

FETAL ANEUPLOIDY TESTING USING CELL-FREE FETAL NUCLEIC ACIDS IN MATERNAL BLOOD

Policy Number: 2018T0560M

Effective Date: January 1, 2018

Table of Contents	Page
INSTRUCTIONS FOR USE	1
BENEFIT CONSIDERATIONS	1
COVERAGE RATIONALE	1
APPLICABLE CODES	2
DESCRIPTION OF SERVICES	3
CLINICAL EVIDENCE	4
U.S. FOOD AND DRUG ADMINISTRATION	12
CENTERS FOR MEDICARE AND MEDICAID SERVICES ..	12
REFERENCES	12
POLICY HISTORY/REVISION INFORMATION	15

Related Commercial Policy
<ul style="list-style-type: none"> Chromosome Microarray Testing (Non-Oncology Conditions)

Community Plan Policy
<ul style="list-style-type: none"> Fetal Aneuploidy Testing Using Cell-Free Fetal Nucleic Acids in Maternal Blood

INSTRUCTIONS FOR USE

This Medical Policy provides assistance in interpreting UnitedHealthcare benefit plans. When deciding coverage, the member specific benefit plan document must be referenced. The terms of the member specific benefit plan document [e.g., Certificate of Coverage (COC), Schedule of Benefits (SOB), and/or Summary Plan Description (SPD)] may differ greatly from the standard benefit plan upon which this Medical Policy is based. In the event of a conflict, the member specific benefit plan document supersedes this Medical Policy. All reviewers must first identify member eligibility, any federal or state regulatory requirements, and the member specific benefit plan coverage prior to use of this Medical Policy. Other Policies and Coverage Determination Guidelines may apply. UnitedHealthcare reserves the right, in its sole discretion, to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

UnitedHealthcare may also use tools developed by third parties, such as the MCG™ Care Guidelines, to assist us in administering health benefits. The MCG™ Care Guidelines are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.

BENEFIT CONSIDERATIONS

Before using this policy, please check the member specific benefit plan document and any federal or state mandates, if applicable.

Essential Health Benefits for Individual and Small Group

For plan years beginning on or after January 1, 2014, the Affordable Care Act of 2010 (ACA) requires fully insured non-grandfathered individual and small group plans (inside and outside of Exchanges) to provide coverage for ten categories of Essential Health Benefits (“EHBs”). Large group plans (both self-funded and fully insured), and small group ASO plans, are not subject to the requirement to offer coverage for EHBs. However, if such plans choose to provide coverage for benefits which are deemed EHBs, the ACA requires all dollar limits on those benefits to be removed on all Grandfathered and Non-Grandfathered plans. The determination of which benefits constitute EHBs is made on a state by state basis. As such, when using this policy, it is important to refer to the member specific benefit plan document to determine benefit coverage.

COVERAGE RATIONALE

DNA-based noninvasive prenatal tests of fetal aneuploidy are proven and/or medically necessary as screening tools for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) or trisomy 13 (Patau syndrome) in ANY ONE of the following circumstances:

- Maternal age of 35 years or older at delivery
- Fetal ultrasound findings indicating an increased risk of aneuploidy
- History of a prior pregnancy with a trisomy

- Positive first- or second-trimester screening test results for aneuploidy
- Parental balanced Robertsonian translocation with an increased risk of fetal trisomy 13 or trisomy 21

DNA-based noninvasive prenatal tests of fetal aneuploidy are unproven and/or not medically necessary for all other indications including, but not limited to, the following:

- Multiple gestation pregnancies
- Screening for microdeletions
- Screening for sex chromosome aneuploidies

Further studies are needed to evaluate the use of these tests in other populations.

Genetic Counseling

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

APPLICABLE CODES

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

CPT Code	Description
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
81420	Fetal chromosomal aneuploidy (e.g., 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 (e.g., DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood
81507	Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy

CPT® is a registered trademark of the American Medical Association

ICD-10 Diagnosis Code	Description
G91.2	(Idiopathic) normal pressure hydrocephalus
O09.291	Supervision of pregnancy with other poor reproductive or obstetric history, first trimester
O09.292	Supervision of pregnancy with other poor reproductive or obstetric history, second trimester
O09.293	Supervision of pregnancy with other poor reproductive or obstetric history, third trimester
O09.299	Supervision of pregnancy with other poor reproductive or obstetric history, unspecified trimester
O09.511	Supervision of elderly primigravida, first trimester
O09.512	Supervision of elderly primigravida, second trimester
O09.513	Supervision of elderly primigravida, third trimester
O09.519	Supervision of elderly primigravida, unspecified trimester
O09.521	Supervision of elderly multigravida, first trimester
O09.522	Supervision of elderly multigravida, second trimester
O09.523	Supervision of elderly multigravida, third trimester
O09.529	Supervision of elderly multigravida, unspecified trimester
O28.3	Abnormal ultrasonic finding on antenatal screening of mother
O28.5	Abnormal chromosomal and genetic finding on antenatal screening of mother
O28.9	Unspecified abnormal findings on antenatal screening of mother

ICD-10 Diagnosis Code	Description
O35.0XX0	Maternal care for (suspected) central nervous system malformation in fetus, not applicable or unspecified
O35.1XX0	Maternal care for (suspected) chromosomal abnormality in fetus, not applicable or unspecified
O35.1XX1	Maternal care for (suspected) chromosomal abnormality in fetus, fetus 1
O35.1XX2	Maternal care for (suspected) chromosomal abnormality in fetus, fetus 2
O35.1XX3	Maternal care for (suspected) chromosomal abnormality in fetus, fetus 3
O35.1XX4	Maternal care for (suspected) chromosomal abnormality in fetus, fetus 4
O35.1XX5	Maternal care for (suspected) chromosomal abnormality in fetus, fetus 5
O35.1XX9	Maternal care for (suspected) chromosomal abnormality in fetus, other fetus
O35.2XX0	Maternal care for (suspected) hereditary disease in fetus, not applicable or unspecified
Q90.0	Trisomy 21, nonmosaicism (meiotic nondisjunction)
Q90.1	Trisomy 21, mosaicism (mitotic nondisjunction)
Q90.2	Trisomy 21, translocation
Q90.9	Down syndrome, unspecified
Q91.0	Trisomy 18, nonmosaicism (meiotic nondisjunction)
Q91.1	Trisomy 18, mosaicism (mitotic nondisjunction)
Q91.2	Trisomy 18, translocation
Q91.3	Trisomy 18, unspecified
Q91.4	Trisomy 13, nonmosaicism (meiotic nondisjunction)
Q91.5	Trisomy 13, mosaicism (mitotic nondisjunction)
Q91.6	Trisomy 13, translocation
Q91.7	Trisomy 13, unspecified
Q92.0	Whole chromosome trisomy, nonmosaicism (meiotic nondisjunction)
Q92.1	Whole chromosome trisomy, mosaicism (mitotic nondisjunction)
Q92.2	Partial trisomy
Q92.5	Duplications with other complex rearrangements
Q92.61	Marker chromosomes in normal individual
Q92.62	Marker chromosomes in abnormal individual
Q92.7	Triploidy and polyploidy
Q92.8	Other specified trisomies and partial trisomies of autosomes
Q92.9	Trisomy and partial trisomy of autosomes, unspecified
Q95.0	Balanced translocation and insertion in normal individual
Q95.1	Chromosome inversion in normal individual
Q95.2	Balanced autosomal rearrangement in abnormal individual
Q95.3	Balanced sex/autosomal rearrangement in abnormal individual
Q95.5	Individual with autosomal fragile site
Q95.8	Other balanced rearrangements and structural markers
Q95.9	Balanced rearrangement and structural marker, unspecified

DESCRIPTION OF SERVICES

Humans have 23 pairs of chromosomes. Aneuploidy is an abnormal number of chromosomes. Trisomy is a type of aneuploidy in which there are three copies of a chromosome instead of two. Trisomy 21, also called Down syndrome, is a chromosomal condition that is associated with intellectual disability, a characteristic facial appearance and poor muscle tone (hypotonia) in infancy. The degree of intellectual disability varies, but it is usually mild to moderate. Individuals with Down syndrome may be born with a variety of birth defects including heart defects and digestive abnormalities. The risk of having a child with trisomy 21 increases with a mother's age (Genetics Home Reference, June 2012). Down syndrome can also be caused by translocation, which occurs when a part of chromosome 21

breaks away and becomes attached to another chromosome. In a balanced translocation, pieces of chromosomes are rearranged but no genetic material is gained or lost in the cell. In these cases, the parent's health is not affected (Genetics Home Reference, November 2017).

Trisomy 18, also called Edwards syndrome, is a chromosomal condition associated with slow growth before birth and a low birth weight. Affected individuals may have heart defects and abnormalities of other organs that develop before birth. Other features of trisomy 18 include a small, abnormally shaped head; a small jaw and mouth; and clenched fists with overlapping fingers. The risk of having a child with trisomy 18 increases with a mother's age (Genetics Home Reference, March 2012).

Trisomy 13, also called Patau syndrome, is a chromosomal condition associated with severe intellectual disability and physical abnormalities in many parts of the body. Individuals with trisomy 13 often have heart defects, brain or spinal cord abnormalities, very small or poorly developed eyes (microphthalmia), extra fingers and/or toes, an opening in the lip (a cleft lip) with or without an opening in the roof of the mouth (a cleft palate) and weak muscle tone (hypotonia). The risk of having a child with trisomy 13 increases with a mother's age (Genetics Home Reference, November 2013). Patau syndrome can also be caused by translocation.

Routine screening tests for trisomies 21, 18 and 13 include first-trimester screening (which involves an ultrasound and a blood test), maternal serum screening (a blood test) and a high-resolution ultrasound evaluation in the second trimester. These tests may identify women with an increased risk of having a child with trisomy 21, 18 or 13, but they cannot diagnose, confirm or exclude the possibility of a chromosomal disorder. Conventional prenatal diagnosis (i.e., chorionic villus sampling (CVS) or amniocentesis) can definitively diagnose fetal trisomies, although these invasive procedures are associated with a risk of miscarriage (Hayes, 2015).

Tests that detect fetal trisomies, without the need for CVS or amniocentesis, analyze cell-free DNA (cfDNA) fragments in maternal blood. During pregnancy, there are cfDNA fragments from both the mother and fetus in maternal circulation. The tests detect an increased amount of chromosomal material in maternal blood and can be offered as early as 10 weeks of pregnancy. Available tests use different methodologies and algorithms for data analysis. Depending on the test, the methodology may involve massively parallel sequencing (MPS), targeted sequencing of specific chromosomal segments or directed sequence analysis of single nucleotide polymorphisms (SNPs) (Hayes, 2015). All tests were validated in high-risk couples. It is unknown whether they are as accurate for low- to average-risk couples.

In addition to screening for common fetal aneuploidies, cfDNA panels are also being used to screen for microdeletion syndromes which are caused by a chromosomal deletion that is too small to be detected by conventional cytogenetic testing.

The Centers for Disease Control and Prevention (CDC) created the ACCE model process for evaluating genetic or genomic-based tests. The 4 main components of the ACCE process include analytic validity, clinical validity, clinical utility and ELSI. Analytic validity refers to how accurately and reliably the test measures the genotype of interest. Clinical validity refers to how consistently and accurately the test detects or predicts the intermediate or final outcomes of interest. Is what's measured associated with the outcome of interest? Clinical utility refers to how likely the test is to significantly improve patient outcomes. What is the clinical value? ELSI refers to the ethical, legal and social implications that may arise in the context of using the test (CDC, 2017).

CLINICAL EVIDENCE

Summary

Limited data is available regarding improvement in patient outcomes if cfDNA is offered as a screening test to all pregnant women. While some study results suggest making cfDNA-based testing available to all pregnant women, regardless of risk, these studies fail to address the fact that a test's positive predictive value (PPV) is highly dependent on the patient's pretest trisomy risk. cfDNA-based testing's very low PPV in patients at low risk for trisomy must be considered in any discussion of expanding current guidelines to offer cfDNA testing as a first-line screen in this population.

Hayes evaluated the clinical utility of cfDNA screening in populations at low risk for aneuploidy. The overall quality of evidence was low for fetal trisomy 21, 18 and 13 in women with low-risk singleton pregnancies and very low in women with multiple gestation pregnancies. Study limitations included varied and small patient populations with few chromosomally abnormal pregnancies detected, incomplete reporting of results and exclusion of women with cfDNA screening failures. Studies directly comparing clinical outcomes of cfDNA screening with those of routine screening strategies for low-risk or general obstetric patients in a real-world setting are needed (Hayes, 2017a).

In a multicenter, observational study, Palomaki et al. (2017) assessed the clinical utility of cfDNA-based screening for aneuploidies offered through primary obstetrical care providers to a general pregnancy population. Among 2,681 tests reported, 16 women (0.6%) were screen-positive for trisomy 21, 18 or 13. Twelve were confirmed (PPV, 75%) and four were false-positives (0.15%). Of 150 test failures (5.6%), 79% had a negative serum or subsequent cfDNA test. There were no reported cases of aneuploidy among cfDNA test failures.

Iwarsson et al. (2017) conducted a systematic review and meta-analysis to assess the performance of cfDNA testing for the detection of trisomy 21, 18 and 13 in a general pregnant population and update the data on high-risk pregnancies. In a general pregnant population, the authors noted moderate evidence of a pooled sensitivity of 0.993 and specificity of 0.999 for the analysis of trisomy 21. Pooled sensitivity and specificity for trisomies 13 and 18 were not calculated in this population due to the low number of studies. In a high-risk pregnant population, the authors noted moderate evidence of pooled sensitivities for trisomies 21 and 18 of 0.998 and 0.977, respectively, and low evidence of a pooled sensitivity for trisomy 13 of 0.975. The pooled specificity for all three trisomies was 0.999. The authors concluded that cfDNA performs well as a screen for trisomy 21 in a general pregnant population; however, more data is needed for trisomies 18 and 13.

Norton et al. (2016) compared the performance of sequential and cfDNA screening for detection of fetal chromosomal abnormalities in a general prenatal cohort. Of 452,901 women who underwent sequential screening, 2575 (0.57%) had a fetal chromosomal abnormality. The DR of sequential screening for all aneuploidies in the cohort was greater than cfDNA. The authors concluded that cfDNA should not be adopted as a primary screening tool without further evaluation of the implications for detection of all chromosomal abnormalities and how to best evaluate no results cases.

Taylor-Phillips et al. (2016) conducted a systematic review and meta-analysis to measure the accuracy of NIPT for Down, Edwards and Patau syndromes using cfDNA. Pooled sensitivity was 99.3% for Down, 97.4% for Edwards and 97.4% for Patau syndrome. The pooled specificity was 99.9% for all three trisomies. Sensitivity was lower in twin than singleton pregnancies, reduced by 9% for Down, 28% for Edwards and 22% for Patau syndrome. Pooled sensitivity was also lower in the first trimester of pregnancy in studies of the general obstetric population. NIPT using cfDNA has very high sensitivity and specificity for Down syndrome, with slightly lower sensitivity for Edwards and Patau syndrome. NIPT should not be used as a final diagnosis for positive cases.

Zhang et al. (2015) reported the clinical performance of MPS-based NIPT in detecting trisomies 21, 18 and 13 and to compare its performance in low-risk and high-risk pregnancies. NIPT was performed and results obtained in 146,958 samples, for which outcome data were available in 112,669 (76.7%). Repeat blood sampling was required in 3213 cases and 145 had test failure. Aneuploidy was confirmed in 720/781 cases positive for trisomy 21, 167/218 cases positive for trisomy 18 and 22/67 cases positive for trisomy 13. Nine false negatives were identified, including six cases of trisomy 21 and three of trisomy 18. The overall sensitivity of NIPT was 99.17%, 98.24% and 100% for trisomies 21, 18 and 13, respectively, and specificity was 99.95%, 99.95% and 99.96% for trisomies 21, 18 and 13, respectively. In the low-risk population, the PPV was 81.36% for trisomy 21. The reduced PPV in the low-risk group as a consequence of lower disease prevalence, reaffirmed that NIPT should not be used as a diagnostic test and that confirmation by invasive testing is still necessary. The authors reported no significant difference in test performance between 72,382 high-risk and 40,287 low-risk pregnancies (sensitivity, 99.21% vs. 98.97% ; specificity, 99.95% vs. 99.95%). The major factors contributing to false-positive and false-negative results were maternal copy number variant and fetal/placental mosaicism. An author noted limitation was the incomplete follow-up of NIPT results, which could introduce bias into the performance evaluation.

Mersy et al. (2013) conducted a systematic review of seven studies that examined noninvasive prenatal testing (NIPT) of fetal trisomy 21 using MPS. The authors reported sensitivities between 98.58% and 100% and specificities between 97.95% and 100%. MPS with or without pre-selection of chromosomes exhibits a negative predictive value (NPV) (100%); however, PPVs were lower, even in high-risk pregnancies (19.7-100%).

Analytic Validity

Verweij et al. (2012) conducted a systematic review on the diagnostic accuracy of noninvasive prenatal diagnosis (NIPD) of fetal trisomies using cfDNA or RNA in maternal blood to detect fetal trisomy 21. With a total of 681 pregnancies included, overall sensitivity was 125/125 (100%) and specificity 552/556 (99.3%). NIPD of fetal trisomy 21, using fetal nucleic acids in maternal plasma, appears to have a high diagnostic accuracy.

MaterniT21

Studies evaluating the analytic validity of the MaterniT21 PLUS assay showed that analytic sensitivity depended on the conditions being tested. For this assay, the test sensitivity was 98.6% to 100% for T21, 100% for T18 and 91.7% to 100% for T13, with a test specificity > 99%. In a single study evaluating the NIPT assay in multiple-gestation pregnancies, the sensitivity was 100% for T21, and the assay was able to detect the single case of fetal T18 (Hayes, 2015).

In a prospective multicenter observational study of pregnant women at high risk for fetal aneuploidy, Porreco et al. (2014) evaluated the clinical performance of MPS of cfDNA in maternal blood. A total of 3430 patients were included in the analysis. There were 137 fetuses with trisomy 21, 39 with trisomy 18 and 16 with trisomy 13. There were no false-negative results for trisomy 21, 3 for trisomy 18 and 2 for trisomy 13. All false-positive results (3) were for trisomy 21. The PPVs for trisomies 18 and 13 were 100% and 97.9% for trisomy 21. The authors concluded that noninvasive prenatal analysis of cfDNA from maternal blood is an accurate advanced screening test with extremely high sensitivity and specificity for trisomy 21 (>99%) but less sensitivity for trisomies 18 and 13. Despite high sensitivity, there was modest positive predictive value for the small number of common sex chromosome aneuploidies because of their very low prevalence rate.

Canick et al. (2012) conducted a study on prenatal testing for Down syndrome (trisomy 21), trisomy 18 and trisomy 13 by massively parallel shotgun sequencing (MPSS) of circulating cfDNA in pregnant women with multiple gestations. Among a cohort of 4664 high-risk pregnancies, maternal plasma samples were tested from 25 twin pregnancies (17 euploid, five discordant and two concordant for Down syndrome; one discordant for trisomy 13) and two euploid triplet pregnancies. Seven twin pregnancies with Down syndrome, one with trisomy 13, and all 17 twin euploid pregnancies were correctly classified [detection rate 100%, false positive rate 0%], as were the two triplet euploid pregnancies. Although study size is limited, the authors concluded that this data provides evidence that MPSS testing can be reliably used as a secondary screening test for Down syndrome in women with high-risk twin gestations. Further studies, with larger patient populations, are needed to confirm these results.

In the largest and most comprehensive study to date, Palomaki et al. (2011) evaluated the analytic validity of a noninvasive prenatal screening test for Down syndrome that measures circulating cfDNA in maternal plasma. Test results were compared to those obtained after chorionic villus sampling or amniocentesis. A total of 4664 pregnant women, each considered at high risk for having a child with trisomy 21 (based on maternal age, screening test results and/or ultrasound results), were recruited from 27 prenatal diagnostic centers and included in this blinded, nested case-control study. Of the 4664 cases, 279 (6%) were excluded from the analysis for various reasons. Of the remaining cases, 218 (5%) fetuses were diagnosed with trisomy 21 based on the results of invasive testing. The first 212 cases were selected for analysis. Fetal karyotyping was compared with an internally validated, laboratory-developed test based on next-generation sequencing. Down syndrome detection rate was 98.6% (209/212), the false-positive rate was 0.20% (3/1471) and the testing failed in 13 pregnancies (0.8%). The authors concluded that, when applied to high-risk pregnancies, measuring maternal plasma DNA detects nearly all cases of Down syndrome at a very low false-positive rate. This method can substantially reduce the need for invasive diagnostic procedures and attendant procedure-related fetal losses.

Using the same cohort of patients, Palomaki et al. (2012) reported additional data indicating that maternal plasma cfDNA sequencing also has the capability to detect other aneuploidies, such as trisomy 18 and trisomy 13. Sixty-two pregnancies with trisomy 18 and 12 with trisomy 13 were included in the analysis. Among the 99.1% of samples interpreted, observed trisomy 18 and 13 detection rates were 100% (59/59) and 91.7% (11/12) with false-positive rates of 0.28% and 0.97%, respectively.

Chiu et al. (2011) validated the clinical efficacy and practical feasibility of massively parallel maternal plasma DNA sequencing to screen for fetal trisomy 21 among high risk pregnancies clinically indicated for amniocentesis or chorionic villus sampling. The diagnostic accuracy was validated against full karyotyping, using prospectively collected or archived maternal plasma samples. Results were available from 753 pregnancies with the 8-plex sequencing protocol and from 314 pregnancies with the 2-plex protocol. The performance of the 2-plex protocol was superior to that of the 8-plex protocol. With the 2-plex protocol, trisomy 21 fetuses were detected at 100% sensitivity and 97.9% specificity, which resulted in a positive predictive value (PPV) of 96.6% and NPV of 100%. The 8-plex protocol detected 79.1% of the trisomy 21 fetuses and 98.9% specificity, giving a PPV of 91.9% and NPV of 96.9%. The authors concluded that multiplexed maternal plasma DNA sequencing analysis could be used to rule out fetal trisomy 21 among high risk pregnancies.

Ehrich et al. (2011) evaluated a multiplexed MPSS assay for noninvasive trisomy 21 detection using circulating cfDNA. A total of 480 plasma samples from high-risk pregnant women were analyzed. Thirteen samples were removed due to insufficient quantity or quality. Eighteen samples failed prespecified assay quality control parameters. In all, 449 samples remained: 39 trisomy 21 samples were correctly classified; 1 sample was misclassified as trisomy 21. The overall classification showed 100% sensitivity and 99.7% specificity. The authors reported that extending the scope of previous reports, these results warrant clinical validation in a larger multicenter study.

verifi

A single case-control study reported the sensitivity of the Verifi assay as 100% for T21, 97.1% for T18 and 78.6% for T13, with a test specificity of 100% for all 3 conditions (Hayes, 2015).

The MELISSA (MatEternal Blood IS Source to Accurately Diagnose Fetal Aneuploidy) trial was conducted as a prospective, multicenter observational study to determine the diagnostic accuracy of MPS (verifi™) to detect fetal aneuploidy from maternal plasma. Blood samples were collected from 2,882 women undergoing invasive prenatal diagnostic procedures. Within an analysis cohort, the following were classified correctly: 89 of 89 trisomy 21 cases (sensitivity 100%, 95% CI), 35 of 36 trisomy 18 cases (sensitivity 97.2%, 95% CI) and 11 of 14 trisomy 13 cases (sensitivity 78.6%, 95% CI). The authors reported high sensitivity and specificity for the detection of trisomies 21, 18, 13 in women with singleton pregnancies at an increased risk for aneuploidy. Further studies are needed to build confidence in the diagnostic performance of the test in low-risk populations and in multiple gestations. ClinicalTrials.gov NCT01122524 (Bianchi et al., 2012).

Sehnert et al. (2011) evaluated an optimized algorithm for use with massively parallel DNA sequencing of cfDNA to detect fetal chromosomal abnormalities. Existing algorithms focus on the detection of fetal trisomy 21 (T21); however, these same algorithms have difficulty detecting trisomy 18 (T18). Blood samples were collected from 1014 patients prior to undergoing an invasive prenatal procedure. The DNA extracted from 119 samples underwent massively parallel DNA sequencing. Fifty-three sequenced samples came from women with an abnormal fetal karyotype. To minimize the intra- and interrun sequencing variation, investigators developed an optimized algorithm by using normalized chromosome values (NCVs) from the sequencing data on a training set of 71 samples with 26 abnormal karyotypes. The classification process was then evaluated on an independent test set of 48 samples with 27 abnormal karyotypes. Sequencing of the independent test set led to 100% correct classification of T21 (13 of 13) and T18 (8 of 8) samples. Other chromosomal abnormalities were also identified. The authors concluded that MPS is capable of detecting multiple fetal chromosomal abnormalities from maternal plasma when an optimized algorithm is used.

In a multicenter comparative study funded by Illumina, Bianchi et al. (2014) evaluated the performance of noninvasive prenatal testing using MPS of cfDNA in maternal blood for detecting fetal aneuploidy in low-risk women. The primary series included 1914 women with an eligible sample, a singleton fetus without aneuploidy, results from cfDNA testing and a risk classification based on standard screening. For trisomies 21 and 18, the false positive rates with cfDNA testing were significantly lower than those with standard screening (0.3% versus 3.6% for trisomy 21; and 0.2% versus 0.6% for trisomy 18). The use of cfDNA testing detected all cases of aneuploidy (5 for trisomy 21, 2 for trisomy 18 and 1 for trisomy 13; NPV, 100%). The PPVs for cfDNA testing versus standard screening were 45.5% versus 4.2% for trisomy 21 and 40.0% versus 8.3% for trisomy 18. Further studies are needed to determine the clinical validity of sequence-based fetal aneuploidy testing in this patient population.

Harmony Prenatal Test

Studies evaluating the analytic validity of the Harmony Prenatal Test indicated that the test sensitivity varied depending on the aneuploidy tested, although the test specificity was consistently > 99%. Among high-risk women with single pregnancies, the test sensitivity was 100% for T21, 94% to 100% for T18 and 80% for T13. In an average-risk population of women with singleton pregnancies, the sensitivity and specificity of the Harmony assay were 100% and 99.9%, respectively, for both T21 and T18. A single study examining the analytic validity of the Harmony assay in twin gestations reported an overall test sensitivity of 90.9% for T21, T18 and T13 (Hayes, 2015).

Gil et al. (2014) performed a meta-analysis of cfDNA in maternal blood in screening for aneuploidies. Weighted pooled detection rates (DR) and false-positive rates (FPR) in singleton pregnancies were 99.0% and 0.08%, respectively, for trisomy 21; 96.8% and 0.15% for trisomy 18; 92.1% and 0.20% for trisomy 13; 88.6% and 0.12% for monosomy X and 93.8% and 0.12% for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR was 94.4% and the FPR was 0% for trisomy 21. The authors concluded that screening for aneuploidies analyzing cfDNA in maternal blood provides effective screening for trisomies. The same group of authors updated the meta-analysis with data from 37 studies published since the initial review. The studies reported cfDNA results in relation to fetal karyotype from invasive testing or clinical outcome. Weighted pooled DR and FPR in singleton pregnancies were 99.2% and 0.09%, respectively, for trisomy 21; 96.3% and 0.613% for trisomy 18; 91.0% and 0.13% for trisomy 13; 90.3% and 0.23% for monosomy X and 93.0% and 0.14% for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR for trisomy 21 was 93.7% and the FPR was 0.23% (Gil et al., 2015). In a 2017 update, weighted pooled DR and FPR in singleton pregnancies were 99.7% and 0.04%, respectively, for trisomy 21; 97.9% and 0.04% for trisomy 18; 99.0% and 0.04% for trisomy 13; 95.8% and 0.14% for monosomy X and 100% and 0.004% for sex chromosome aneuploidies other than monosomy X. For twin pregnancies, the DR for trisomy 21 was 100% and the FPR was 0.0% (Gil et al., 2017).

Ashoor et al. (2013) assessed the performance of the Harmony Prenatal Test for the detection of trisomy 13. A two-phase, blinded, case-control study was performed to optimize and validate the trisomy 13 algorithm. In the first phase, trisomy 13 risk scores were given for 11 cases of trisomy 13 and 145 euploid cases at 11-13 weeks' gestation. The test identified seven (63.6%) cases of trisomy 13 with no false positives. The trisomy 13 algorithm was subsequently modified and the trisomy 13 risk score was > 99% in all 11 cases of trisomy 13 and < 0.01% in all 145 euploid cases. In the second phase, the new algorithm was used to generate trisomy 13 risk scores for 10 cases of trisomy 13 and 1939 euploid cases. The trisomy 13 risk scores were > 99% in eight (80.0%) cases of trisomy 13. In the 1939 euploid

cases the risk score for trisomy 13 was < 0.01% in 1937 (99.9%), 0.79% in one and > 99% in one. Therefore, at the predefined risk cut-off of 1% for classifying a sample as high or low risk, the false-positive rate (FPR) was 0.05%.

Nicolaides et al. (2012) conducted a cohort study of 2049 pregnant women undergoing routine screening for aneuploidies at 11-13 weeks' gestation. Plasma cfDNA analysis using chromosome-selective sequencing was used. Laboratory testing on a single plasma sample was carried out blindly and results were provided as risk score (%) for trisomies 21 and 18. Trisomy risk scores were given for 95.1% (1949 of 2049) of cases including all 8 with trisomy 21 and 2 of the 3 with trisomy 18. The trisomy risk score was >99% in the 8 cases of trisomy 21 and 2 of trisomy 18 and <1% in 99.9% (1937 of 1939) of euploid cases. The authors concluded that noninvasive prenatal testing (NIPT) using chromosome-selective sequencing in a routinely screened population identified trisomies 21 and 18 with a false-positive rate of 0.1%. However, the authors cautioned that because the sensitivity and specificity of NIPT is not 100%, the test should not be considered a diagnostic replacement for invasive testing in high-risk pregnancies.

Norton et al. (2012) conducted a multicenter cohort study evaluating the performance of a noninvasive prenatal test for fetal trisomy 21 (T21) and trisomy 18 (T18) using cfDNA from maternal plasma. Chromosome-selective sequencing was performed with reporting of an aneuploidy risk (high risk or low risk) for each subject. Of the 81 T21 cases, all were classified as high risk for T21 and there was 1 false-positive result among the 2888 normal cases, for a sensitivity of 100% and a false-positive rate of 0.03%. Of the 38 T18 cases, 37 were classified as high risk and there were 2 false-positive results among the 2888 normal cases, for a sensitivity of 97.4% and a false-positive rate of 0.07%. The authors concluded that chromosome-selective sequencing of cfDNA and application of an individualized risk algorithm is effective in the detection of fetal T21 and T18.

Sparks and colleagues (2012a) described the initial development of the Harmony Prenatal Test. Blood samples were obtained from 298 women with singleton pregnancies at 10 weeks or later. Invasive prenatal diagnosis identified 39 fetuses with trisomy 21 and 7 fetuses with trisomy 18. Samples were analyzed using a novel, highly multiplexed assay, referred to as digital analysis of selected regions (DANSR™). All (100%) aneuploid samples were correctly identified.

Sparks et al. (2012b) evaluated a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 (T21) and trisomy 18 (T18) using cfDNA obtained from maternal blood. In a blinded analysis of 167 pregnant women, digital analysis of selected regions (DANSR), in combination with a novel algorithm, fetal-fraction optimized risk of trisomy evaluation (FORTE), correctly identified all 36 cases of T21 and 8 cases of T18. The investigators assayed cfDNA from a training set and a blinded validation set of pregnant women, comprising 250 disomy, 72 T21 and 16 T18 pregnancies. 163/171 subjects in the training set passed quality control criteria. FORTE produced an individualized trisomy risk score for each subject, and correctly discriminated all T21 and T18 cases from disomic cases. All 167 subjects in the blinded validation set passed quality control and FORTE performance matched that observed in the training set correctly discriminating 36/36 T21 cases and 8/8 T18 cases from 123/123 disomic cases. The authors concluded that while DANSR and FORTE enable accurate noninvasive fetal aneuploidy detection in a high-risk population, larger studies that include low- and average-risk pregnancies are needed.

In a nested case-control study, Ashoor et al. (2012) assessed the prenatal detection rate of trisomy 21 and 18 by chromosome-selective sequencing of maternal plasma cfDNA. cfDNA was examined in stored plasma that was obtained at 11-13 weeks before chorionic villous sampling from 300 euploid pregnancies, 50 pregnancies with trisomy 21 and 50 pregnancies with trisomy 18. The authors reported that the sensitivity for detecting trisomy 21 was 100% (50/50 cases); the sensitivity for trisomy 18 was 98% (49/50 cases) and the specificity was 100% (297/297 cases).

Panorama Prenatal Test

Data regarding the analytic validity of the Panorama Prenatal Test consistently showed a high sensitivity for T21 (100%), T18 (96% to 100%) and T13 (100%) when evaluating the assay in high-risk singleton pregnancies. The test specificity was consistently ≥ 99.9%. In a single study that included average-risk women with singleton pregnancies, the overall sensitivity and specificity of the Panorama assay was 100% (Hayes, 2015).

Pergament et al. (2014) evaluated the analytic validity of a SNP-based NIPT in a high- and low-risk population. A total of 1064 maternal blood samples from 7 weeks of gestation and beyond were included. Of these, 1,051 were within specifications and 518 (49.3%) were low risk. Nine hundred sixty-six samples (91.9%) successfully generated a cfDNA result. Among these, sensitivity was 100% for trisomy 21 (58/58) and trisomy 13 (12/12) and 96.0% for trisomy 18 (24/25). Specificity was 100% for trisomies 21 and 13 and 99.9% for trisomy 18. However, 16% (20/125) of samples did not return a result. Sensitivity and specificity did not differ in low-risk and high-risk cohorts. Level of evidence: II.

Nicolaides et al. (2013) validated the Panorama Prenatal Test (Natera Inc.) in a population of 242 women with singleton pregnancies undergoing chorionic villus sampling (CVS) at 11 to 13 weeks of gestation. Women were referred because first-trimester screening indicated an increased risk for trisomy 21, 18 or 13. Maternal blood samples were sent to the Natera laboratory, where testing involved the targeted sequencing of 19,488 SNPs. Sequencing data

were analyzed using the NATUS algorithm. Results were provided for 229 (94.6%) of the 242 cases. Thirty-two cases were correctly identified as aneuploid, including trisomy 21 [n = 25; sensitivity = 100%, specificity = 100%], trisomy 18 (n = 3), trisomy 13 (n = 1), Turner syndrome (n = 2) and triploidy (n = 1), with no false positive or false negative results.

Zimmermann et al. (2012) evaluated a noninvasive prenatal test using targeted sequencing of SNPs (Panorama Prenatal Test). Blood samples were obtained from 166 pregnant women at 9 weeks of gestation or later. It was not specified whether these pregnancies were high- or average-risk. Of the 166 fetuses, 11 had trisomy 21, 3 had trisomy 18 and 2 had trisomy 13. Twenty-one samples did not pass the DNA quality test and were excluded from the analysis. All aneuploidies were correctly identified for a sensitivity and specificity of 100%.

Clinical Validity

Studies evaluating the clinical validity of the Harmony Prenatal Test involved more than 4600 singleton pregnancies and 68 multiple-gestation pregnancies. Among women with singleton pregnancies, the test was able to detect 94.4% to 100% of fetal T21 cases, with a clinical specificity $\geq 99.9\%$. In addition, 2 studies reported that all known cases of T18 and T13 were detected, with a specificity of 99.9%. For twin-gestation pregnancies, the test was able to detect 90.9% of all tested aneuploidies (T21, T18 and T13) (Hayes, 2015).

Studies assessing the clinical validity of the MaterniT21 PLUS test (which involved >110,000 pregnant women) indicated that the clinical sensitivity of the assay varied depending on the aneuploidy evaluated. Overall, MaterniT21 PLUS was able to detect $\geq 95.9\%$ of T21 pregnancies, with a false-positive rate of 0.1%. One study reported a PPV of 97.9% for T21. For T18, the assay was able to detect 81.0% to 92.3% of cases, with a false-positive rate of 0.1% and a PPV of 100%. For T13, the test was able to detect up to 87.5% of cases, with a specificity and PPV both of 100%. In an analysis of more than 3500 women with multiple-gestation pregnancies, 99.9% of T21 pregnancies (i.e., T21 in at least 1 fetus), 95.2% of T18 pregnancies (i.e., T18 in at least 1 fetus) and 99.9% of T13 pregnancies (i.e., T13 in at least 1 fetus) were detected by the MaterniT21 PLUS assay, with a false-positive rate < 0.1% (Hayes, 2015).

Studies evaluating the clinical validity of the Panorama Prenatal test suggested that the clinical sensitivity of NIPT is highest for T21 (100%). In addition, a study involving >31,000 pregnant women tested on a clinical basis, the PPV was 90.9% for T21 and 82.9% for all other aneuploidies (Hayes, 2015).

Two studies evaluated the clinical validity of the Verifi Prenatal test. In the first study, which involved 6123 patients with blood samples submitted for clinical testing, there were 14 (0.2%) known cases with discordant (likely false-positive) results. The second study involved 1914 women with singleton pregnancies who were undergoing first-trimester screening. In this population, the sensitivity, specificity, PPV, and NPV for T21 were 100%, 99.7%, 45.5% and 100%, respectively. The sensitivity, specificity, PPV, and NPV for T18 were 100%, 99.8%, 40.0% and 100%, respectively (Hayes, 2015).

Dar et al. (2014) reported clinical experience of a SNP-based NIPT in high- and low-risk women. Samples were received from 31,030 patients, 30,705 met study criteria and 28,739 passed quality-control metrics and received a report detailing aneuploidy risk. Samples that passed quality control were analyzed for trisomy 21, trisomy 18, trisomy 13 and monosomy X. Results were reported as high or low risk for fetal aneuploidy for each chromosome. Follow-up on outcome was sought for a subset of high-risk cases. False-negative results were reported voluntarily by providers. PPV was calculated from cases with an available prenatal or postnatal karyotype or clinical evaluation at birth. In all, 507 patients received a high-risk result for any of the 4 tested conditions (324 trisomy 21, 82 trisomy 18, 41 trisomy 13, 61 monosomy X; including 1 double aneuploidy case). Within the 17,885 cases included in follow-up analysis, 356 were high risk, and outcome information revealed 184 (51.7%) true positives, 38 (10.7%) false positives, 19 (5.3%) with ultrasound findings suggestive of aneuploidy, 36 (10.1%) spontaneous abortions without karyotype confirmation, 22 (6.2%) terminations without karyotype confirmation and 57 (16.0%) lost to follow-up. This yielded an 82.9% PPV for all aneuploidies, and a 90.9% PPV for trisomy 21. The overall PPV for women aged ≥ 35 years was similar to the PPV for women aged <35 years. Two patients were reported as false negatives. The authors reported that the results suggest the clinical performance of this SNP-based NIPT in a mixed high- and low-risk population is consistent with performance in validation studies. However, the authors also noted that the main limitation of the study is the incomplete follow-up data, particularly on low-risk patients. This lack of follow-up precludes precise calculation of sensitivity and specificity and determination of NPV.

In a prospective, multicenter, blinded study, Norton et al. (2015) evaluated cfDNA testing in the assessment of risk for trisomy 21 in a large, routine prenatal screening population. Pregnant women presenting for aneuploidy screening at 10 to 14 weeks of gestation were assigned to undergo both standard screening (with measurement of nuchal translucency and biochemical analytes) and cfDNA testing. The study also evaluated the performance of cfDNA testing and standard screening in the assessment of risk for trisomies 18 and 13. Determination of the birth outcome was based on diagnostic genetic testing or newborn examination. Of 18,955 women who were enrolled, results from 15,841 were available for the primary analysis. The mean maternal age was 30.7 years, and the mean gestational age

at testing was 12.5 weeks. Trisomy 21 was detected in 38 of 38 women (100%) in the cfDNA-testing group, as compared with 30 of 38 women (78.9%) False positive rates were 0.06% in the cfDNA group and 5.4% in the standard-screening group. The PPV for cfDNA testing was 80.9%, as compared with 3.4% for standard screening. In a secondary analysis of 11,994 women with low-risk pregnancies based on a maternal age <35, cfDNA testing identified 19 of 19 women with trisomy 21, with 6 false positive results. Among the 14,957 women for whom standard screening showed a risk of less than 1 in 270, cfDNA testing identified 8 of 8 women with trisomy 21, with 8 false positive results. The PPV for cfDNA testing was 76.0% for women under the age of 35 years and 50.0% for those with a negative result on standard screening. Of the 10 cases of trisomy 18 in the primary analysis population, cfDNA testing identified 9 and standard screening identified 8. cfDNA testing had a false positive rate of 0.01% and a PPV of 90.0%, as compared to a false positive rate of 0.31% and a PPV of 14.0% with standard screening. Among the 11,185 women who underwent both cfDNA testing and standard screening for trisomy 13, there were 2 confirmed cases. Of these, cfDNA testing identified 2 and standard screening identified 1. cfDNA testing had a false positive rate of 0.02% compared to 0.25% for standard screening.

In the first study of 284 obstetrical patients who were evaluated by both the Harmony Prenatal Test and traditional first-trimester screening, all patients received NIPT results indicating a low likelihood (< 0.01%) of fetal trisomies 21, 18 or 13. A single patient, who had a first-trimester screening result of 1 in 5 for trisomy 21, elected to have invasive prenatal diagnosis, which revealed a normal fetal karyotype. The clinical outcomes for the remaining pregnancies were not known at the time of publication, although the NIPT results were consistent with the risk estimations provided by first-trimester screening (Fairbrother et al., 2013).

In a second study involving the Harmony Prenatal Test, 1005 pregnant women were tested with both NIPT and traditional first-trimester screening. The detection rates for trisomies 21 and 18 were 100% and 80%, respectively. In addition, assuming the pregnancies that had not yet delivered did not have an undiagnosed aneuploidy (missed by both NIPT and first-trimester screening), the false-positive rate was 0.1% for NIPT and 3.4% for first-trimester screening (Gil et al., 2013).

Another study, which involved 6123 patients with blood samples submitted for testing with the Verifi Prenatal Test, 155 (2.6%) fetuses were identified as having trisomy 21, 66 (1.1%) with trisomy 18, 19 (0.3%) with T13 and 40 (0.7%) with Turner syndrome (45,X). Unclassifiable results were obtained in up to 1% of cases for each of the chromosomes analyzed. Overall, of 280 fetuses with aneuploidy detected by NIPT, 94 (33.6%) were confirmed or the pregnancies resulted in miscarriage, and 14 (0.2%) yielded discordant (likely false-positive) results. Among cases with euploid NIPT results, there were 5 (0.08%) known false-negative cases (2 trisomy 21, 2 trisomy 18, and 1 45,X). Follow-up information was not available for the remaining cases at the time of publication (Futch et al., 2013).

Clinical Utility

Published evidence regarding the clinical utility of noninvasive prenatal testing is limited. Prospective data is needed in which test results are acted upon clinically, showing that results lead to a change in patient management and/or outcomes (Simpson, 2012). For example, data must demonstrate that physicians have sufficient confidence in both positive and negative test results to refrain from performing more invasive testing, e.g., amniocentesis, for the purpose of confirming the previously obtained test results.

Microdeletions

Lo et al. (2016) reported limited clinical utility of NIPT as a screening test for subchromosomal abnormalities such as microdeletion and microduplication syndromes. Because standard NIPT can only detect the majority of larger chromosomal rearrangements and requires knowledge of fetal fraction, it is not yet ready for routine clinical implementation.

Wapner et al. (2015) estimated the performance of a SNP-based NIPT for 5 microdeletion syndromes. After validation of the SNP-targeted assay, a cohort of 469 test samples was evaluated. Detection rates were 97.8% for a 22q11.2 deletion (45/46) and 100% for Prader-Willi (15/15), Angelman (21/21), 1p36 deletion (1/1) and cri-du-chat syndromes (24/24). False-positive rates were 0.76% for 22q11.2 deletion syndrome (3/397) and 0.24% for cri-du-chat syndrome (1/419). No false positives occurred for Prader-Willi (0/428), Angelman (0/442) or 1p36 deletion syndromes (0/422). Further validation studies are needed to determine the sensitivity and specificity of this screening test for microdeletion syndromes.

Sex Chromosome Aneuploidies

Hayes evaluated the clinical utility of cfDNA screening for fetal sex chromosome aneuploidy in women with singleton or multiple gestation pregnancies. The overall quality of evidence was very low. Current clinical utility studies demonstrate a wide variability in the impact of cfDNA sex chromosome aneuploidy screening in decision-making outcomes. Study limitations include varied and small patient populations with few women with abnormal cfDNA screening results for individual sex chromosome aneuploidies, conflicting results or information that limited available data, confusing results presentation and limited patient follow-up. Additional studies with large numbers of women

with abnormal cfDNA sex chromosome aneuploidy screening are needed. No studies were identified evaluating cfDNA screening for sex chromosome aneuploidy in multiple gestation pregnancies (Hayes, 2017).

Professional Societies

American College of Medical Genetics and Genomics (ACMG)

An ACMG position statement addresses the advantages and disadvantages of noninvasive prenatal screening for fetal aneuploidy and offers recommendations for pre- and post-test genetic counseling. The statement includes the following recommendations:

- Provide up-to-date, balanced and accurate information early in gestation to optimize patient decision making, independent of the screening approach used.
- Laboratories should work with public health officials, policymakers and private payers to make NIPT, including the pre- and post-test education and counseling, accessible to all pregnant women.
- Allow patients to select diagnostic or screening approaches for the detection of fetal aneuploidy and/or genomic changes that are consistent with their personal goals and preferences.
- Inform all pregnant women that diagnostic testing (CVS or amniocentesis) is an option for the detection of chromosome abnormalities and clinically significant copy-number variants.
- Inform all pregnant women that NIPT is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes).

ACMG emphasizes that all genetic screening has residual risk and there are pros and cons to any screening approach (Gregg et al, 2016).

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

ACOG and SMFM jointly published a practice bulletin on screening for fetal aneuploidy. The document states that no one screening test is superior to other screening tests in all test characteristics. Screening for aneuploidy should be an informed patient choice with shared decision making. The guidelines state that because cfDNA is a screening test with the potential for false-positive and false-negative test results, such testing should not be used as a substitute for diagnostic testing. Most published experience is based on studies conducted on high-risk populations. The sensitivity and specificity in the general obstetric population are similar to the levels published for the high-risk population. However, cfDNA screening cannot have the same accuracy in low-risk pregnancies because the positive predictive value is affected by the prevalence of the disorder in the population. The positive predictive value is lower in the general obstetric population because of the lower prevalence of aneuploidy in this population. All women with a positive cfDNA test result should have a diagnostic procedure before any irreversible action is taken. cfDNA screening tests for microdeletions have not been validated clinically and are not recommended at this time (ACOG, 2016).

ACOG and SMFM also jointly published a committee opinion stating that noninvasive prenatal screening using cfDNA from the plasma of pregnant women offers potential as a screening method for fetal aneuploidy. A number of laboratories have validated different techniques and all tests have a high sensitivity and specificity for trisomy 18 and trisomy 21, regardless of which molecular technique is used. Women whose results are not reported, indeterminate or uninterpretable should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. Patients should be counseled that cfDNA screening does not replace the precision obtained with diagnostic tests, such as CVS or amniocentesis and, therefore, is limited in its ability to identify all chromosome abnormalities. The cfDNA screening test should not be considered in isolation from other clinical findings and test results. Management decisions should not be based on the results of the cfDNA screening alone. Patients should be counseled that a negative cfDNA test result does not ensure an unaffected pregnancy. Given the performance of conventional screening methods and the limitations of cfDNA screening performance, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population. Screening for microdeletions has not been validated in clinical studies, and the true sensitivity and specificity of this screening test is uncertain. Routine cfDNA screening for microdeletion syndromes should not be performed (ACOG, 2015).

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

In a joint statement, ASHG and ESHG present different scenarios for NIPT-based screening for common autosomal aneuploidies. The statement suggests that trade-offs involved in these scenarios should be assessed in light of the aim of screening, the balance of benefits and burdens for pregnant women and their partners. The statement includes the following recommendations:

- NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as combined first-trimester screening. However, a positive NIPT result should not be regarded as a final diagnosis: false positives occur for a variety of reasons. Women should be advised to have a positive result confirmed through diagnostic testing if they are considering a possible termination of pregnancy.
- Expanding NIPT-based prenatal screening to also report on sex chromosomal abnormalities and microdeletions is not recommended.

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

National Society of Genetic Counselors (NSGC)

An NSGC practice guideline (Wilson et al., 2013) provides a summary of screening and diagnostic testing options for chromosome aneuploidy. A decision tree and comparison tables are included to help providers select the test which best suits their patient's needs. The guideline also states that referral to a genetic counselor or other qualified provider may be appropriate if a patient could benefit from additional discussion prior to making a decision regarding screening and diagnostic testing options. NIPT is only recommended for patients from high-risk populations, including advanced maternal age, positive screening test, abnormal ultrasound suggestive of aneuploidy or prior pregnancy with chromosome aneuploidy. It is recommended that a positive NIPT be followed by confirmatory diagnostic testing prior to making pregnancy decisions.

In a separate position statement, the NSGC supports NIPT screening as an option for pregnant patients. Because cfDNA screening cannot definitively diagnose or rule out genetic conditions, qualified providers should communicate the benefits and limitations of cfDNA screening to patients prior to testing. Many factors influence cfDNA screening performance, therefore it may not be the most appropriate option for every pregnancy. Prior to undergoing cfDNA screening, patients should have the opportunity to meet with qualified prenatal care providers who can facilitate an individualized discussion of patients' values and needs, including the option to decline all screening or proceed directly to diagnostic testing. Clinicians with expertise in prenatal screening, such as genetic counselors, should provide post-test genetic counseling to patients with increased-risk screening results. Diagnostic testing should be offered to patients with increased-risk results to facilitate informed decision making. Released October 2016 (NSGC website).

Society for Maternal-Fetal Medicine (SMFM)

In a statement clarifying recommendations regarding cfDNA testing, SMFM asserted that it does not recommend that cfDNA aneuploidy screening be offered to all pregnant women. Limited data on the effectiveness and clinical utility for improving patient outcomes preclude a recommendation that cfDNA be actively offered to all pregnant women. However, SMFM believes that the option should be available to women who request additional testing beyond what is currently recommended by professional societies (SMFM, 2015).

U.S. FOOD AND DRUG ADMINISTRATION (FDA)

Laboratories that perform DNA-based prenatal tests for trisomy 21, 18 and 13 are regulated by the FDA under the Clinical Laboratory Improvement Amendments. See the following website for more information:

<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124105.htm>.

(Accessed November 7, 2017)

Additional Product Information

- MaterniT21[®] PLUS (LabCorp[®]/Sequenom Laboratories)
- VisibiliT[™] (LabCorp[®]/Sequenom Laboratories)
- veriFi[®] Prenatal Test (Illumina[®], Inc.)
- Harmony[™] Prenatal Test (Ariosa[®] Diagnostics/Roche)
- Panorama[™] Prenatal Test (Natera[™] Inc.)

CENTERS FOR MEDICARE AND MEDICAID SERVICES (CMS)

Medicare does not have a National Coverage Determination (NCD) for DNA-based noninvasive prenatal tests of fetal aneuploidy. Local Coverage Determinations (LCDs) exist; see the LCDs for [Biomarkers Overview](#), [Molecular Diagnostic Tests \(MDT\)](#), [MolDX: Molecular Diagnostic Tests \(MDT\)](#) and [Molecular Pathology Procedures](#).

(Accessed October 13, 2017)

REFERENCES

American College of Obstetricians and Gynecologists (ACOG). Committee Opinion No. 640. Cell-free DNA screening for fetal aneuploidy. *Obstet Gynecol*. 2015 Sep;126(3):e31-7.

American College of Obstetricians and Gynecologists (ACOG). Practice Bulletin No. 162. Prenatal diagnostic testing for genetic disorders. *Obstet Gynecol*. 2016 May;127(5):e108-22.

American College of Obstetricians and Gynecologists (ACOG). Practice Bulletin No. 163. Screening for fetal aneuploidy. *Obstet Gynecol*. 2016 May;127(5):e123-37.

Ariosa[®] Diagnostics website. Harmony[™] Prenatal Test. Available at: <http://sequencing.roche.com/harmony.html>. Accessed November 7, 2017.

Ashoor G, Syngelaki A, Wagner M, et al. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol*. 2012 Apr;206(4):322.e1-5.

Ashoor G, Syngelaki A, Wang E, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. *Ultrasound Obstet Gynecol*. 2013 Jan;41(1):21-5.

Bianchi DW, Parker RL, Wentworth J, et al.; CARE Study Group. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014 Feb 27;370(9):799-808.

Bianchi DW, Platt LD, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol*. 2012 May;119(5):890-901.

Canick JA, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn*. 2012 Aug;32(8):730-4.

Centers for Disease Control and Prevention (CDC). Genomic Testing. July 2017. Available at: <https://www.cdc.gov/genomics/gtesting/>. Accessed November 7, 2017.

Chiu RW, Akolekar R, Zheng YW, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ*. 2011 Jan 11;342:c7401.

Dar P, Curnow KJ, Gross SJ, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based noninvasive prenatal aneuploidy testing. *Am J Obstet Gynecol*. 2014 Nov;211(5):527.e1-e17.

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

Driscoll DA, Gross S. Clinical practice. Prenatal screening for aneuploidy. *N Engl J Med*. 2009 Jun 11;360(24):2556-62.

Ehrich M, Deciu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol*. 2011 Mar;204(3):205.e1-11.

Fairbrother G, Johnson S, Musci TJ, Song K. Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. *Prenat Diagn*. 2013 Jun;33(6):580-3.

Futch T, Spinosa J, Bhatt S, et al. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn*. 2013 Jun;33(6):569-74.

Genetics Home Reference website. Can changes in the structure of chromosomes affect health and development? November 2017. Available at: <http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/structuralchanges>. Accessed November 9, 2017.

Genetics Home Reference website. Down syndrome. June 2012. Available at: <http://ghr.nlm.nih.gov/condition/down-syndrome>. Accessed November 9, 2017.

Genetics Home Reference website. Trisomy 18. March 2012. Available at: <http://ghr.nlm.nih.gov/condition/trisomy-18>. Accessed November 9, 2017.

Genetics Home Reference website. Trisomy 13. November 2013. Available at: <http://ghr.nlm.nih.gov/condition/trisomy-13>. Accessed November 9, 2017.

Gil MM, Akolekar R, Quezada MS, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther*. 2014;35(3):156-73.

Gil MM, Quezada MS, Bregant B, et al. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. *Ultrasound Obstet Gynecol*. 2013 Jul;42(1):34-40.

Gil MM, Quezada MS, Revello R, et al. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2015 Mar;45(3):249-66.

Gil MM, Accurti V, Santacruz B, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2017 Sep;50(3):302-314.

Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2016 Oct;18(10):1056-65.

Hayes, Inc. Hayes Genetic Test Evaluation Report. Cell-free DNA (cfDNA) [formerly NIPS, NIPT] screening for fetal trisomy 21, 18 and 13 in low-risk women. Lansdale, PA: Hayes, Inc.; October 2017a.

Hayes, Inc. Hayes Genetic Test Evaluation Report. Cell-free DNA (cfDNA) [formerly NIPS, NIPT] screening for fetal sex chromosome aneuploidy. Lansdale, PA: Hayes, Inc.; October 2017b.

Hayes, Inc. Hayes Genetic Test Evaluation Report. Noninvasive prenatal testing (NIPT). Lansdale, PA: Hayes, Inc.; March 2015.

Hayes, Inc. Hayes Genetic Test Evaluation Synopsis. VisibiliT (Sequenom). Lansdale, PA: Hayes, Inc.; August 2016.

Illumina® website. veriFi® Prenatal Test. Available at: <http://www.illumina.com/clinical/reproductive-genetic-health.html>. Accessed November 7, 2017.

Iwarsson E, Jacobsson B, Dagerhamn J, et al. Analysis of cell-free fetal DNA in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population – a systematic review and meta-analysis. *Acta Obstet Gynecol Scand*. 2017 Jan;96(1):7-18.

Lo KK, Karampetsou E, Boustred C, et al. Limited clinical utility of non-invasive prenatal testing for subchromosomal abnormalities. *Am J Hum Genet*. 2016 Jan 7;98(1):34-44.

Mersy E, Smits LJ, van Winden LA, et al. Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012. *Hum Reprod Update*. 2013 Jul-Aug;19(4):318-29.

Natera™ website. Panorama™ Prenatal Test. Available at: <http://www.panoramatest.com/>. Accessed November 7, 2017.

National Society of Genetic Counselors (NSGC). Position statement. Prenatal cell-free DNA screening. 2016. Available at: <https://www.nsgc.org/p/bl/et/blogaid=805>. Accessed December 8, 2017.

Nicolaides KH, Syngelaki A, Ashoor G, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol*. 2012 Nov;207(5):374.e1-6.

Nicolaides KH, Syngelaki A, Gil M, et al. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn*. 2013 Jun;33(6):575-9.

Norton ME, Baer RJ, Wapner RJ, et al. Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities. *Am J Obstet Gynecol*. 2016 Jun;214(6):727.e1-6.

Norton ME, Brar H, Weiss J, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol*. 2012 Aug;207(2):137.e1-8.

Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. *N Engl J Med*. 2015 Apr 23;372(17):1589-97

Palomaki GE, Kloza EM, O'Brien BM, et al. The clinical utility of DNA-based screening for fetal aneuploidy by primary obstetrical care providers in the general pregnancy population. *Genet Med*. 2017 Jul;19(7):778-786.

Palomaki GE, Deciu C, Kloza EM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med*. 2012 Mar;14(3):296-305.

Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med*. 2011 Nov;13(11):913-20.

Pergament E, Cuckle H, Zimmermann B, et al. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstet Gynecol*. 2014 Aug;124(2 Pt 1):210-8.

Porreco RP, Garite TJ, Maurel K, et al. Noninvasive prenatal screening for fetal trisomies 21, 18, 13 and the common sex chromosome aneuploidies from maternal blood using massively parallel genomic sequencing of DNA. *Am J Obstet Gynecol*. 2014 Oct;211(4):365.e1-12.

Sehnert AJ, Rhee B, Comstock D, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. *Clin Chem*. 2011 Jul;57(7):1042-9.

Sequenom® Laboratories website. MaterniT21® PLUS. Available at: <https://www.sequenom.com/>. Accessed November 7, 2017.

Simpson JL. Is cell-free fetal DNA from maternal blood finally ready for prime time? *Obstet Gynecol*. 2012 May;119(5):883-5.

Society for Maternal-Fetal Medicine (SMFM). Consult Series #36. Prenatal aneuploidy screening using cell-free DNA. *Am J Obstet Gynecol*. 2015 Jun;212(6):711-6.

Society for Maternal-Fetal Medicine (SMFM). SMFM statement: clarification of recommendations regarding cell-free DNA aneuploidy screening. *Am J Obstet Gynecol*. 2015 Dec;213(6):753-4.

Sparks AB, Struble CA, Wang ET, Song K, Oliphant A. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol*. 2012b Apr;206(4):319.e1-9.

Sparks AB, Wang ET, Struble CA, et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. *Prenat Diagn*. 2012a Jan;32(1):3-9.

Taylor-Phillips S, Freeman K, Geppert J, et al. Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis. *BMJ Open*. 2016 Jan 18;6(1):e010002.

Verweij EJ, van den Oever JM, de Boer MA, et al. Diagnostic accuracy of noninvasive detection of fetal trisomy 21 in maternal blood: a systematic review. *Fetal Diagn Ther*. 2012;31(2):81-6.

Wapner RJ, Babiarez JE, Levy B, et al. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am J Obstet Gynecol*. 2015 Mar;212(3):332.e1-9.

Wilson KL, Czerwinski JL, Hoskovec JM, et al. NSGC practice guideline: prenatal screening and diagnostic testing options for chromosome aneuploidy. *J Genet Couns*. 2013 Feb;22(1):4-15.

Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies. *Ultrasound Obstet Gynecol*. 2015 May;45(5):530-8.

Zimmermann B, Hill M, Gemelos G, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn*. 2012 Dec;32(13):1233-41.

POLICY HISTORY/REVISION INFORMATION

Date	Action/Description
06/01/2018	<ul style="list-style-type: none"> • Updated list of related policies to reflect title change for <i>Chromosome Microarray Testing (Non-Oncology Conditions)</i>; previously titled <i>Chromosome Microarray Testing</i>
01/01/2018	<ul style="list-style-type: none"> • Updated coverage rationale; replaced language indicating: <ul style="list-style-type: none"> ○ “DNA-based noninvasive prenatal tests of fetal aneuploidy are proven <i>and</i> medically necessary as screening tools for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) or trisomy 13 (Patau syndrome) in any one of the circumstances [listed in the policy]” with “DNA-based noninvasive prenatal tests of fetal aneuploidy are proven <i>and/or</i> medically necessary as screening tools for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) or trisomy 13 (Patau syndrome) in any one of the circumstances [listed in the policy]” ○ “DNA-based noninvasive prenatal tests of fetal aneuploidy are unproven <i>and</i> not medically necessary for all other indications including, but not limited to, the [indications listed in the policy]” with “DNA-based noninvasive prenatal tests of fetal aneuploidy are unproven <i>and/or</i> not medically necessary for all other indications including, but not limited to, the [indications listed in the policy]” • Updated supporting information to reflect the most current clinical evidence and references • Archived previous policy version 2017T0560L