNVs, confirmatory testing was frequently unavailable and complete ascertainment of cases was lacking. Sample sizes in each study were relatively small and sensitivities varied greatly. Additionally, it was often difficult to distinguish between low- and high-risk cohort in individual studies. The study authors concluded that the performance of NIPS was significantly poorer when targeting CNVs than the common trisomies and additional outcome studies are needed to understand the unique clinical value of NIPS for CNVs when compared with other approaches.

Zaninovic et al (2022) conducted a systematic review of NIPS for CNVs and microdeletions.[23] A total of 32 studies were identified with literature searches conducted through February 2022. Of these, 21 studies concerned screening for microdeletion syndromes. Meta-analyses were not conducted due to study heterogeneity. Although a comprehensive quality assessment of studies was not conducted, the study authors described notable limitations of the included studies. Most studies did not define indications for screening and some included only high-risk pregnancies. Negative predictive values could not be determined because none of the studies performed systematic confirmatory analysis by chromosomal microarray analysis for negative/low-risk cases, mostly relying on clinical follow-up. The study authors concluded that given the limited follow-up and validation data available, NIPT for microdeletions and CNVs should be used with caution.

Table 12. Systematic Review of Cell-Free DNA Screening for Microdeletions and Microduplications- Characteristics and Results

Study	Literature Search Dates	Study Inclusion/Exclusion Criteria	Studies Included	Pooled Results
Familiari et al (2021)[22]	2000-January 2020	Inclusion: Retrospective and prospective cohort studies where all patients underwent one or more cfDNA methods and the reference standard; >5000 cases; full text, published in English language Exclusion: method tested only for common aneuploidies (T21, 18, 13, and sex chromosome aneuploidies). Studies reporting the diagnostic performance of cell-free DNA screening for microdeletions and microduplications, more than 5000 cases	N=7 studies; published 2015-2019 474,189 pregnancies 210 cases of microdeletions/microduplications	Diagnostic verification of screen positive cases is available in 486 of 678 cases (71.7%) Screen positive rate: 0.19% (95% CI 0.09 to 0.33; range 0.03% to 0.63%); I^2 98.8% FP rate: 0.07% (95% CI 0.02 to 0.15; range 0.002% to 0.28%); I^2 98.1% PPV: 44.1% (95% CI 31.49 to 63.07; range 28.9% to 90.6%); I^2 91.7% Detection rate not assessed
Rose et al (2022)[14]	Through March 2021	Population: general-risk pregnant individuals Interventions: NIPS used as primary or secondary screening for T21, T18, T13, RATs, CNVs, and maternal conditions Outcomes: diagnostic performance, psychosocial outcomes, uptake of invasive diagnostic testing subsequent to NIPS, economic implications of NIPS	(For CNVs) N=17 studies	Data not pooled due to heterogeneity; narrative synthesis only
Zaninovic et al (2022)[23]	2013-February 2022	Studies with information about the validity or utility of cfDNA-based NIPT for fetal CNVs and microdeletions Exclusions: reports in which the validity of the test was not confirmed by invasive testing or statistically expressed	N = 32 studies	Data not pooled due to heterogeneity; narrative synthesis only

cfDNA: cell-free DNA; CI: confidence interval; FP: false positive; N: sample size; NIPT: noninvasive prenatal testing; PPV: positive predictive value; T: trisomy;

Nonrandomized Studies

Studies reporting on the clinical validity of NIPS for detecting microdeletion syndromes not included in the systematic reviews discussed above are shown in Tables 13 and 14. Study limitations are shown in Tables 15 and 16.

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.[24] Diagnostic testing results were available in 42.5% (n = 1,142) of screen-positive samples, and 0.82% of screen-negative samples, with an overall 2.98% of samples with diagnostic outcomes. Test characteristics for microdeletions are shown in Table 14. Data on false negatives were not reported because follow-up after negative screening results was voluntary and/or not available from the retrospective review of de-identified data.

Wang et al (2021) conducted a prospective analysis of 39,002 pregnant women who received NIPS in a single center between 2018 and 2020.[25] There were 473 (1.21%) pregnancies that tested positive for fetal chromosome abnormalities, of which 95 were microdeletion/microduplication syndrome cases. Limitations of this study include variable types of diagnostic testing and specimen types, a large number of patients who refused to receive a prenatal diagnosis (n=135) and then were lost to follow-up (n=128), and low percentage of overall specimens that had diagnostic testing results available.

Dar et al (2022) conducted a prospective analysis of 20,887 women who underwent NIPS testing at 21 centers in 6 countries.[26] A genetic outcome result was available for 18,289 women (87.6%), and 12 cases of 22q11.2 deletion syndrome were confirmed in the cohort. Limitations of the study include the low number of overall confirmed cases, wide confidence intervals for sensitivity, positive and false positive values, and varied indications for testing.

Tian et al. (2023) conducted a retrospective analysis of 452 pregnancies in China who had previously undergone chromosomal microarray analysis following amniocentesis or chorionic villus sampling.[35] Participants also had NIPS with microdeletion and microduplication analysis performed and compared the testing results. Several syndromes due to copy number variants were identified with sensitivities ranging from 33% to 100%. Limitations of the study include the low number of overall confirmed cases, absence of confidence intervals for sensitivity, and a lack of statistical reporting for other test characteristics such as specificity, positive predictive value, negative predictive value, and uncertain indications for testing.

Table 13. Nonrandomized Studies of Noninvasive Screening for Microdeletion Syndromes - Characteristics

Study	Test	Copy Number Variant, Syndrome	Population	Reference Test
Soster et al (2021)[24],	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; population was a mix of high risk and no known high risk indications for testing.	Karotype (58.5%); microarray (10.8%), FISH (1.6%), other or unspecified (16.7%), multiple tests (12.5%).
Wang et al (2021)[25],	MPS	Multiple microdeletion/microduplication syndromes	39,002 samples; indications for testing varied (e.g., high-risk due to prior screening or maternal age, patient request, abnormal ultrasound, IVF, twin pregnancy)	Karotype on 51 of 95 cases (53.6%)
Dar et al (2022)[26], NCT02381457	Natera	22q11.2, DiGeorge	20,887 (54.8% in the US, 45.2% in Europe enrolled 18,289 (87.6%) had both cfDNA and DNA confirmation results for 22q11.2DS	DNA from neonates' cord blood, buccal smear. or dried blood spot obtained by state health departments for routine neonatal screening

Tian et al (2023)	NIPT-PLUS	1p36 microdeletion, 5p15.2-13.3 (cri du cat syndrome), Williams-Beuren syndrome, Chromosome 9p deletion syndrome, Angelman/Prader-Willi syndrome, Renal cysts and diabetes syndrome, 22q11.2 (DiGeorge syndrome)	452 pregnancies in China enrolled to have NIPS with microdeletion and microduplications	Prenatal testing with chromosomal microarray by amniocentesis or chorionic villus sampling

cfDNA: cell-free DNA; FISH: fluorescence in-situ hybridization; MPS: massively sequencing

Table 14. Nonrandomized Studies of Noninvasive Screening for Copy Number Variants-Results

Study	Initial N	Final N	Excluded Samples	Positive Tests, n (%)	Clinical Validity						
					TP, n	Sensitivity, % (95% CI)	Specificity	PPV, %	NPV	FP	FN
Soster et al (2021)[24,]			s								
Overall	55,517	1569	Samples without diagnostic results for microdeletion	2687 (5.06%)							
22Q					38	88.4% (74.1 to 95.6%)	99.9% (99.6–100%)	97.4% (84.9–99.9%)	1	5	
1p36					7	100% (56.1–100%)	100% (99.7–100%)	100% (56.1–100%)	0	0	
15q					8	100% (59.8–100%)	100% (99.7–100%)	100% (59.8–100%)	0	0	
4p					9	100% (62.9–100%)	100% (99.7–100%)	100% (62.9–100%)	0	0	
5p					6	100% (51.7–100%)	99.9% (99.5–100%)	75.0% (35.6–95.5%)	2	0	
11q					5	100% (46.3–100%)	100% (99.7–100%)	100% (46.3–100%)	0	0	

8q					2	100% (19.8–100%)	100% (99.7–100%)	100% (19.8–100%)	0	0	
Wang et al (2021)[25,]				25	Of 25 cases confirmed: 10 pathogenic, 3 likely pathogenic, 9 VOUS			49.02 (CI NR)		26	
Dar et al (2022)[26,] NCT02381457	20,887	18,289	N = 2598 (12.4%) 296 (1.4%) pregnancy loss without genetic confirmation 1110 (5.3%)	12 confirmed cases	10	updated algorithm: 10/12 83.3% (51.56% to 97.9%)	updated algorithm: 10/12 83.3% (51.56% to 97.9%)	updated algorithm 10/19 52.6% (28.9% to 75.6%)	updated algorithm: 18,022/19,024 99.98% (99.95 to 100%)	original algorithm: N = 29 (0.16%) updated algorithm: N = 9 (0.5%)	original algorithm: N = 3 updated algorithm: N = 2
Tian et al (2023)											
Overall	452										
1p36 microdeletion					2	2/2 (100%)					
5p15.2-13.3 (cri du cat syndrome)					2	2/2 (100%)					
Williams-Beuren syndrome					3	1/3 (33.3%)					
Chromosome 9p deletion syndrome					4	4/4 (100%)					
Angelman/Prader-Willi syndrome					3	2/3 (66.7%)					
Renal cysts and diabetes syndrome (RCAD)					15	11/15 (73.3%)					
22q11.2 (DiGeorge syndrome)					13	12/13 (92.31%)					

CI: confidence interval; FN: false-negatives; FP: false-positives; NPV: negative predicted value; NR: not reported; PPV: positive predictive value; TP: true-positives; VOUS: variant of unknown significance.

Table 15. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Soster et al (2021)[24,]	4. Indications for NIPS varied				
Wang et al (2021)[25,]	4. Indications for NIPS varied				

Dar et al (2022)[26] NCT02381457	4. Indications for NIPS varied				
Tian et al (2023)	4. Indications for NIPS unclear			3. Only sensitivity reported	

NIPT: noninvasive prenatal testing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 16. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Soster et al (2021)[24]	2. Convenience sample				3. Outcome data on confirmed results collected via 2 methods: clinician feedback reported voluntarily and matching of cfDNA results with diagnostic specimens	
Wang et al (2021)[25]	2. Convenience sample				3. Large number lost to follow-up (n=128)	1. Confidence intervals not reported
Dar et al (2022) NCT02381457						2. Comparison to other tests not reported
Tian et al (2023)	2. Convenience sample					1. Confidence intervals not reported

cfDNA: cell-free DNA.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Clinical Utility

Direct Evidence

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

Chain of Evidence

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.

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.

Section Summary: Noninvasive Screening for Fetal Microdeletions Using Cell-Free Fetal DNA

Multiple nonrandomized studies of the clinical validity of microdeletion testing have been published. Recent systematic reviews of these studies have identified limitations that preclude drawing conclusions about clinical validity. The number of cases of microdeletions is small, leading to imprecise estimates of test performance. Few studies reported complete follow-up data to confirm diagnostic confirmation.

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 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 microdeletion syndromes is low, and not all individuals with a microdeletion will have clinical symptoms.

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 transfusion syndrome (TTTS) and other monochorioinc (MC) twin-related abnormalities.

The following PICO was used to select literature to inform this review.

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 one 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 MC and 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 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).

REVIEW OF EVIDENCE

Observational Study
Norwitz et al (2019) conducted a validation study of a single-nucleotide polymorphism-based NIPT in twin pregnancies (Table 17).[4] Twin zygosity results from this study are shown in Table 18. 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 are summarized in Tables 19 and 20. A major limitation was a lack of information on timing of the index test and the use of different methods to confirm zygosity.

Table 17. Validation Study of Cell-Free Fetal DNA Testing for Twin Zygosity - Study Characteristics

Study	Study Population	Design	Reference Standard	Timing of Reference and Index Tests	Blinding of Assessors
Norwitz et al (2019)[4]	95 twin pregnancies	Prospective, unclear if random or consecutive	Confirmed zygosity, MZ or DZ determined by molecular genetic testing by an external laboratory (n = 47), presence of twins with different fetal sex (n = 36, only valid for DZ), SNP-based analysis of buccal samples from children (n = 8), clinical presentation of twin-to-twin transfusion syndrome (n = 3), or single embryo transfer plus monochorionic/monoamniotic observation by ultrasound (n = 1).	Timing of reference test not described	Yes

DZ: dizygotic; MA: monozygotic; SNP: single nucleotide polymorphism.

Table 18. Validation Study of Cell-Free Fetal DNA Testing for Twin Zygosity – Results

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity	
					MZ Sensitivity/DZ Specificity	MZ Specificity/DZ Sensitivity
Norwitz et al (2019)[4]	95	93	Overall 2.1% (no result due to low fetal fraction) MZ: 1/30 (3.3%) DZ: 1/65 (1.5%)	29 MZ, 64 DZ	100% (29/30) (95% CI 88.1%-100%)	100% (64/65) (95% CI 94.4%-100%)

CI: confidence interval; DZ: dizygotic; MA: monozygotic; N: sample size.

Table 19. Validation Study of Cell-Free Fetal DNA Testing for Twin Zygosity- Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Norwitz et al (2019)[4]			3. Techniques to confirm zygosity varied		

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 20. Validation Study of Cell-Free Fetal DNA Testing for Twin Zygosity-Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Norwitz et al (2019) [4]	1. Unclear if random or consecutive samples		1,2. Unclear when index testing occurred			

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

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

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.

Chain of Evidence

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

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 cfDNA 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 women 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 patients who have singleton pregnancy is to inform a decision whether to proceed with diagnostic testing.

The following PICO was used to select literature to inform this review.

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).

REVIEW OF EVIDENCE
In a proof of concept study, Vanadis NIPT analyzed chromosome 21.[27] For the case-control study two sample sets were collected; confirmed trisomy 21 pregnancies samples were collected from pregnant women carrying one 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.[28] 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 five 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
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.

Chain of Evidence
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: Noninvasive Prenatal Screening Using Vanadis NIPT for Chromosomal Trisomies in Singleton Pregnancies
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 one proof of concept study and one clinical validation study.

Noninvasive Prenatal Testing for Single-Gene Disorders

Clinical Context and Test Purpose
The purpose of single-gene NIPT using cfDNA (e.g. Vistara or UNITY Fetal Risk Screen™) is to screen for disorders caused by a single gene. The purpose of UNITY carrier screening is to identify if the mother carries genes for five autosomal recessive single-gene disorders: cystic fibrosis, spinal muscular atrophy, sickle cell disease, alpha thalassemia, and beta thalassemia. If the mother is found to be a carrier, reflex confirmatory single-gene NIPT with fetal risk assessment is provided (UNITY Fetal Risk Screen™). UNITY additionally includes two separate tests, the UNITY aneuploidy test, and fetal Rh antigen test, which are ordered independently.

The following PICO was used to select literature to inform this review.

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

Interventions

The intervention of interest is NIPT using analysis of cfDNA (e.g. Vistara or UNITY Fetal Risk Screen™) for detection of single-gene disorders.

Vistara screens for 25 autosomal dominant and X-linked conditions across 30 genes, including Noonan syndrome, osteogenesis imperfecta, craniosynostosis syndromes, achondroplasia, and Rett syndrome. The UNITY Carrier Screen™ for maternal carrier status for cystic fibrosis, spinal muscular atrophy, alpha thalassemia, beta thalassemia, and sickle cell disease, with reflex fetal single-gene NIPT when a maternal carrier is identified (UNITY Fetal Risk Screen™). A proprietary, personalized fetal risk score ranging from > 9 in 10 to 1 in 20,0000 is reported when performing single-gene NIPT.

Comparators

The following tests are currently being used to make decisions about identifying single-gene disorders: conventional serum and ultrasound screening followed by invasive diagnostic testing, as well as standard of care without screening.

It is unclear if Vistara or UNITY are intended to replace other screening modalities such as ultrasound, or an add-on test.

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.

Review of Evidence

Clinical Validity

Vistara NIPT

The performance characteristics of the Vistara NIPT were evaluated in a validation study conducted by Zhang et al (2019) (Table 21).[29] Most of the study participants were high risk due to prenatal ultrasound findings or a family history of genetic disease. The validation cohort included 76 cases (3 positive and 73 negative) and the clinical study included 422 samples (32 positive and 390 negative). Pregnancy outcome data were obtained for 26 of 35 (74.2%) positive tests and 198 of 463 (42.7%) negative tests from both the validation and clinical studies.

Mohan et al (2022) reported on the clinical experience of Vistara NIPT in a series of 2208 pregnancies.[6] Of 2416 initial tests, 132 (5.5%) tests were ineligible and 76 (3.1%) did not pass quality control. Indications for NIPT included family history (6.0%), abnormal US finding (23.3%), advanced paternal age (41.3%), and unspecified/other/advanced maternal age (29.4%). Overall, the test positive rate was 125 of 2208 (5.7%). In cases without abnormal ultrasound findings or family history, the test positive rate was 6 of 52 (0.4% (6/52).

Study results are summarized in Table 22. Study limitations are summarized in Tables 23 and 24. Major limitations included a lack of confirmatory testing and selection bias. Because of missing data, it is not possible to determine accurate estimates of true positive and true negative tests. In addition, a large proportion of participants in both studies had a previous screening with findings suggestive of a potential disorder. It is unclear if the Vistara test is intended to be an adjunct to or replacement for other screening tests such as ultrasound. More clarity on the proposed use of the test would be needed to adequately evaluate performance characteristics.

UNITY Fetal Risk Screen™

Westin et al (2022) published a retrospective clinical validation study of the UNITY single-gene NIPT for 77 pregnant women who had previously been identified as beta hemoglobinopathy carriers.[36] Single-gene NIPT was performed from October 2018 to December 2019 and returned a fetal beta hemoglobinopathy genotype prediction for 68 of the 77 pregnancies, with 9 undetermined (11.7%). The UNITY Fetal Risk Screen™ accurately distinguished heterozygous from homozygous fetuses with 100% sensitivity (95% CI, 90.8% to 100%) and 96.5% specificity (95% CI, 82.2% to 99.9%) compared to confirmatory newborn chart review or genotyping of umbilical cord blood. The predicted fetal genotype concorded with the newborn genotype in 67 out of 68 pregnancies (98.5%). Using single-gene NIPT data and a priori risk adjustments, residual risk could classify fetuses as 'low risk,' 'decreased risk,' or 'high risk' in 75 of 77 pregnancies with a 2.6% no-call rate. Two fetuses affected with sickle cell disease were correctly classified as high risk (>9 in 10 residual disease risk), and one fetus, which had a previously undetermined homozygosity score, was also affected and has an elevated residual risk score of 1 in 20.

The performance characteristics of the UNITY Fetal Risk Screen™ were evaluated in a clinical validation study conducted by Hoskovec et al (2023).[37] The study participants comprised a general population not at high risk for cystic fibrosis, hemoglobinopathies, and spinal muscular atrophy, who were screened with UNITY from August 2019 to May 2021. All pregnancies were ≥ 10 weeks gestation, were singleton pregnancies and were not conceived with a donor egg or gestational carrier. The cohort included 9151 pregnancies seen by 240 providers. A total of 1669 (18.2%) of women were found to be heterozygous carriers for a pathogenic variant of at least one condition (4.47% were heterozygous for a CFTR pathogenic variant, 4.64% for an HBB variant, 8.65% for HBA1/HBA2 variant, and 2.26% for SMN1) and underwent reflex single-gene NIPT. Newborn outcomes data was available for 201 (12%) pregnancies with an identified positive maternal carrier, and of these, 10 (4.9%) had no call sing-gene NIPS results and were excluded from the analysis. Single-gene NIPT identified 14 out of 15 affected fetuses as 'high risk' for one of the screened conditions on the panel, which resulted in a sensitivity of 93.3% (95% CI, 68.1% to 99.8%), a positive predictive value of 48.3% (95% CI, 36.1% to 60.1%) and a negative predictive value of 99.4% (95% CI, 96% to 99.9%). Newborn outcomes by proprietary personalized fetal risk score across all screened conditions showed that 4 out of 4 (100%) pregnancies with >9 in 10 risk were affected, 8 out of 17 (47%) with risks between 1 in 2 and 2 in 3 risk were affected, 2 out of 8 (25%) with risks between 1 in 10 and 1 in 100 were affected, and 1 out of 162 (0.6%) with risks <1 in 100 were affected. The authors also modeled the end-to-end clinical analytics of carrier screening with UNITY versus standard NGS carrier screening. The authors reported that in a real-world scenario accounting for the sensitivity of carrier screening and single-gene NIPT, the end-to-end sensitivity of carrier screening with UNITY was 90% (95% CI, 71.8% to 98.9%), which was higher than that for conventional carrier screening.

Wynn et al (2023) also evaluated the UNITY Fetal Risk Screen™ in a general population of 42067 pregnant individuals who underwent UNITY carrier screening.[38] A total of 7538 (17.92%) carriers were identified and underwent reflex single-gene NIPT. Only 3299 were able to be contacted for follow-up. The outcomes cohort consisted of 528 neonates and fetuses who were able to be assessed for single-gene disorders across 253 centers in the U.S.

The authors calculated that in this cohort, the sensitivity of the UNITY Fetal Risk Screen™ was 96.0% (95% CI, 79.65% to 99.90%), with a specificity of 95.2% (95% CI, 92.98%to 96.92%), PPV of 50.0% (95% CI, 35.23% to 64.77%), and an NPV of 99.8% (95% CI, 98.84% to 99.99%). Single-gene NIPT identified 9 of 10 pregnancies affected by cystic fibrosis, 11 of 11 affected HBB, 4 of 4 affected by spinal muscular atrophy, and none affected by HBA as high risk. The authors also modeled the performance characteristics of maternal carrier screening followed by single-gene NIPT with the UNITY Fetal Risk Screen™. They found an end-to-end sensitivity of 92.4% with a specificity of 99.9% and PPV and NPV values of 50.7% and 99.9%, respectively of the full cohort of 42067 pregnancies; this was higher than conventional carrier screening and would result in a greater number of fetuses being characterized as high risk.

Study results are summarized in Table 22. Study limitations are summarized in Tables 23 and 24. Major limitations included missing data, a lack of consistent confirmatory testing methods, and selection bias. Because of missing data, it is not possible to determine accurate estimates of true positive and true negative tests. Three studies examined testing for single-gene disorders with UNITY Fetal Risk Screen™; sensitivity and specificity across these studies was high and few samples resulted in a no-call result. The available studies on clinical validity have limitations, and the added benefit of Unity Fetal Risk Screen™compared with current approaches is unclear. Information on the clinical utility of the test was not evaluated in published studies.

Table 21. Clinical Validity of Non-invasive Prenatal Testing for Single-Gene Disorders - Study Characteristics

Study	Study Population	Design	Reference Standard
Zhang et al (2019)	Individuals seeking prenatal diagnosis or genetic disease risk assessment for their pregnancies due to family history of genetic disease (10.2%), prenatal ultrasound findings indicative of a fetal developmental abnormality (35.8%), previous abnormal serum screening result (0.7%), advanced paternal or maternal age, or parental concerns Average gestational age at the time of collection was 16.8 weeks (range 9.0 to 38.3 weeks)	Retrospective cohort	Pathogenic or likely pathogenic variants confirmed using a secondary NGS assay. Sanger sequencing used to confirm positive findings if an invasive specimen (eg, amniotic fluid) or a postnatal sample was available.
Mohan et al (2022)	Indication for NIPT: family history (6.0%); abnormal US finding (23.3%), advanced paternal age (41.3%), unspecified/other/advanced maternal age (29.4%)	Retrospective cohort	Positive variants were confirmed by a secondary amplicon-based NGS assay using deeper sequencing (> 10 000×). Variants of unknown significance were not reported. Confirmatory prenatal or postnatal diagnostic testing was recommended for all screen-positive patients.
Westin et al (2022)	Individuals seeking a prenatal diagnosis or genetic disease risk assessment for their pregnancies with the UNITY Fetal Risk Screen™ who were known to be carriers for the HBB allele. Gestational age at the time of collection ranged from 16.4 weeks to collection at delivery, with a median fetal fraction of 9.3%.	Retrospective cohort	Sickle cell status of newborns was determined by newborn screening chart review, or genotyping of umbilical cord blood.

Hoskovec et al (2023)	Individuals seeking a prenatal diagnosis or genetic disease risk assessment for their pregnancies with the UNITY Fetal Risk Screen™; the cohort was drawn from the general population and is not deemed to be at high risk for single- gene disorders. The average gestational age at the time of collection was a mean of 16.8 weeks ± 6.1 weeks standard deviation. The mean fetal fraction was 6.8%.	Retrospective cohort	Fetal or neonatal outcomes were determined by state newborn screening program data, additional testing related to the condition of interest, post-natal molecular testing, newborn and pediatric symptoms of concern, and reports or referrals to pediatric specialists.
Wynn et al (2023)	Individuals seeking a prenatal diagnosis or genetic disease risk assessment for their pregnancies with the UNITY Fetal Risk Screen™; the cohort was drawn from the general population and is not deemed to be at high risk for single- gene disorders. The average gestational age at the time of collection was 16.4 weeks (median 13.9 weeks; range 10 to 37 weeks). The mean fetal fraction was 7.8%.	Retrospective cohort	Fetal or neonatal outcomes were determined by newborn screening results, molecular testing (prenatally or postnatally), and diagnostic laboratory testing.

NGS: next generation sequencing; NIPT: non-invasive prenatal testing; US: ultrasound.

Table 22. Clinical Validity of Non-invasive Prenatal Testing for Single-Gene Disorders - Study Results

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Results
Zhang et al (2019) [32,]	458	422	n =36 8 did not meet fetal fraction or sequence coverage cutoff 11 did not meet sample acceptance requirement 3 had maternal pathogenic/likely pathogenic variants 2 had ovum-donor status 2 had twins	35 positive results	20/35 cases had a confirmed diagnosis Pregnancy outcome data were obtained for 26 of 35 (74.2%) positive cases with 1 of 35 (2.9%) spontaneous abortion, 8 of 35 (22.9%) elective terminations, 7 of 35 (20%) neonatal demise, and 10 of 35 (28.6%) delivery with neonatal survival.
Mohan et al (2022) [6,]	2416	2208	132 (5.5%) tests ineligible 76 (3.1%) did not pass quality control	125 of 2208 (5.7%)	Of 125 positive cases, follow-up information was available for 67 (53.6%), with none classified as false positive. Positive tests in cases without abnormal

					ultrasound findings or family history: 6/52 (0.4%)
Westin et al (2022)[33,]	77	77	None	All mothers had at least 1 pathogenic HBB allele. Informative information on fetal disease risk was available for 97.4% of individuals, and a determination of beta hemoglobinopathy genotype was available in 88.3% of fetuses. Risk Category and status: High: 2 (2.6%) Decreased: 1 (1.3%) Low: 72 (93.5%) No-Call: 2 (2.6%) *Both high-risk NIPT* *individuals were affected, and one individual who had a no-call was determined to be affected.*	Distinguish homozygous from heterozygous fetuses: Sensitivity: 100% (90.8% to 100%) Specificity: 96.5% (82.2% to 99.9%) No-result available: 2 (2.6%)
Hoskovec et al (2023)[34,]	9151	201	n=7482 negative carrier screen n=171 did not have reflex single-gene NIPT due to inadequate contact information n=1297 newborns did not have outcome data No-call rate: 1.3%	Of the 201 newborns with outcome data, pathogenic variants were found for: Cystic fibrosis: 66 (32.8%) Beta-hemoglobinopathy: 45 (22.4%) Alpha-hemoglobinopathy: 43 (21.4%) Spinal muscular atrophy: 47 (23.4%)	Single-gene NIPT by Fetal Risk Category, n: High risk - Affected: 14 High risk - Unaffected: 15 Low risk - Affected: 1 Low risk - Unaffected: 161 Single-gene Clinical Performance, % (95% CI): Sensitivity: 93.3% (68.1% to 99.8%) PPV: 48.3% (36.1% to 60.1%) NPV: 99.4% (96% to 99.9%) End-to-end Clinical Analytic Estimate for Carrier Screening with

					Reflex Single-Gene NIPT, % (95% CI): Specificity: 99.8% (99.5% to 99.9%)
Wynn et al (2023)[35,]	42067	528	n=41621 negative carrier screen n= 62 single-gene NIPT had an uninformative result n= 3046 solicitations for newborn outcome data were not responded to No-call rate: 0.9%	Of the 526 newborns with outcome data, pathogenic variants were found for: Cystic fibrosis: 91 (17.3%) Beta-hemoglobinopathy: 157 (29.9%) Alpha-hemoglobinopathy: 205 (39%) Spinal muscular atrophy: 75 (14.3%)	Fetal Risk Score, n (%): High:34 (6.4%) Increased: 17 (3.23%) Decreased: 12 (2.28%) Low: 465 (88.4%) Single-gene NIPT Clinical Performance, % (95% CI): Sensitivity: 96% (79.7% to 99.9%) Specificity: 95.2% (93% to 96.9%) PPV: 50% (35.2% to 64.7%) NPV: 99.8% (98.4% to 99.9%) End-to-end Clinical Analytic Estimate for Carrier Screening with Reflex Single-Gene NIPT, % (95% CI): Sensitivity: 92.4% Specificity: 95.2%

CI: confidence interval; NIPT: non-invasive prenatal testing; NPV: negative predictive value; PPV: positive predictive value;

Table 23. Study Relevance Limitations

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Zhang et al (2019)[29,]	1. most had abnormal ultrasound findings or family history of genetic disease; unclear is test is intended to be used as adjunct or replacement for other screening				

Mohan et al (2022)[6,]	1. 23% had abnormal ultrasound findings; unclear is test is intended to be used as adjunct or replacement for other screening				
Westin et al (2022)	1. All mothers undergoing screening were previously determined to have at least one pathogenic HBB allele; Gestational age at single-gene NIPS not reported				
Hoskovec et al (2023)					
Wynn et al (2023)					

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 24. Study Design and Conduct Limitations

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Zhang et al (2019)[29,]	2. convenience sample				20/35 positive tests had confirmed diagnosis; 71 of 198 negative tests unknown outcome	
Mohan et al (2022)[6,]	2. convenience sample				Missing follow-up data	
Westin et al (2022)	2. convenience sample					
Hoskovec et al (2023)	2. convenience sample				2. 171 of 1669 positive maternal carriers did not receive single-gene NIPT; 1297of 1498 newborn outcomes not available	

Wynn et al (2023)	2. convenience sample				2. 4239 of 7538 positive maternal carriers did not receive single-gene NIPT; 2773 of 3299 carriers with single-gene NIPT did not have newborn outcomes available.	

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Clinical Utility

Direct Evidence
There is no direct evidence evaluating the clinical utility of NIPS for single-gene disorders.

Chain of Evidence
It is not possible to construct a chain of evidence for clinical utility due to the lack of sufficient evidence on clinical validity.

Summary: Noninvasive Prenatal Screening for Single-Gene Disorders
There is no direct evidence of clinical utility for either the Vistara or UNITY Fetal Risk Screen™, and concerns regarding the evidence for clinical validity. There is a potential that prenatal identification of pregnancies with single-gene disorders 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. Additionally, given the variability of single-gene disorders identified by the tests, there is a lack of experience with routine genetic screening for some of these disorders, with uncertainty regarding clinical decision-making based on the NIPT results.

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. The relevant outcomes are test accuracy and validity, morbid events, and resource utilization. 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 individuals at high risk of T21, but several studies have reported similar levels of diagnostic accuracy in average-risk individuals. 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 an improvement in the net health outcome.

For individuals who have a singleton pregnancy who receive NIPS for sex chromosome aneuploidies using cell-free fetal DNA, the evidence includes observational studies, mainly in high-risk pregnancies, and systematic reviews. The relevant outcomes are test accuracy and validity, morbid events, and resource utilization. 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. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have a twin pregnancy who receive NIPS for aneuploidies using cell-free fetal DNA, the evidence incudes 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. According to the American Congress of Obstetrics and Gynecologists (ACOG, 2020), cell free DNA screening can be performed in twin gestations. Sensitivity for trisomy 21 using cell free DNA for twin pregnancy is similar to singleton pregnancy although test failure may be higher. Because each fetus in a single pregnancy contribute different amounts of cell free DNA into the maternal circulation it is possible that an aneuploidy fetus would contribute less fetal DNA, masking the aneuploid test result. Nonetheless, noninvasive prenatal testing using cell-free DNA is considered an appropriate noninvasive prenatal screening option.

For individuals with pregnancy(ies) who receive NIPS for microdeletions using cell-free fetal DNA, the evidence includes several observational studies. The relevant outcomes are test accuracy and validity, morbid events, and resource utilization. The available studies on clinical validity have limitations (eg, missing data on confirmatory testing, false-negatives), and the added benefit of NIPS compared with current approaches is unclear. Moreover, the clinical utility of NIPS for microdeletions remains unclear and has not been evaluated in published studies. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who have twin pregnancy who receive noninvasive prenatal testing (NIPT) for twin zygosity using cell-free fetal DNA, the evidence includes an observational study. Relevant outcomes are test accuracy and validity, morbid events, and resource utilization. Sensitivity and specificity were high (100%) in one validation study conducted in 95 twin gestations. 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 that the technology results in an improvement in the net health outcome.

For individuals who have a singleton pregnancy who receive NIPS for T21, T18, and T13 using Vanadis NIPT, the evidence includes two industry sponsored studies. Relevant outcomes are test accuracy and validity, morbid events, and resource utilization. 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. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with pregnancies who receive NIPS for single-gene disorders the evidence base includes two commercially available tests. Using Vistara Single-Gene NIPT, the evidence includes 1 validation study and a case series of 2208 pregnancies. For the UNITY Fetal Risk Screen™ for autosomal recessive single-gene disorders, the evidence includes 1 retrospective validation study in a high-risk cohort of pregnancies with known HBB carrier status and two retrospective validation studies in a cohort of general pregnancies not at high risk for alpha-or beta-thalassemia, cystic fibrosis, sickle cell disease or spinal muscular atrophy. In the two cohorts of general-risk pregnancies, sensitivity ranged from 93.3% to 96%, specificity was reported as 95.2%, PPV ranged from 48.3% to 50%, and NPV was between 99.5 % and 99.9%. No-call results rates ranged from 0.9% to 1.3%. Relevant outcomes are test accuracy and validity, morbid events, and resource utilization. There is no direct evidence of clinical utility and a chain of evidence cannot be conducted due to insufficient evidence on clinical validity. There is a potential that prenatal identification of pregnancies with single-gene disorders 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 single-gene disorders identified by the test and the lack of experience with routine genetic screening for single-gene disorders, clinical decision making based on the Vistara NIPT is not well defined. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

PRACTICE GUIDELINES AND POSITION STATEMENTS

Guidelines or position statements will be considered for inclusion in ‘Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

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

The American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine (2020) released a joint practice bulletin summary (No. 226) on the screening for fetal aneuploidy.[30]

The following recommendations on cell-free DNA were based on “good and consistent” scientific evidence (Level A):

- "Prenatal genetic screening (serum screening with or without nuchal translucency ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling 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."

- "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."

The following recommendations were 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."

The following recommendations 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."

Cell-free DNA Screening for Single-Gene Disorders

In a practice advisory on cell-free DNA screening for single-gene disorders published in 2019 and reaffirmed in 2022, ACOG stated, "Although this technology is available clinically and marketed as a single-gene disorder prenatal screening option for obstetric care providers to consider in their practice, often in presence of advanced paternal age, there has not been sufficient data to provide information regarding accuracy and positive and negative predictive value in the general population. For this reason, single-gene cell-free DNA screening is not currently recommended in pregnancy."[31]

American College of Medical Genetics and Genomics

In 2023, the American College of Medical Genetics and Genomics (ACMG) published a practice guideline on NIPS for fetal chromosome abnormalities in the general-risk population.[32] The recommendations were informed by the systematic evidence review conducted by Rose et al (2022).[14] The guideline included the following relevant recommendations:

- "ACMG recommends NIPS over traditional screening methods for all pregnant patients with singleton gestation for fetal trisomies 21, 18, and 13 (Strong recommendation, based on high certainty of evidence)."
- "ACMG recommends NIPS over traditional methods for trisomy screening in twin gestations (Strong recommendation, based on high certainty of evidence)."
- "ACMG recommends that NIPS be offered to patients with a singleton gestation to screen for fetal SCA (Strong recommendation, based on high certainty of evidence)."
- "ACMG suggests that NIPS for 22q11.2 deletion syndrome be offered to all patients (Conditional recommendations, based on moderate certainty of the evidence)."
- "At this time, there is insufficient evidence to recommend routine screening for CNVs other than 22q11.2 deletions (No recommendation, owing to lack of clinically relevant evidence and validation)."
- "At this time, there is insufficient evidence to recommend or not recommend NIPS for the identification of RATs [rare autosomal trisomies] (No recommendation, owing to lack of clinically relevant evidence."

U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS

Not applicable.

ONGOING AND UNPUBLISHED CLINICAL TRIALS

Some currently ongoing and unpublished trials that might influence this evidence review are listed in Table 25.

Table 25. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			

NCT05618431[a]	Prospective Biological Sample Collection Aiming to Validate Non-invasive Prenatal Tests by Analyzing Fetal DNA Present in Maternal Blood Using a Next-generation Digital PCR Technique	1790	Jun 2024 (Recruiting)
Unpublished			
NCT03559374[a]	Study of Vanadis NIPT for Non-Invasive Prenatal Screening of Trisomies (T21, T18, andT13)	1200	Aug 2020 (status unknown, last update August 2018)
NCT03375359	First Trimester Screening for Trisomy 21, 18, 13 and 22q11.2 Deletion Syndrome - ReFaPo02	1000	1000 (status unknown, last update August 2022)
NCT05312814[a]	Clinical Utility of the Addition of a SNP-based NIPT Zygosity Determination in Twin Pregnancy Management.	700	Study completed on 5/15/23, no results posted

NCT: national clinical trial.
[a]Denotes industry-sponsored or cosponsored trial.

Government Regulations

National:

There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.

Local:

Wisconsin Physicians Service Insurance Corporation (WPS)
Local Coverage Determination (LCD): MolDX: Molecular Diagnostic Tests (MDT) (L36807)
Original Effective Date: For services performed on or after 02/16/2017
Revision Effective Date: For services performed on or after 04/27/2023

Coverage Indications, Limitations, and/or Medical Necessity
This coverage policy provides the following information:

- defines tests required to register for a unique identifier
- defines tests required to submit a complete technical assessment (TA) for coverage determination
- defines the payment rules applied to covered tests that are not reported with specific CPT codes
- lists specific covered tests that have completed the registration and TA process and meets
  Medicare's reasonable and necessary criteria for coverage.

Tests evaluated through the application process and/or technical assessment will be reviewed to answer the following questions:

- Is the test performed in the absence of clinical signs and symptoms of disease?
- Will the test results provide the clinician with information that will improve patient outcomes and/or change physician care and treatment of the patient?
- Will the test results confirm a diagnosis or known information?
- Is the test performed to determine risk for developing a disease or condition?

- Will risk assessment change management of the patient?
- Is there a diagnosis specific indication to perform the test?
- Is the test performed to measure the quality of a process or for Quality Control/Quality Assurance (QC/QA), i.e., a test to ensure a tissue specimen matches the patient?

Molecular Diagnostic Test (MDT) Policy Specific Definitions
MDT: Any test that involves the detection or identification of nucleic acid(s) (DNA/RNA), proteins, chromosomes, enzymes, cancer chemotherapy sensitivity and/or other metabolite(s). The test may or may not include multiple components. An MDT may consist of a single mutation analysis/identification, and/or may or may not rely upon an algorithm or other form of data evaluation/derivation.

LDT: Any test developed by a laboratory developed without FDA approval or clearance.

Applicable Tests/Assays
In addition to the MDT definition, this coverage policy applies to all tests that meet at least one of the following descriptions:

- All non-FDA approved/cleared laboratory developed tests (LDT)
- All modified FDA-approved/cleared kits/tests/assays
- All tests/assays billed with more than one CPT code to identify the service, including combinations of method-based, serology-based, and anatomic pathology codes
- All tests that meet the first three bullets and are billed with an NOC code

Covered Tests
Please refer to the MolDX website www.palmettogba.com/MolDX for covered and excluded tests' specific coding and billing information. www.palmettogba.com/moldx

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

- First-Trimester Screening for Down Syndrome Using Fetal Ultrasound Assessment of Nuchal Translucency Combined with Assessment of Markers in Maternal Serum – Retired 3/1/2010.
- Genetic Testing and Counseling
- Genetic Testing for Fetal RHD Genotyping Using Maternal Plasma
- Invasive Prenatal (Fetal) Diagnostic Testing
- Genetic Testing - Carrier Screening for Genetic Diseases

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through September 12, 2024, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
5/1/13	2/19/13	3/4/13	Joint policy established
9/1/13	6/19/13	6/26/13	Code update, added 0005M to the policy
5/1/14	2/24/14	3/3/14	Routine maintenance, code update, added CPT code 81507 to the policy
1/1/16	10/13/15	11/5/15	• Routine maintenance • Title changed to "Genetic Testing-Noninvasive Prenatal Testing for Fetal Aneuploidies and Microdeletions Using Cell-Free DNA" • Added codes 81420, 81599, 81479 and 0009M • Updates to mirror BCBSA: Description/Background, Regulatory Status, Inclusions/Exclusions, Rationale, Practice Guidelines/Position Statements & References • Medical Policy Statement updated (no position change)
3/1/17	12/13/16	12/13/16	• Routine maintenance • Code update-Addition ○ 81422 • MPS re: Microdeletions added – no change in position
1/1/18	10/19/17	10/19/17	• Routine maintenance • Rationale and references updated • Added WPS article information regarding excluded tests
1/1/19	10/16/18	10/16/18	• Routine maintenance
1/1/20	10/15/19		• Routine maintenance
1/1/21	10/20/20		Routine maintenance
1/1/22	10/19/21		• Routine maintenance • References updated • Language revised under Summary of Evidence in

			Indication 3 to clarify the evidence review refers to twin, not higher order multiple, gestations per BCBSA update. • Policy statements unchanged.
3/1/22	12/14/21		• 3/25/22 This revision is added for clarification purpose under Exclusion: • The first bullet is updated from Nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with multiple gestation pregnancies to Nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with pregnancies of multiple gestations of 3 or more fetuses. • Several changes made to the Inclusions and Exclusions section. • Inclusions: • Added twin: Nucleic acid sequencing-based testing of maternal plasma to screen for trisomy 21 in women with singleton and **twin** pregnancies. (Karyotyping would be necessary to exclude the possibility of a false positive nucleic acid sequencing-based test.) • Nucleic acid sequencing-based testing of maternal plasma for fetal sex or fetal sex chromosome aneuploidy only when certain fetal abnormalities are noted on ultrasound such as cases of ambiguous genitalia or cystic hygroma when the determination of fetal sex is necessary to help guide medical management. • Exclusions: • Removed **twins** under the first bullet: Nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with multiple gestation pregnancies.

			• Added the below bolded language: • Nucleic acid sequencing-based testing of maternal plasma for fetal sex determination and/or fetal sex chromosome aneuploidies other than the situation specified above. • For other aneuploidies or genetic disorders not specified above • Added the below language to both unlisted codes 81599 and 81479 for clarification: [when specified as cell-free fetal DNA-based prenatal testing involving multianalyte assays and an algorithmic analysis for fetal aneuploidy]
3/1/23	12/20/22		• Added new indication and investigational policy statement under Exclusions: • Vistara NIPT of maternal plasma to screen for single-gene disorders. Updated title to include single-gene disorders. • The words women and patients changed to individuals. • Per code update: added code 0327U EFD 7/1/22 as E/I. • Code 0168U deleted as code was deleted EFD 10/1/21. • References updated. (ky)
3/1/24	12/19/23		• Routine maintenance • BCBSM received multiple emails from company requesting JUMP to review the Unity Screen (BillionToOne) test. After evaluation, Unity Screen (BillionToOne) test added as E/I. o Unity Screen (BillionToOne) test added to the Description section and added under the Regulatory section. o Removed the last bullet for other aneuploidies or genetic disorders not

			specified above under Exclusions and added NIPT for other aneuploidies, genetic disorders, or single-gene disorders using cell-free DNA. • Vendor: N/A • Added code 81479 under exclusion if the code represents the test Vistara. (ky)
7/1/24	4/16/24		• Routine maintenance • Per code update added code 0449U effective 4/1/24 for Unity Screen by BillionToOne, Inc. under E/I. • Aligning with BCBSA – moved code 0327U for the test Vasistera TM from E/I to EST. • Updated the first bullet under Inclusions to align with BCBSA with the below bold 18, and 13: o Nucleic acid sequencing-based testing of maternal plasma to screen for trisomy 21, 18, and 13 in women with singleton and twin pregnancies. (Karyotyping would be necessary to exclude the possibility of a false positive nucleic acid sequencing-based test.) • Removed the 2nd bullet below under Inclusions: o Concurrent nucleic acid sequencing-based testing of maternal plasma for trisomy 13 and/or 18 in women who are eligible for and are undergoing nucleic acid sequencing-based testing of maternal plasma for trisomy 21.

			• Updated the first bullet under Exclusions with the below bold **18, and 13**: o Nucleic acid sequencing-based testing of maternal plasma for trisomy 21, **18, and 13** in individuals with pregnancies of multiple gestations of 3 or more fetuses. • Removed the 2nd bullet under Exclusions as the above statement was adjusted to include 18 and 13: o Nucleic acid sequencing-based testing of maternal plasma for trisomy 13 and/or 18, other than in the situations specified above. • Updated the below bullet under Exclusions: o NIPT of maternal plasma to screen for single-gene disorders (e.g. Vistara or UNITY NIPTs). • Vendor: N/A Post JUMP • Added or any combination of the three to the below first bullet under Inclusions: Nucleic acid sequencing-based testing of maternal plasma to screen for trisomy 21, 18, and 13 in individuals **or any combination of the three** with singleton and twin pregnancies. (Karyotyping would be necessary to exclude the possibility of a false positive nucleic acid sequencing-based test. (ky)
1/1/25	10/15/24		• Routine maintenance: this JUMP policy is coming early because of coding issues. Per

			code update added code 0489U EFD 10/1/24 as E/I. • Code 0449U is removed from this JUMP policy and will be put on the GT-carrier screen policy as E/I. • Deleted NIPTs and added Fetal Risk Screen™ under Exclusion in the below bullet. ○ NIPT of maternal plasma to screen for single-gene disorders (e.g. Vistara or UNITY Fetal Risk Screen™). • Policy edited to remove UNITY NIPT and replace with Fetal Risk Screen™ throughout the policy. • This JUMP policy will now come to October, JUMP. • Vendor: N/A ky

Next Review Date: 4th Qtr, 2025

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING – NONINVASIVE PRENATAL SCREENING FOR FETAL ANEUPLOIDIES, MICRODELETIONS, SINGLE-GENE DISORDERS AND TWIN ZYGOSITY USING CELL-FREE FETAL DNA

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered, criteria apply
BCNA (Medicare Advantage)	See Government Regulations section.
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member's contract must be active at the time the service is rendered.
- Coverage is based on each member’s certificate and is not guaranteed. Please consult the individual member’s certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member's PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.