Medical Policy

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Title: Noninvasive Prenatal Screening for Fetal Aneuploidies and Microdeletions Using Cell-Free Fetal DNA

Professional
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Populations	Interventions	Comparators	Outcomes
Individuals: • With a singleton pregnancy	Interventions of interest are: • Noninvasive prenatal screening for trisomies 21, 18, and 13 using cell-free fetal DNA	Comparators of interest are: • Conventional serum screening • Diagnostic testing • Standard of care without screening	Relevant outcomes include: • Test accuracy • Test validity • Morbid events • Resource utilization

Populations	Interventions	Comparators	Outcomes
Individuals: • With a singleton pregnancy	Interventions of interest are: • Noninvasive prenatal screening for sex chromosome aneuploidies using cell-free fetal DNA	Comparators of interest are: • Conventional serum screening • Diagnostic testing • Standard of care without screening	Relevant outcomes include: • Test accuracy • Test validity • Morbid events • Resource utilization
Individuals: • With twin or multiple pregnancies	Interventions of interest are: • Noninvasive prenatal screening for aneuploidies using cell-free fetal DNA	Comparators of interest are: • Conventional serum screening • Diagnostic testing • Standard of care without screening	Relevant outcomes include: • Test accuracy • Test validity • Morbid events • Resource utilization
Individuals: • With pregnancy (ies)	Interventions of interest are: • Noninvasive prenatal screening for microdeletions using cell-free fetal DNA	Comparators of interest are: • Diagnostic testing • Standard of care without screening	Relevant outcomes include: • Test accuracy • Test validity • Morbid events • Resource utilization

DESCRIPTION

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 (T21), 18 (T18), and 13 (T13) are the most common forms of fetal aneuploidy. Fetusus with T18 and T13 generally do not survive to birth. There are numerous limitations to standard screening for these disorders using maternal serum and fetal ultrasound. Noninvasive prenatal screening (NIPS) analyzing cell-free fetal DNA in maternal serum is a potential complement or alternative to conventional serum screening. NIPS using cell-free fetal DNA has also been proposed to screen for microdeletions.

OBJECTIVE

The objective of this policy is to determine whether noninvasive testing for cell-free fetal DNA to screen for aneuploidies of chromosomes 13, 18, or 21, or sex chromosome aneuploidies, or microdeletions improves the net health outcome in individuals compared with standard of care.

BACKGROUND

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 3 copies of 1 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]

Trisomy 21 is the most common cause of human birth defects and provides the impetus for current maternal serum screening programs. Other trisomy syndromes include trisomy 18 (Edwards syndrome), and trisomy 13 (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 and increases detection of T21, T18, and T13 has the potential to improve outcomes. Confirmation of positive noninvasive screening tests with amniocentesis or CVS is recommended; with more accurate tests, fewer individuals would receive positive screening results.

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

Cell-Free DNA Analysis Methods

Sequencing-based tests use 1 of 2 general approaches to analyzing cell-free DNA. The first category of tests uses quantitative or counting methods. The most widely used technique to date uses massively parallel shotgun sequencing (MPS; also known as next-generation or “next gen” sequencing). DNA fragments are amplified by polymerase chain reaction; during the sequencing process, the amplified fragments are spatially segregated and sequenced simultaneously in a massively parallel fashion.

Sequenced fragments can be mapped to the reference human genome in order to obtain numbers of fragment counts per chromosome. The sequencing-derived percent of fragments from the chromosome of interest reflects the chromosomal representation of the maternal and fetal DNA fragments in the original maternal plasma sample. Another technique is direct DNA analysis, which analyzes specific cell-free DNA fragments across samples and requires approximately a tenth the number of cell-free DNA fragments as MPS. The digital analysis of selected regions (DANSR™) is an assay that uses direct DNA analysis.

The second general approach is single nucleotide variant-based methods. These use targeted amplification and analysis of approximately 20,000 SNPs on selected chromosomes (eg, 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.

Copy Number Variants and Clinical Disorders

Microdeletions (also known as submicroscopic deletions) are defined as chromosomal deletions that are too small to be detected by microscopy or conventional cytogenetic methods. They can be as small as 1 and 3 megabases (Mb) long. Microdeletions, along with microduplications, are collectively known as copy number variations (CNVs). CNVs can lead to disease when the change in copy number of a dose-sensitive gene or genes disrupts the ability of the gene/s to function and effects the amount of protein produced. A number of genomic disorders associated with microdeletion have been identified. The disorders have distinctive and, in many cases, serious clinical features, such as cardiac anomalies, immune deficiency, palatal defects, and developmental delay as in DiGeorge syndrome. Some of the syndromes such as DiGeorge have complete penetrance yet marked variability in clinical expressivity. Reasons for the variable clinical expressivity are not entirely clear. 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. Risk of a fetus with a microdeletion syndrome is independent of maternal age. There is little population-based data and most studies published to date base 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 microdeletion syndromes in the prenatal population because the population of mutation 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).[2]

Routine prenatal screening for microdeletion syndromes is not recommended by national organizations. Current practice is to offer invasive prenatal diagnostic testing in selected cases to women when a prenatal ultrasound indicates anomalies (eg, heart defects, cleft palate) that could be associated with a particular microdeletion syndrome. Samples are analyzed using fluorescence in situ hybridization (FISH), chromosomal microarray analysis (CMA), or karyotyping. In addition, families at risk (eg, those known to have the deletion or with a previous 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 (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories offering LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of noninvasive prenatal screening tests using cell-free fetnal DNA.

Commercially available tests include, but are not limited to, the following:

- VisibiliT™ (Sequenom Laboratories, now LabCorp) tests for T21 and T18, and tests for sex.
- MaterniT21™ PLUS (Sequenom Laboratories) tests for trisomy 21, 18, and 13 and fetal sex aneuploidies. The enhanced sequencing series includes testing for trisomies 16 and 22 and 7 microdeletions: 22q deletion syndrome (DiGeorge syndrome), 5p (cri du chat syndrome), 15q (Prader-Willi and Angelman syndromes), 1p36 deletion syndrome, 4p (Wolf-Hirschhorn syndrome), 8q (Langer-Giedion syndrome), and 11q (Jacobsen syndrome). The test uses massive parallel sequencing (MPS) and reports results as positive or negative. The enhanced sequencing series is offered on an opt-out basis.

- Harmony™ (Ariosa Diagnostics was acquired by Roche in 2015) tests for trisomies 21, 18, and 13. Uses directed DNA analysis, results reported as risk score.
- Panorama™ (Natera) is a prenatal test for detecting trisomy 21, 18, and 13, as well as select sex chromosome abnormalities. It uses single-nucleotide polymorphisms technology; results reported as risk score. An extended panel tests for 5 microdeletions: 22q deletion syndrome (DiGeorge syndrome), 5p (cri du chat syndrome), 15q11-13 (Prader-Willi and Angelman syndromes), and 1p36 deletion syndrome. Screening for 22q11.2 will be included in the panel unless the opt-out option is selected; screening for the remaining 4 microdeletions is offered on an opt-in basis.
- Verifi® (Illumina; formerly Verinata Health, which it acquired) is a prenatal test for trisomy 21, 18, and 13. The test uses MPS and calculates a normalized chromosomal value [NPS], reporting results as 1 of 3 categories: no aneuploidy detected, aneuploidy detected, or aneuploidy suspected.
- InformaSeq℠ (Integrated Genetics) is a prenatal test for detecting trisomy 21, 18, and 13, with optional additional testing for select sex chromosome abnormalities. It uses the Illumina platform and reports results in similar manner.
- QNatal™ Advanced (Quest Diagnostics) tests for trisomies 21, 18, and 13.

POLICY

A. Nucleic acid sequencing-based testing of plasma for trisomy 21, 18, and 13 may be considered **medically necessary** in individuals with singleton pregnancies.

B. Nucleic acid sequencing-based testing of plasma for trisomy 21, 18, and 13 is considered **experimental / investigational** in individuals with twin or multiple pregnancies.

D. Nucleic acid sequencing-based testing of plasma for fetal sex chromosome aneuploidies is considered **experimental / investigational**.

E. Nucleic acid sequencing-based testing of plasma for microdeletions is considered **experimental / investigational**.

Policy Guidelines

1. Karyotyping would be necessary to exclude the possibility of a false-positive, nucleic acid sequencing–based test. Before testing, women 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.

2. Studies published to date report rare but occasional false positives. In these studies, the actual false-positive test results were not always borderline; some were clearly above the assay cutoff value, and no processing or biological explanations for the false-positive results were reported. False-positive findings have been found to be associated with factors including placental mosaicism, vanishing twins, and malignancies. In its 2015 committee opinion, ACOG recommended diagnostic testing to confirm positive cell-free fetal DNA tests, and that management decisions not be based solely on the results of cell-free DNA testing. ACOG further recommends that patients with indeterminate or uninterpretable (ie, "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.
3. Cell-free fetal DNA screening does not assess risk of anomalies such as neural tube defects. Patients should continue to be offered ultrasound or serum alpha-fetoprotein screening, regardless of the type of serum screening selected.

Genetics Nomenclature Update

Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Human Genome Variation Society nomenclature is recommended by the Human Genome Variation Society, the Human Variome Project, and the Human Genome Organisation.

The American College of Medical Genetics and Genomics and Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, and the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

Genetic Counseling

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

RATIONALE

The evidence review is based on literature reviews, most recently through June 4, 2018.

The review was informed by 2 TEC Assessments. One TEC Assessment (2013) focused on detection of trisomy 21 (T21),[3] and the other TEC Assessment (2014) addressed detection of fetal aneuploidies other than T21 (specifically trisomies 13 [T13] and 18 [T18], and fetal sex chromosome aneuploidies).[4]

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 noninvasive prenatal screening (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 patients who have singleton pregnancy is to inform a decision whether to proceed with diagnostic testing.

The question addressed in this evidence review is as follows: In pregnant individuals, does NIPS for chromosomal aneuploidies lead to improvements in health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is women 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 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.

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

Setting
The test would be used in the primary care or specialty care setting (ie, obstetrics-gynecology). Genetic counseling may also be necessary.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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).

A Cochrane review by Badeau et al (2017) included 65 studies on the screening of women with singleton pregnancy (see Table 2).[5] None of the studies was rated as at low risk of bias, although they were considered to have 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 (see 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 (see Table 4). Of the 94,149 unaffected women, 94 would undergo an unnecessary invasive test. Reviewers concluded that the performance of 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)[5]	65	Women with singleton pregnancy	RCTs, cohort studies, case-control	Fetal karyotyping or neonatal clinical examination	0	41	24

RCT: randomized controlled trial.

Table 3. Systematic Reviews 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	

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, 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 Reviews 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.

Section Summary: Clinical Validity

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. Calculation indicated that 64 to 70 affected cases would be missed out of 100000 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.

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 randomized controlled trials.

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.[3,4] The model in the 2013 TEC Assessment focused on T21. In this model, the primary health outcomes of interest included the number of cases of aneuploidy correctly identified, number of cases missed, the number of invasive procedures potentially avoided (ie, with a more sensitive test), and the number of miscarriages potentially avoided as a result of fewer invasive procedures. The results were calculated for a high-risk population of women 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 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 United States per year and two-thirds of the population of average-risk pregnant women (2.8 million) accepted screening, the following outcomes would occur for the 3 screening strategies under consideration:

- Standard screening: Of the 2.8 million screened with the stepwise sequential screen, 87,780 would have an invasive procedure (assuming 60% uptake after a positive screening test and a recommendation for confirmation), 448 would have a miscarriage, and 3976 (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 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 2 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 (ie, as a screening test) and without confirmatory testing (ie, as a diagnostic test).[6] Results of the model concluded that using sequencing-based tests with confirmatory test results in fewer losses of normal pregnancies compared with sequencing-based tests used without a confirmatory test. The model assumed estimates using the total population of 520,000 high-risk women presenting for first trimester care each year in the United States. Sequencing-based tests used with confirmatory testing resulted in 1441 elective terminations (all with Down syndrome). Without confirmatory testing, sequencing-based 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: Clinically Useful
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 (ie, Down syndrome) cases detected and when included in the model, a large decrease in the number of invasive tests and associated miscarriages.

NIPS 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 question addressed in this evidence review are as follows: In pregnant individuals, does NIPS for sex chromosome aneuploidies lead to improvements in health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is women 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.

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

Setting
The test would be used in the primary care or specialty care setting (ie, obstetrics-gynecology). Genetic counseling may also be necessary.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid

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

The Cochrane review by Badeau et al (2017) evaluated the diagnostic accuracy of NIPS for sex chromosome anomalies.[5] 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% (see Table 5). Reviewers calculated that of 100000 pregnancies, 1039 would be affected by 45, X. 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 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 Pregnant Women

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	

MPS: massively parallel sequencing; TMPS: targeted massively parallel sequencing.
[a] Chromosomes 45, X, 47, XXX, 47, XXY and 47, XYY combined.

Section Summary: Clinically Valid

There are less data on the diagnostic performance of sequencing-based tests for detecting sex chromosome aneuploidies. The available data have suggested that diagnostic performance for detecting these other fetal aneuploidies is not as high as it is for detection of T21, T18, and T13 and there is a higher rate of false-positive tests.

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 randomized controlled trials.

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 an evaluation of diminished fertility. Alternatively, sex chromosome aneuploidies may be diagnosed incidentally during invasive karyotype testing of pregnant women at high risk for Down syndrome. It 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: Clinically Useful
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.

NIPS for Fetal Aneuploidies in Twin and Multiple Pregnancies
Clinical Context and Test Purpose
The purpose of NIPS using analysis of cell-free fetal DNA in patients who have a twin or other multiple pregnancy is to inform a decision whether to proceed with diagnostic testing.

The questions addressed in this evidence review are as follows: In patients who have a twin or multiple pregnancy, does NIPS for aneuploidies lead to improvements in health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is women with first- and second-trimester twin or other multiple 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 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 use of other noninvasive and invasive tests received by the pregnant individuals.

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

Setting
The test would be used in the primary care or specialty care setting (ie, obstetrics-gynecology). Genetic counseling may also be necessary.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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).

A meta-analysis by Liao et al (2017) identified 10 studies published through July 2016 that reported on the diagnostic performance of NIPS for identifying aneuploidies in twin pregnancies (see Table 6).[7] Only 1 of the studies (12 patients) was rated as low risk of bias. Risk of bias was highest for the domains of patient selection, flow and timing, and reference standard. There were no applicability concerns.

Of 2093 cases included in the analysis, there were 69 cases of T21, 13 cases of T18, and 3 cases of T13. Of the 69 cases of T21, there was 1 false-negative and 1 false-positive test (see Table 7). A limitation of this systematic review was the exclusion of 23% of cases, including a loss to follow-up of 483 patients and failure of the test in 70 patients. Evaluation of diagnostic accuracy for T13 was limited by the small number of cases.

Table 6. Characteristics Systematic Reviews for Fetal Aneuploidies

					No. of Studies Rated as "High" or "Unclear" Risk of Bias		
Study	No. of Studies	Study Populations	N (N Excluded)	Reference Standard of Studies	No Domains	1-2 Domains	>2 Domains
Liao et al (2017)[7]	10	Women with twin pregnancy	2711 (618 excluded from analysis)	Fetal karyotyping or neonatal clinical examination	1	7	2

Table 7. Systematic Review Results for Fetal Aneuploidies

Trisomy	Affected Pregnancies	Sensitivity (95% CI), %	Specificity (95% CI), %	FP	FN	Diagnostic Odds Ratio (95% CI)
21	69	99 (92 to 100)	100 (99 to 100)	1	1	1298 (438 to 3844)
18	13	85 (55 to 98)	100 (99 to 100)			334 (35 to 3171)
13	3	100	100	0	0	

Adapted from Liao et al (2017).[7]
CI: confidence interval.

Two other studies were published after the search date of the Liao meta-analysis. Du et al (2017) included 92 women with twin pregnancies.[8] Cell-free fetal DNA testing correctly identified two T21 pregnancies, and there was 1 false-positive T13 test. No cases of T18 were identified. Fosler et al (2017) evaluated 2 sets of blood samples from women pregnant with twins.[9] In the first set of samples (n=115), 3 cases of T21 and 1 case of T18 were correctly identified. In the second set (n=487), 6 of 9 cases of suspected of being affected by T21 were confirmed by invasive testing or birth outcomes to be true positives in at least 1 twin.

Section Summary: Clinically Valid

A meta-analysis identified 10 studies assessing the clinical validity of NIPS for detecting aneuploidies in twin pregnancies, and 2 additional studies were published in 2017. The total number of cases of T21 identified was less than 100 and there were even fewer cases of T18 and T13. This quantity of evidence is insufficient for drawing conclusions about clinical validity.

Clinically Useful

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

Direct Evidence

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

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Direct evidence is not available for the evaluation of noninvasive prenatal testing to detect fetal aneuploidies in women pregnant with twins or multiples.

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.
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: Clinically Useful
There is a lack of direct evidence of clinical utility, and a chain of evidence cannot be constructed due to insufficient evidence on clinical validity.

Noninvasive Screening for Fetal Microdeletions Using Cell-Free Fetal 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 questions addressed in this evidence review are as follows: In pregnant individuals, does NIPS for fetal microdeletions have better diagnostic accuracy than standard approaches and does NIPS lead to improvements in health outcomes?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population of interest is women who are pregnant.

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 microdeletions: 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 use of other noninvasive and invasive tests received by the pregnant individuals.

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

Setting
The test would be used in the primary care or specialty care setting (ie, obstetrics-gynecology). Genetic counseling may also be necessary.

Technically Reliable

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid

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

Several studies have reported on the clinical validity of NIPS for detecting microdeletion syndromes (see Table 8). Gross et al (2016) and Helgeson et al (2015) reported on positive NIPS results for high-risk women.[10,11] Petersen et al (2017) compared test results from amniotic or chorionic samples of unselected women referred for diagnostic testing due to a positive NIPS result. The positive predictive value of NIPS to identify a microdeletion syndrome ranged from 13% in Petersen et al (2017) and 18% in Gross et al (2017) to 77% in Helgeson et al (2015). The basis for the large variance in the positive predictive value (PPV) is unclear, although Helgeson et al (2015) reported that, in 25 of the 55 microdeletions identified by NIPS, a maternal component was identified. In at least 5 cases, deletions were confirmed in the pregnant woman but not in the fetus. Gross et al (2017) reported that 8 (73%) of the 11 true-positive cases in their study could have been identified without NIPS (ie, by ultrasound followed by invasive testing). A limitation of all studies is the lack of reporting on false- negatives, because follow-up after negative screening results was voluntary and/or not available from the retrospective review of deidentified data.

Table 8. Systematic Review Characteristics for Microdeletions

Study	Test	Syndrome		Population	Reference Test	Comment
Gross et al (2016)[10]	Natera	22q11.2	DiGeorge	20,776 samples from high-risk pregnant women submitted for screening	Diagnostic testing in 61	
Petersen et al (2017)[12]	Various	• 1p36 • 5p- • 15q- • 22q11.2	• 1p36 • Cri du chat • Prader-Willi • DiGeorge	52 cases referred for diagnostic testing following positive NIPS for MDS	Diagnostic CMA, FISH, or karyotyping	
Helgeson et al (2015)[11]	Sequenom MPS-based test	• 1p36 • 5p- • 15q- • 22q11.2	• 1p36 • Cri du chat • Prader-Willi • DiGeorge	175,393 samples from high-risk pregnant women submitted for screening	Diagnostic CMA, FISH, karyotyping, or clinical suspicion	In at least 5 cases, deletions were confirmed in the pregnant woman but not in the fetus

CMA: chromosomal microarray; FISH: florescence in-situ hybridization; MDS: microdeletion syndromes; MPS: massively sequencing; NIPS: noninvasive prenatal screening.

Table 9. Systematic Review Results for Microdeletion Syndromes

Study	Initial N	Final N	Excluded Samples	Positive Tests, n (%)	Clinical Validity			
					TP, n (%)	PPV, %	FP	FN
Gross et al (2016)[10]	21,949	20,776	1172	97 (0.46)	11 (0.05)	18	86	Unknown
Petersen et al (2017)[12]	52	52	NR	52	7	13	45	Unknown
Helgeson et al (2015)[11]	175,393		NR	55 (0.03)	41	77.4%[a]	3	Unknown

FN: false negatives; FP: false positives; NR: not reported; PPV: positive predictive value; TP: true positives.
[a] An additional 9 cases did not have confirmatory testing but had clinical features consistent with one of the microdeletions.

Section Summary: Clinically Valid
Several studies on the clinical validity of microdeletion testing have been published; they are based on large numbers of samples submitted to the testing companies. The PPV in these studies ranged from 18% to 77%. Another study evaluated diagnostic test results for women who had received a positive NIPS result, finding a PPV of only 13%. These studies have limitations (eg, missing data on confirmatory testing, lack of complete data on false-negatives). Many (up to 73%) of the cases of microdeletion syndromes may also be detected by characteristic anomalies seen on prenatal ultrasound.

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 randomized controlled trials.

There are no direct data on whether sequencing-based testing for microdeletions 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.

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

There is a potential that prenatal identification of individuals with microdeletion syndromes could improve health outcomes due to the ability to allow for informed reproductive decision making and/or initiate earlier treatment; however, data demonstrating improvement are unavailable. Given the variability of expressivity of microdeletion syndromes and the lack of

experience with routine genetic screening for microdeletions, clinical decision making based on genetic test results is not well defined. It is not clear what follow-up testing or treatments might be indicated for screen-detected individuals.

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

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

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

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. 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 women at high risk of T21, but several studies have reported similar levels of diagnostic accuracy in average-risk women. Compared with standard serum screening, both the sensitivity and specificity of cell-free fetal DNA screening are considerably higher. As a result, screening with cell-free fetal DNA for T21 will result in fewer missed cases of Down syndrome, fewer invasive procedures, and fewer cases of pregnancy loss following invasive procedures. Screening for T18 and T13 along with T21 may allow for preparation for fetal demise or termination of the pregnancy prior to fetal loss. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have a 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. 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 the effects of the technology on health outcomes.

For individuals who have a twin or multiple pregnancy who receive NIPS for aneuploidies using cell-free fetal DNA, the evidence includes observational studies and a systematic review. Relevant outcomes are test accuracy and validity, morbid events, and resource utilization. The total number of cases of aneuploidy identified in these studies is small and is insufficient to draw conclusions about clinical validity. There is a lack of direct evidence of clinical utility, and a chain of evidence cannot be conducted due to the paucity of evidence on clinical validity. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with pregnancy(ies) who receive NIPS for microdeletions using cell-free fetal DNA, the evidence includes several observational studies. 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 the effects of the technology on health outcomes.

CLINICAL INPUT RECEIVED THROUGH PHYSICIAN SPECIALTY SOCIETIES AND ACADEMIC MEDICAL CENTERS

While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

In response to requests, input was received from 3 physician specialty societies and 4 academic medical centers while this policy was under review in 2013. There was a consensus that sequencing-based tests to determine trisomy 21 (T21) from maternal plasma cell-free fetal DNA may be considered medically necessary in women with high-risk singleton pregnancies undergoing screening for T21. Input was mixed on whether sequencing-based tests to determine T21 from maternal plasma DNA may be considered medically necessary in women with average-risk singleton pregnancies. An American College of Obstetricians and Gynecologists genetics committee opinion, included as part of the specialty society's input, did not then recommend the new tests for women with singleton pregnancies who were not at high risk of aneuploidy. There was a consensus that sequencing-based tests to determine T21 from maternal plasma DNA are investigational for women with multiple pregnancies. Regarding an appropriate protocol for using sequencing-based testing, there was a consensus that testing should not be used as a single-screening test without confirmation of results by karyotyping. There was mixed input on the use of the test as a replacement for standard screening tests with karyotyping confirmation and on use as a secondary screen in women with screen positive on standard screening tests with karyotyping confirmation. Among the 5 reviewers who responded to the questions (which did not include the American College of Obstetricians and Gynecologist), there was a consensus that the modeling approach is sufficient to determine the clinical utility of the new tests and near-consensus there is no need for clinical trials comparing

a screening protocol using the new tests to a screening protocol using standard serum screening before initiation of clinical use of the tests.

PRACTICE GUIDELINES AND POSITION STATEMENTS

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 (2016) released a joint practice bulletin summary (No. 163) on the screening for fetal aneuploidy.[13] The following recommendations on cell-free DNA were based on "good and consistent" scientific evidence:

- "Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result."
- "Because cell-free DNA is a screening test with the potential for false-positive and false-negative results, such testing should not be used as a substitute for diagnostic testing."
- "All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken."
- "Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy."

The following recommendations were based on "limited or inconsistent" scientific evidence:

- "Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time."
- "No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies."

The following recommendations are based "primarily on consensus and expert opinion":

- "Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing."
- "This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy."
- "Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost effective and should not be performed."

American College of Medical Genetics and Genomics

In 2016, the American College of Medical Genetics and Genomics (ACMG) published a position statement on NIPS for fetal aneuploidy.[14] Relevant ACMG recommendations are as follows:

- "Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes)."
- "Referring patients to a trained genetics professional when an increased risk of aneuploidy is reported after NIPS."
- "Offering diagnostic testing when a positive screening test result is reported after NIPS."

- "Providing accurate, balanced, up-to-date information, at an appropriate literacy level when a fetus is diagnosed with a chromosomal or genomic variation in an effort to educate prospective parents about the condition of concern. These materials should reflect the medical and psychosocial implications of the diagnosis."

ACMG did not recommend "NIPS to screen for autosomal aneuploidies other than those involving chromosomes 13, 18, and 21."

International Society for Prenatal Diagnosis

In 2015, the International Society for Prenatal Diagnosis published a position statement on the prenatal diagnosis of chromosomal abnormalities, updating its 2013 statement.[15,16] (Note that a number of the authors of the 2015 report had financial links to industry.) The following summarizes the Society's recommendations:

"I. High sensitivities and specificities are potentially achievable with cfDNA [cell-free DNA] screening for some fetal aneuploidies, notably trisomy 21.

II. Definitive diagnosis of Down syndrome and other fetal chromosome abnormalities can only be achieved through testing on cells obtained by amniocentesis or CVS [chorionic villous sampling].

III. The use of maternal age alone to assess fetal Down syndrome risk in pregnant women is not recommended.

IV. A combination of ultrasound NT [nuchal translucency] measurement and maternal serum markers in the first trimester should be available to women who want an early risk assessment and for whom cfDNA screening cannot be provided.

V. A four-marker serum test should be available to women who first attend for their prenatal care after 13 weeks 6 days of pregnancy and where cfDNA screening cannot be provided.

VI. Protocols that combine first trimester and second trimester conventional markers are valid.

VII. Second trimester ultrasound can be a useful adjunct to conventional aneuploidy screening protocols.

VIII. When cfDNA screening is extended to microdeletion and microduplication syndromes or rare trisomies the testing should be limited to clinically significant disorders or well defined severe conditions. There should be defined estimates for the detection rates, false-positive rates, and information about the clinical significance of a positive test for each disorder being screened."

U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS

The U.S. Preventive Services Task Force does not currently address screening for Down syndrome. This topic had been addressed in the 1990s, but the topic is no longer listed on the USPSTF website.

ONGOING AND UNPUBLISHED CLINICAL TRIALS

Some currently unpublished trials that might influence this policy are listed in Table 10.

Table 10. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT03200041[a]	Clinical Evaluation of the IONA Test for Non-invasive Pre Natal Screening in Twin Pregnancies	1000	Jul 2019
NCT01545674[a]	Prenatal Non-invasive Aneuploidy Test Utilizing SNPs Trial (PreNATUS)	1000	Dec 2019
Unpublished			
NCT01925742	PEGASUS: PErsonalized Genomics for Prenatal Aneuploidy Screening Using Maternal Blood	3819	Jun 2017 (completed)

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

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

81420	Fetal chromosomal aneuploidy (eg, trisomy 21, monosomy X) genomic sequence analysis panel, circulating cell-free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18 and 21
81422	Fetal chromosomal microdeletion(s) genomic sequence analysis (eg, DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood
81479	Unlisted molecular pathology procedure
81507	Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each
81599	Unlisted multi analyte assay with algorithmic analysis
88271	Molecular cytogenetics; DNA probe, each (eg, FISH)
0009M	Fetal aneuploidy (trisomy 21 and 18) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy

- There is a specific MAAA CPT code for the Ariosa Diagnostics Harmony™ Prenatal Test: 81507.
- If the test is run as a genomic sequence analysis panel that includes analysis of all 3 chromosomes and does not involve an algorithmic analysis, the following CPT code is available: 81420.
- There is a multianalyte assays with algorithmic analyses (MAAA) administrative code specific to the VisibiliT™ test: 0009M.
- If the codes above do not apply and the test involves multianalyte assays and an algorithmic analysis, it would be reported with the unlisted MAAA code: 81599.
- If the codes above do not apply, the unlisted molecular pathology code 81479 is available when the test does not involve an algorithmic analysis.

- There are reports that the Natera Panorama panel is reported with CPT code 88271.
- Code 81420 is not specific to the depth of sequencing; therefore, it would include deeper sequencing such as for microdeletion syndromes. Hence, it would be incorrect to additionally report code 88271. Some laboratories are reporting this testing with multiple units of 88271.
- There is specific for testing blood for fetal chromosomal microdeletion(s): 81422.

ICD-10 Diagnoses

O09.511	Supervision of elderly primigravid, first trimester
O09.512	Supervision of elderly primigravida, second trimester
Z31.5	Encounter for procreative genetic counseling
Z36.0	Encounter for antenatal screening for chromosomal anomalies

REVISIONS

06-10-2013	Policy added to the bcbsks.com web site on 05-10-2013. Effective on 06-10-2013, 30 days after posting.
06-10-2013	(Posted 06-07-2013) In Coding section ▪ Added CPT code: 81479 ▪ Updated Coding information
12-31-2013	In Coding section: ▪ Added CPT codes: 81504 and 81507 *(New codes, effective January 1, 2014)* ▪ Added ICD-10 Diagnosis codes *(Effective October 1, 2014)*
	Updated Reference section
12-24-2014	In Policy title: ▪ Changed Policy title from "Sequencing-Based Tests to Determine Trisomy 21 from Maternal Plasma DNA"
	Updated Description section.
	In Policy section: ▪ Added "B. Concurrent Nucleic acid sequencing-based testing of maternal plasma for trisomy 13 and/or 18 may be considered medically necessary in women who are eligible for and are undergoing nucleic acid sequencing-based testing of maternal plasma for trisomy 21." In Policy Guideline section: ▪ Removed, "This policy focuses on detection of trisomy 21, as it is the most common cause of human birth defects and provides the impetus for current maternal serum screening programs. Detection of trisomy 21 by DNA-based sequencing methods would likely be representative of the testing technology and interpretation for autosomal trisomy detection such as trisomy 18 and 13 (but not for aneuploidies of sex chromosomes). However, screening for these other trisomy syndromes is not currently the main intent of prenatal screening programs. The prevalence of other trisomy syndromes is much lower than the prevalence of trisomy 21. Also, the clinical implications of identifying trisomy 18 and 13 are unclear, as most fetuses with trisomy 18 and 13 do not survive to term."
	Updated Rationale section.
	Updated Summary section.
	In Coding section:

	▪ Added CPT code 81420, Fetal chromosomal aneuploidy (eg, trisomy 21, monosomy X) genomic sequence analysis panel, circulating cell-free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18 and 21 *(New code, effective January 1, 2015)* ▪ Added CPT code 88271, Molecular cytogenetics; DNA probe, each (eg, FISH) ▪ Added "Effective in 2015, if the test is run as a genomic sequence analysis panel that includes analysis of all 3 chromosomes and does not involve an algorithmic analysis, the code 81420 is available" ▪ Added "There are reports that the Natera Panorama panel is reported with CPT code 88271"
	Updated References section.
	Added Appendix section.
07-01-2015	Updated Description section.
	In Coding section: ▪ Added HCPCS code: 0009M. ▪ Removed CPT code: 81504. ▪ Removed ICD-10 codes: O09.513 and O09.519.
09-29-2015	Updated Description section.
	In Policy section: ▪ In Item A, removed "high-risk", to read "Nucleic acid sequencing-based testing of maternal plasma for trisomy 21 may be considered medically necessary in women with singleton pregnancies ..." ▪ Removed Item C, "Nucleic acid sequencing-based testing of maternal plasma for trisomy 21 is considered not medically necessary in women with average-risk singleton pregnancies." In Policy Guidelines: ▪ In Item 1, removed "High-risk singleton pregnancies, as defined by the American College of Obstetricians and Gynecologists (ACOG) Committee Opinion, Number 454, December 2012, include women who meet at least one of the following criteria: a. Maternal age 35 years or older at delivery; b. Fetal ultrasonographic findings indicating increased risk of aneuploidy; c. History of previous pregnancy with a trisomy; d. Standard serum screening test positive for aneuploidy; or e. Parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21." and replaced with "In a 2015 committee opinion, the American College of Obstetricians and Gynecologists (ACOG) recommends that all patients receive information on the risks and benefits of various methods of prenatal screening and diagnostic testing, including the option of no testing." ▪ In Item 2, removed " In the decision model conducted for the 2012 TEC Assessment, using an overall estimate for predictive value calculations, even in a high risk population, the predictive value of a positive result was only 83%. Thus, in the absence of substantial data to confidently characterize the false-positive rate, a karyotyping test would be necessary to confirm a positive result." and added " False-positive findings have been found to be associated with factors including placental mosaicism, vanishing twins, and maternal malignancies. In its 2015 committee opinion, ACOG recommended diagnostic testing to confirm positive cell-free DNA tests, and that management decisions not be based solely on the results of cell-free DNA testing. ACOG further recommends that patients with indeterminate or uninterpretable (ie, “no call”) cell-free 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."

	▪ Added Item 3, "As noted in the 2015 ACOG committee opinion, cell-free DNA screening does not assess risk of anomalies such as neural tube defects. Patients should continue to be offered ultrasound or maternal serum alpha-fetoprotein screening, regardless of the type of serum screening selected."
	Updated Rationale section.
	Updated References section.
01-07-2016	Revised title from "Noninvasive Prenatal Testing for Fetal Aneuploidies Using Cell-Free Fetal DNA"
	Updated Description section.
	In Policy section: ▪ Added Item F, "Nucleic acid sequencing-based testing of maternal plasma for fetal sex chromosome aneuploidies is considered experimental / investigational." In Policy Guidelines: ▪ Added Item 5. ▪ Added paragraph on Genetic Counseling.
	Updated Rationale section.
	In Coding section: ▪ Added bullet under CPT/HCPCS codes.
	Updated References section.
11-22-2016	Updated Description section.
	In Policy section: ▪ In Item A, removed "maternal" and "women" and added "individuals" to read, "Nucleic acid sequencing-based testing of plasma for trisomy 21 may be considered medically necessary in individuals with singleton pregnancies undergoing screening for trisomy 21. (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 [see Policy Guidelines])." ▪ In Item B, removed "maternal" and "women" and added "individuals" to read, "Concurrent nucleic acid sequencing-based testing of plasma for trisomy 13 and/or 18 may be considered medically necessary in individuals who are eligible for and are undergoing nucleic acid sequencing-based testing of plasma for trisomy 21." ▪ In Item C, removed "maternal" and "women" and added "individuals" to read, "Nucleic acid sequencing-based testing of plasma for trisomy 21 is considered experimental / investigational in individuals with twin or multiple pregnancies. ▪ In Item D, removed "maternal" to read, "Nucleic acid sequencing-based testing of plasma for trisomy 13 and/or 18, other than in the situations specific above, is considered experimental / investigational." ▪ In Item E, removed "maternal" to read, "Nucleic acid sequencing-based testing of plasma for fetal sex chromosome aneuploidies is considered experimental / investigational." ▪ In Item F, removed "maternal" to read, "Nucleic acid sequencing-based testing of plasma for microdeletions is considered experimental / investigational." ▪ In Policy Guidelines Items 2 and 3, removed "maternal" from verbiage. ▪ Removed Policy Guidelines Item 4, "In some cases, tissue samples from chorionic villous sampling (CVS) or amniocentesis may be insufficient for karyotyping; confirmation by specific fluorescent in situ hybridization (FISH) assay is acceptable for these samples."
	Updated Rationale section.
	In Coding section: ▪ Added CPT code: 81422.

	▪ Updated coding bullets.
	Updated References section.
10-01-2017	Updated Description section.
	In Policy section: ▪ In Policy Guidelines, added "Genetics Nomenclature Update" information.
	Updated Rationale section.
	In Coding section: ▪ Updated coding bullets. ▪ Added ICD-10 code: Z36.0. ▪ Revised nomenclature to ICD-10 code: Z31.5. ▪ Removed ICD-10 code: Z36.
	Updated References section.
10-01-2018	Updated Description section.
	In Policy section: ▪ In Item A, added “18, and 13” and removed “undergoing screening for trisomy 21. (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 [see Policy Guidelines])” to read, “Nucleic acid sequencing-based testing of plasma for trisomy 21, 18, and 13 may be considered medically necessary in individuals with singleton pregnancies.” ▪ Removed previous Item B, “Concurrent nucleic acid sequencing-based testing of plasma for trisomy 13 and/or 18 may be considered medically necessary in individuals who are eligible for and are undergoing nucleic acid sequencing-based testing of plasma for trisomy 21.” ▪ Removed previous Item C, “Nucleic acid sequencing-based testing of plasma for trisomy 21 is considered experimental / investigational in individuals with twin or multiple pregnancies.” ▪ Removed previous Item D, “Nucleic acid sequencing-based testing of plasma for trisomy 13 and/or 18, other than in the situations specified above, is considered experimental / investigational.” ▪ Added new Item B, “Nucleic acid sequencing-based testing of plasma for trisomy 21, 18, and 13 is considered experimental / investigational in individuals with twin or multiple pregnancies.” ▪ Updated Policy Guidelines.
	Updated Rationale section.
	In Coding section: ▪ Removed ICD-9 codes.
	Updated References section.
	Removed Appendix section.

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