Sequencing-based Tests to Determine Fetal Aneuploidies and Microdeletions from Maternal Plasma DNA

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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Fetal cell-free DNA fragments present in the plasma of pregnant women can be used for fetal screening, including testing for fetal sex chromosome aneuploidies (e.g., Turners, Klinefelter syndrome), fetal sex determination, and microdeletion syndromes (e.g., Prader-Willi/Angelman syndrome)

MEDICAL POLICY CRITERIA

Note: This policy does not address fetal trisomy aneuploidy screening (Trisomy 13, 18, 21).

I. Nucleic acid sequencing-based testing of maternal plasma for fetal sex chromosome aneuploidies (e.g. sex chromosome aneuploidy (SCAs) or sex chromosome aneuploidy panel (SCAP) testing) is considered investigational.

II. Nucleic acid sequencing-based testing of maternal plasma for fetal sex determination is considered not medically necessary.

III. Nucleic acid sequencing-based testing of maternal plasma for microdeletion syndromes is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.
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. Therefore, before testing, women should be counseled about the risk of a false positive test and that karyotyping via invasive prenatal diagnostic testing would be necessary to exclude the possibility of a false positive test.

**CROSS REFERENCES**

1. [Evaluating the Utility of Genetic Panels](#), Genetic Testing Policy No. 64
2. [Fetal RHD Genotyping Using Maternal Plasma](#), Genetic Testing No. 74
3. [Invasive Prenatal (Fetal) Diagnostic Testing Using Chromosomal Microarray Analysis (CMA)](#), Genetic Testing, Policy No. 78
4. [Chromosomal Microarray Analysis (CMA) for the Evaluation of Products of Conception and Pregnancy Loss](#), Genetic Testing, Policy No. 79
5. [Carrier Screening for Genetic Diseases](#), Genetic Testing, Policy No. 81

**BACKGROUND**

Historically, karyotype testing was an optional test used to examine chromosomes in a sample of fetal cells to help identify genetic disorders. Karyotype testing is an invasive, and requires either an amniocentesis or a chorionic villi sampling test (CVS). Newer non-invasive prenatal screening tests have been developed that analyzes fetal cell-free DNA (cfDNA) circulating in maternal blood. Most DNA is contained within cells, but a small amount circulates freely in the bloodstream, called cfDNA. This non-invasive prenatal screening test (NIPT) analyzes the maternal serum for fetal trisomy aneuploidies, and can also include testing for fetal sex chromosomes aneuploidies, microdeletions, and fetal sex determination.

**FETAL TRISOMY ANEUPLOIDY TESTING**

National guidelines recommend that all pregnant women be offered screening for fetal chromosomal abnormalities, the majority of which are aneuploidies (an abnormal number of chromosomes).[1] Fetal chromosomal abnormalities occur in approximately 1 in 160 live births. The trisomy syndromes are aneuploidies involving three copies of one chromosome. Trisomies 21 (Down syndrome, T21), 18 (Edwards syndrome, T18) and 13 (Patau syndrome, T13) are the most common forms of fetal aneuploidy that survive to birth. The most important risk factor for Down syndrome is maternal age, with an approximate risk of 1/1500 in young women that increases to nearly 1/10 by age 48.[2] When NIPT tests indicate a high risk of a trisomy syndrome, direct karyotyping of fetal tissue obtained by amniocentesis or CVS is required to confirm that T21 or another trisomy is present. Both amniocentesis and CVS are invasive procedures and have an associated risk of miscarriage. Therefore, sequenced based testing of maternal serum for fetal T21, T18, and T13 may reduce unnecessary amniocentesis and CVS procedures and has the potential to improve health outcomes.

**SEX CHROMOSOME ANEUPLOIDY**

Some of the cfDNA from the NIPT prenatal tests also include testing for sex chromosome aneuploidies (e.g. sex chromosome aneuploidy (SCAs) or sex chromosome aneuploidy panel (SCAP) testing) which examine x and y-linked disorders. Abnormalities in the number of X or Y chromosomes result in the following syndromes:
Sex chromosome aneuploidies occur in approximately one 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. Potential benefits of early identification (e.g., the opportunity for early management of the manifestations of the condition), must be balanced against potential harms that can include stigmatization.

MICRODELETION SYNDROMES

Microdeletion syndromes are defined as a group of clinically recognizable disorders characterized by a small (< 5Mb) deletion of a chromosomal segment spanning multiple disease genes, each potentially contributing to the phenotype independently. The phenotype is defined as the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment. Microdeletion testing can include, but is not limited to the following conditions or syndromes:

- 22q deletion syndrome (DiGeorge)
- 22q11 deletion syndrome (Shprintzen syndrome)
- 15q11.2 (Prader-Willi/Angelman syndromes)
- 5p deletion (Cri-du-chat syndrome)
- 1p36 deletion syndrome
- 4p deletion (Wolf-Hirschhorn syndrome)

Clinical implications of prenatal testing for microdeletions are not well defined. It is unclear whether prenatal diagnosis is appropriate given the inherent difficulty in accurately predicting the phenotype for the myriad of microdeletion syndromes. Though laboratories may offer screening for microdeletion syndromes, screening for these microdeletion syndromes is not currently the main intent of NIPT screening tests.

FETAL SEX DETERMINATION

Sequencing-based testing of maternal serum for determination of fetal sex in the first trimester of pregnancy is possible. However, the current standard of care for fetal sex is ultrasound. Fetal sex includes:

- Male (XX)
- Female (XY)

REGULATORY STATUS

None of the commercially available sequencing assays listed above have been submitted to or reviewed by the U.S. Food and Drug Administration (FDA). 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. The NIPT panels vary significantly in the base components and additional options a provider may choose on the requisition form. Commercial tests include, but are not limited to, the following:

- **Harmony™ Prenatal Test** (Ariosa Diagnostics, a division of LabCorp).
  
  Tests for fetal aneuploidy trisomies.
  
  Additional options for testing fetal sex chromosome aneuploidies, fetal gender, and monosomy X.

- **InformaSeq℠ Prenatal Test** (Integrated Genetics)
  
  Tests for fetal aneuploidy trisomies.
  
  Optional testing includes fetal sex chromosome and fetal gender.

- **Prelude™ Prenatal Screen** (Counsyl, Inc.).
  
  Tests for fetal aneuploidy trisomies.
  
  Optional items include fetal sex chromosome aneuploidies, fetal gender, and microdeletions.

- **MaterniT Genomıe** (Sequenom)
  
  Tests for genome wide aneuploidies

- **MaterniT21™ Plus** (Sequenom Laboratories).
  
  Tests for fetal aneuploidy trisomies and fetal gender.
  
  Additional items to include microdeletions, other chromosomes (T16, T22), and sex chromosomes aneuploidies.

- **Panorama Prenatal Panel** (Natera).
  
  Tests for fetal aneuploidy trisomies, fetal sex chromosome aneuploidies, triploidy, microdeletions, and fetal gender.

- **Panorama Extended Panel** (Natera)
  
  Tests for fetal aneuploidy trisomies, fetal sex chromosome aneuploidies, triploidy, microdeletions, and fetal gender.

- **Progenity Innatal Prenatal Screen** (Progenity)
  
  Tests for fetal aneuploidy trisomies, fetal sex chromosome aneuploidies, and an option for fetal gender.

- **Verifi® Prenatal Test** (Illumina, formerly Verinata Health).

  There are two options for these tests which may include fetal aneuploidy trisomies, fetal sex chromosomes aneuploidies, and fetal gender.
EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[3] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Assessment of a diagnostic technology such as maternal plasma DNA sequencing tests typically focuses on three parameters:

1. Analytic validity;
2. Clinical validity (includes calculations of sensitivity and specificity in appropriate populations of patients); and
3. Clinical utility (demonstration that the diagnostic information can be used to improve patient health outcomes).

The focus of this evidence summary below is on the investigational and not medically necessary indications in the policy criteria. Systematic reviews that report on the performance of testing for detection of various conditions are summarized at the beginning of each section below.

The evidence regarding these three questions was addressed in the 2012 and 2014 BlueCross BlueShield Association Technology Evaluation Center (TEC) Assessments.[4,5] The initial Assessment, published in 2012, focused on detection of T21/Down syndrome because the majority of published data at the time was concentrated on this trisomy. Additionally, large numbers of cases were included in several publications, and all companies had published data regarding the detection of T21. The subsequent Assessment, published in 2014, reviewed the available data for detection of T18, T13, and sex chromosome aneuploidies (SCAs). The scope of both TEC Assessments was limited to the evaluation of tests that are available in the United States. Additional literature published after publication the TEC Assessments is also addressed in the analysis below.

ANALYTIC VALIDITY

Fetal Sex Chromosome Aneuploidies

No studies were identified that provided direct evidence on the analytic validity of cfDNA testing for fetal sex chromosome aneuploidies. Each of the commercially available tests uses next generation sequencing (NGS). On June 23, 2011, the FDA held an exploratory, public meeting on the topic of NGS in preparation for the goal of developing “a transparent evidence-based regulatory pathway for evaluating medical devices/products based on NGS that would assure safety and effectiveness of devices marketed for clinical diagnostics.” The discussion pointed out the differences among manufacturers’ sequencing platforms and the diversity of applications, making it difficult to generate specific regulatory phases and metrics. It was suggested that “the process may need to be judged by the accuracy and fidelity of the final
A consistent discussion trend was that validation be application-specific. Thus, technical performance may need to be more closely linked to intended use and population, and may not be generalizable across all sequencing applications. Each of the companies currently offering a maternal plasma DNA sequencing test has developed a specific procedure for its private, CLIA-licensed laboratory where all testing takes place.

Microdeletion syndromes

A study published by Wapner (2015) evaluated the ability of the Natera single nucleotide polymorphisms (SNP)-based cfDNA test to identify microdeletions.\[6\] The study estimated test performance for identifying five microdeletions: 22q11.2, 1p36, cri du chat, Prader-Willi, and Angelman deletions. After initial validation that the SNP-based assay was capable of detecting the five microdeletions, a cohort of 469 test samples were evaluated. Included were six samples from pregnant women known to have microdeletions, 362 unaffected samples from pregnant women, and 111 artificial DNA mixtures (PlasmArts). The PlasmArts samples mimicked the fetal fraction found in cfDNA from pregnant plasma and were enriched with microdeletions (in half of the samples). Twenty-three (6.4%) of the pregnancy samples and three of the PlasmArts samples failed quality control; all pregnancy samples were from unaffected pregnancies. A total of 82 of 83 microdeletions were identified. The analytic detection rate was 45 of 46 for 22q11.2 deletions (97.8%; 95% CI, 88.5 to 99.9%) and 100% for each of the other microdeletions. There were three false positives, three of 397 pregnancies unaffected with 22q11.2 deletion (false-positive rate, 0.76%; 95% CI, 0.1% to 2.2%) and one of 419 pregnancies unaffected with cri du chat (false-positive rate, 0.24%; 95% CI, not reported).

This study was limited by a number of factors. First, the population studied was not a clinical population and the samples tested were artificially constructed. Also, all patients did not receive a gold standard test for microdeletions, so it is not possible to accurately identify all false negatives and all false positives. Finally, more data are needed to determine if the sequencing-based tests have the ability to identify microdeletions of different sizes (e.g., 10 Mb vs 3 Mb) and the ability to identify microdeletions of fetal origin by the fetal fraction of DNA present in the maternal plasma sample.

Conclusions

Although all currently available commercial tests use NGS for fetal sex chromosome aneuploidy testing, the actual performance and interpretive procedures vary considerably. Clinical sequencing in general is not standardized or regulated by the FDA or other regulatory agencies, and neither the routine quality control procedures used for each of these tests, nor the analytic performance metrics have been published. Finally, the analytic validity of microdeletion testing is limited to one study, which significant limitations. Therefore, more studies are needed to determine the accuracy of testing for fetal sex chromosome aneuploidies and microdeletion syndromes.

CLINICAL VALIDITY

Systematic Reviews Addressing Multiple Conditions

Gil (2017) published a systematic review with meta-analysis which evaluated the performance of screening for fetal trisomies 21, 18 and 13 and sex chromosome aneuploidies.\[7\] This summary will only focus on the results for sex chromosome aneuploidies. There were 36 total
cases of monosomy X and 7,677 unaffected singleton pregnancies. The pooled weighted
detection rate and false positive rate were 95.8% (95% CI: 70.3 to 99.5%) and 0.14% (95% CI:
0.05 to 0.38%), respectively. Also, there were 17 cases of sex chromosome abnormalities that
were not monosomy X and 5,383 unaffected singleton pregnancies. The pooled weighted
detection rate and false positive rate were 100% (95% CI: 83.6 to 100%) and 0.003% (95% CI:
0 to 0.07%), respectively. The authors concluded that the number of cases for sex
chromosome aneuploidy was too small to calculate overall screening performance.

Norton (2016) conducted a high quality systematic review with meta-analysis which evaluated
cohort studies comparing sequential screening to cell free DNA detection rates for fetal
chromosomal abnormalities.[8] A total of 452,901 women underwent sequential screening and
out of those women, 2575 (0.57%) had a fetal chromosomal abnormality. Of those
abnormalities, the detection rate was 81.6% (total of 2101). Additionally, 19,929 euploid
fetuses had positive sequential screening resulting in a detection rate of 4.5%. The authors
concluded that cfDNA testing has good performance for fetal sex and the detection rate of
sequential screening for all aneuploidies was significantly greater than cfDNA (P<.0001).

Mackie (2016) conducted a systematic review with meta-analysis evaluating the performance
of cell free fetal DNA testing for all conditions (singleton pregnancies only).[9] A total of 117
studies addressing 18 conditions were included. The meta-analysis showed that for fetal sex
(60 studies with 11,179 tests), the sensitivity and specificity were 0.989 (95% CI: 0.980 to
0.994) and 0.996 (95% CI: 0.989 to 0.998), respectively. For monosomy X (80 studies and
6,712 tests), the sensitivity was 0.929 (95% CI: 0.741 to 0.984) and specificity 0.999 (95% CI:
0.995 to 0.999). The authors concluded that fetal sex can be considered diagnostic but that
testing for aneuploidies should only be considered as screening.

**Fetal Sex Chromosome Aneuploidies**

Gil (2015) published results from a systematic review and meta-analysis that examined the
analysis of cfDNA in maternal blood in screening for fetal aneuploidies between January 2011
and January 2015.[10] Thirty-seven articles were included in the review; however, just 28 of
these studies reported on sex chromosome aneuploidy testing.

Sixteen of the 28 studies addressed the detection of monosomy X (Turners syndrome). The
authors found, that of the 177 singleton pregnancies with fetal monosomy X, the detection rate
varied between 66.7% and 100% and the false-positive rate varied between 0% and 0.52%.
The pooled weighted detection rate was 90.3% (95% CI, 85.7-94.2%), and the false-positive
rate was 0.23% (95% CI, 0.14-0.34%). The remaining 12 studies reported on the performance
of sex chromosome abnormalities other than monosomy X (i.e.47XXX, 47 XXY, 47 XYY), in a
combined total of 56 affected and 6,699 non-sex chromosome aneuploidy singleton
pregnancies. The pooled detection rate was 93.0% (95% CI, 85.8-97.8% and the false-positive
rate was 0.14% (95% CI, 0.06-0.24%). This study has significant methodological limitations,
which include but are not limited to, very small sample sizes, high risk of bias in relation to flow
and timing (i.e. consecutive cases), testing performed in selected populations, and a lack of
clarity about karyotyping, and the studies did not clearly define the patient’s risk category.

The 2014 BCBSA TEC Assessment included a meta-analysis of sequencing-based studies
published through April 15, 2014 that included a report on sex chromosome anomalies.[5] The
largest number of studies (14 studies, total of 152 cases) published on sex chromosome
aneuploidies addressed detection of monosomy X. Pooled sensitivity for detecting monosomy
X was 83% (95% CI, 74% to 90%) and pooled specificity was 100% (95% CI, 100% to 100%).
In addition, 11 studies with a total of 51 cases were identified on the performance of sequencing-based tests in identifying other sex chromosome anomalies. Pooled sensitivity was 89% (95% CI, 50% to 98%) and pooled specificity was 100% (100% to 100%). The meta-analysis of studies on sex chromosome aneuploidies did not differentiate between high and low-risk populations.

A study published by Wang (2015), which was not included in the above systematic reviews, examined the concordance of NIPT results among 109 consecutive cases with positive or negative NIPT results and compared those findings with the cytogenetic prenatal and/or postnatal karyotype results.[11] Sixteen of these cases were tested for fetal sex chromosome aneuploidies. The authors found that of these, the true positive rate was 38% (6/16 cases), and the false positive rate to be 62% (10/16 cases). This study has methodological limitations, including small sample size and the design, which was limited to testing at just one of the four main laboratories performing NIPT in the U.S., all of which use different methodologies or algorithms.

A similar study by Petersen (2017) evaluated patient samples sent to a single diagnostic genetic laboratory to confirm NIPT results.[12] Confirmatory testing included fluorescence in situ hybridization, chromosomal microarray analysis, and/or G-banded karyotype, from CVS or postnatal blood samples. Of the 712 patient samples submitted, 138 had positive screens for sex chromosome abnormalities. The positive predictive values (PPVs) for monosomy X, 47XXX, and 47XXY were 26%, 50%, and 86%.

Bevilacqua (2017) reported on patient choice and performance of cfDNA testing for sex chromosome aneuploidy (SCA).[13] Of the 3,162 patients undergoing cfDNA testing at a single institution, 1,957 (61.9%), opted for SCA screening. There were 161 positive screening results, 118 (73.3%) of which had available follow-up data. Of the 61 positive screens for monosomy X, 46 were false positives (PPV = 24.6%), and a similar PPV (22.7%) was seen for the 22 positive screens for 47XXX. Eleven of the 30 positive screens for 47XXY were false positives, for a PPV of 63.3%, and all five cases of 47XYY were true positives.

Reiss (2017) compared NIPT to nuchal translucency screening for SCA among patients at a single prenatal diagnosis center. Of the 2,851 patients, 18 were positive for an SCA by NIPT. There were no false positives among the five cases that screened positive for 47XXX or the two cases that screened positive for 47XXY. Among the 11 positive screens for monosomy X, only one was a true positive. Four additional cases of monosomy X were identified due to cystic hygromas, one of which had a negative NIPT result.

**Microdeletion syndromes**

Microdeletion testing is currently offered commercially by two companies. Studies from both companies offering microdeletion testing have been published evaluating data from clinical samples submitted for screening. Gross (2015) published a study evaluating clinical validity of the Natera cfDNA test to identify 22q11.2 deletion syndrome.[14] The study was a retrospective analysis of 21,949 samples submitted for screening. After 1172 cases were excluded (919 failed quality control, 46 were twins/triploidy, 207 were out of specification), 20,776 cases were evaluated for the microdeletion. A total of 97 of the 20,776 cases (0.46%) were considered high risk for 22q11.2 deletion. One of these was confirmed to be a 22q11.2 microdeletion in the mother, not in the fetus, and one other was suspected of being a maternal deletion. Diagnostic testing results were available for 61 of the 95 suspected fetal deletions (64%) (invasive prenatal testing in 48 cases, postnatal testing in 11 cases, products of conception...
testing following a miscarriage in two cases). Eleven cases were confirmed to be true positives. The PPV, based on the subgroup of screening tests with confirmatory information is 11 of 61 (18%). A total of 11 of 20,776 samples (0.05% [1/2000]) were true positives.

Prenatal ultrasound data were available for 77 of 95 high-risk cases (81%); anomalies were identified in 26 of these (33.8%). Nine cases with abnormal ultrasounds were true positives. All had anomalies associated with 22q11.2 deletion syndrome and eight of the nine had abnormal ultrasounds prior to NIPS. Therefore, eight of the 11 true-positive cases (73%) could have been identified without NIPS (i.e., by ultrasound followed by invasive testing). Limitations of the analysis include a lack of diagnostic information in 34 cases (36% of cases that were considered high risk based on NIPS results) and lack of complete information on false-negative tests. (Voluntary reporting of false negatives was encouraged, but none was reported.)

A study published by Helgeson (2015) used the Sequenom MPS-based test. The investigators analyzed 175,393 blood samples from high-risk pregnant women between October 2013 and July 2014 and included 123,096 samples. The sample were tested for four microdeletions: 1p36, 5p, 15q-, and 22q11.2. From August 2014 to October 2014, 52,297 samples were tested for those four plus an additional three microdeletions: 4p-, 8q-, and 11q-. The preferred reference standard was diagnostic testing (CMA, FISH, or karyotype analysis). Cases were considered “confirmed” if the deletion was detected in the pregnant woman and/or fetus, and considered “false-positive” if diagnostic testing was negative for the deletion in either the fetus or pregnant woman. (Maternal plasma samples contain DNA fragments from both the pregnant woman and the fetus; microdeletions detected could be in either or both of them). In the absence of diagnostic testing, cases were considered “suspected” if diagnostic testing was not performed and phenotypic data were consistent with the clinical presentation common to the deletion.

Fifty-five (0.03%) of the samples were found to have one of the tested microdeletions. Nearly half (48%) of the positive tests were in pregnancies referred for testing due to ultrasound findings. Two patients were lost to follow-up, and diagnostic testing and/or clinical phenotype information was available for the remaining 53 patients. Microdeletions were confirmed (in the pregnant woman and/or fetus) in 41 of 53 cases (77.4%) and an additional nine cases did not have confirmatory testing but had clinical features consistent with one of the microdeletions. There were three false-positive cases, one case of 1p36 deletion and two cases of 5p deletion. The PPVs ranged from 60% to 100% for cases with diagnostic and/or clinical follow-up information. The false-positive rate was 0.0017% for confirmed cases; if cases lost to follow-up were all false positives, the rate would be 0.0029%. In 25 of the 55 microdeletions identified by NIPS, a maternal component was identified. Twenty of these cases were associated with 22q11.2 deletion, four with 15q deletion, and one with 8q deletion. In at least five cases, deletions were confirmed in the pregnant woman and not confirmed in the fetus. Clinical outcomes were unavailable for most pregnancies in which a deletion was not detected. Three false negatives were reported, all for 22q11.2 based on phenotypic presentation, but data on false negatives were incomplete. Not all patients had confirmatory testing, so it is not possible to accurately identify all false-negatives and all false positives.

Schwartz (2018) published the follow-up results of positive microdeletion cfDNA tests at a single laboratory. This study included 349 individuals who screened positive for a microdeletion by NIPT and had follow-up testing by either amniocentesis or CVS. The authors reported a PPV of 9.2%. In the study by Petersen (2017) described earlier, 52 patients had
positive screens for microdeletion syndromes, and PPVs ranged from 0% for Cri-du-chat and Prader-Willi/Angelman syndromes to 21% for 22q11.2 deletion syndrome.

**Fetal Sex Determination**

The current standard of care for fetal sex determination is ultrasound.

Three reviews report on the use of cfDNA for fetal sex determination. Davaney (2011) published results from a systematic review and meta-analysis to determine if noninvasive prenatal determination of fetal sex using cfDNA provides an alternative to invasive techniques for some heritable disorders. From 57 selected studies, 80 data sets (representing 3524 male-bearing pregnancies and 3017 female-bearing pregnancies) were analyzed. Authors reported that despite inter-study variability, performance was high using maternal blood. Sensitivity and specificity for detection of Y chromosome sequences was greatest using RTQ-PCR after 20 weeks' gestation. Tests using urine and tests performed before seven weeks' gestation were unreliable.

Wright (2012) published results from a review and meta-analysis of the published literature to evaluate the use of cfDNA for prenatal determination of fetal sex. The authors reviewed 90 studies, incorporating 9,965 pregnancies and 10,587 fetal sex results. Overall mean sensitivity was 96.6% (95% confidence interval [CI] 95.2% to 97.7%) and mean specificity was 98.9% (95% CI, 98.1% to 99.4%). The authors identified one limitation of their study as the inability to properly evaluate the proportion of inconclusive or uncertain results, which is known to be problematic with this technique and may vary with gestational age. Further, literature-based reviews are at risk of publication bias due to the suppression of unwanted findings. The authors concluded that fetal sex can be determined with a high level of accuracy by analyzing cfDNA.

Colmant (2013) published a review of the published literature evaluating the use of cfDNA and ultrasound for prenatal determination of fetal sex during the first trimester of pregnancy. The authors identified 16 reports of the determination of fetal sex in maternal blood and 13 reports of the determination by ultrasound. Authors determined a sensitivity and specificity of nearly 100% from eight weeks of gestation for cfDNA and from 13 weeks of gestation for ultrasound respectively. Authors concluded that fetal sex can be determined with a high level of accuracy by analyzing cfDNA and at an earlier gestation than ultrasound.

**Conclusion**

Studies assessing the clinical validity of cfDNA sequencing for fetal sex chromosome aneuploidies are inconclusive. Although some of the studies show a high detection rate and a low false positive rate, other studies have shown a low detection rate and a high false positive rate. Furthermore, the studies that show a high detection rate have significant limitations, which include but are not limited to, very small sample sizes, high risk of bias in relation to flow and timing (i.e. consecutive cases), testing performed in selected populations, a lack of clarity about follow-up karyotyping, and the studies did not clearly define the patient’s risk category. More studies are needed to determine the clinical validity of NIPT screening for fetal sex chromosome aneuploidies.

Several studies on clinical validity of microdeletion testing have been published, based on large numbers of samples submitted to the testing companies. These studies have methodological limitations which include, but are not limited to, substantial missing data on
confirmatory testing and lack of complete data on false-negatives, and, as demonstrated in one of the studies, many of the cases of microdeletion syndromes are currently initially detected via characteristic anomalies seen on prenatal ultrasound. Given the gaps in the evidence, conclusions cannot be drawn about the impact of the microdeletion testing on the net health outcome.

While there is high diagnostic accuracy on the use of cfDNA for fetal sex determination, evidence does not demonstrate how the use of nucleic acid sequencing-based testing for fetal sex determination is more beneficial than fetal ultrasound, the current clinical standard for fetal sex identification.

**CLINICAL UTILITY**

**Fetal Sex Chromosome Aneuploidies**

The impact of screening for sex chromosome aneuploidies has not been modeled in published studies. Fetal sex chromosome aneuploidies were not included in the decision analysis of the 2014 BCBSA TEC Assessment because the implications of a screen-positive finding and diagnostic confirmation were considered to differ significantly when compared to T13 and T18.\(^5\) Finally, fetal sex aneuploids are generally diagnosed postnatally, in association with specific health problems, including delayed puberty, or diminished fertility or infertility. Therefore, the balance of benefits and harms of cfDNA prenatal screen and subsequent diagnosis of sex chromosome fetal aneuploidies, each of which has variable and uncertain prognosis, is unclear.

**Microdeletion syndromes**

The clinical utility of testing for any particular microdeletion or any panel of microdeletions is uncertain. There is no direct data on whether sequencing-based testing for microdeletions improve outcomes compared with standard care. The incidence of microdeletions in otherwise normal pregnancies is extremely low, lower than the threshold level of testing established for carrier testing (generally 1%). Further, the incidence of clinical disease is likely lower than the incidence of microdeletion variants because not all individuals with a microdeletion will have clinical symptoms. Thus, the yield of testing is very low, requiring testing of many patients to identify a small number of cases.

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 to 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. Routine prenatal screening may identify a small percentage of fetuses with microdeletion variants earlier in pregnancy than would otherwise have occurred (e.g., by ultrasound evaluation and diagnostic testing). At the same time, routine prenatal screening for microdeletions would also result in false-positive tests and a larger number of invasive confirmatory tests. The large number of confirmatory tests could lead to a net harm because of pregnancy loss.

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

Conclusions

The impact of screening for fetal sex chromosome aneuploidies has not been modeled in published studies. Furthermore, there is no published direct evidence that the results from the fetal sex chromosome NIPT test improve improves patient health outcomes. Finally, fetal sex aneuploids are generally diagnosed postnatally, in association with specific health problems, including delayed puberty, or diminished fertility or infertility. Therefore, the balance of benefits and harms of cfDNA prenatal screen and subsequent diagnosis of sex chromosome fetal aneuploidies, each of which has variable and uncertain prognosis, is unclear. More studies are needed to determine the effects of fetal sex chromosome testing on health outcomes.

The clinical utility of NIPS for microdeletions remains unclear and has not been evaluated in published studies. The incidence of microdeletions syndromes is low, and not all individuals with a microdeletion will have clinical symptoms. Clinical followup of screen detected microdeletions is unclear and screening has potential associated harms (e.g. pregnancy loss associated with confirmatory tests for positive screens.) Given the gaps in the evidence, conclusions cannot be drawn about the impact of the technology on the net health outcome.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS

The American College of Obstetricians and Gynecologists (ACOG) released an updated joint committee opinion\[20\] with the Society for Maternal and Fetal Medicine (SMFM) on cell-free DNA screening for fetal aneuploidy. This document replaces the November 2012 committee opinion.[21] It is important to note that this is a joint committee opinion, and not guideline recommendation based on a systematic review of the evidence.

The complete list of recommendations in the 2015 updated committee opinion is as follows:

- “A discussion of the risks, benefits, and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing, should occur with all patients.
- Given the performance of conventional screening methods, the limitations of cfDNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.
- Although any patient may choose cfDNA analysis as a screening strategy for common aneuploidies regardless of her risk status, the patient choosing this testing should understand the limitations and benefits of this screening paradigm in the context of alternative screening and diagnostic options.
- The cfDNA test will screen for only the common trisomies and, if requested, sex chromosome composition.
- Given the potential for inaccurate results and to understand the type of trisomy for recurrence-risk counseling, a diagnostic test should be recommended for a patient who
has a positive cfDNA test result.

- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost effective and should not be performed.
- Management decisions, including termination of the pregnancy, should not be based on the results of the cfDNA screening alone.
- Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cfDNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.
- Routine cfDNA screening for microdeletion syndromes should not be performed.
- cfDNA screening is not recommended for women with multiple gestations.
- If a fetal structural anomaly is identified on ultrasound examination, diagnostic testing should be offered rather than cfDNA screening.
- Patients should be counseled that a negative cfDNA test result does not ensure an unaffected pregnancy
- cfDNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects; patients who are undergoing cfDNA screening should be offered maternal serum alpha-fetoprotein screening or ultrasound evaluation for risk assessment.
- Patients may decline all screening or diagnostic testing for aneuploidy.”

In May, 2016, ACOG and SMFM released a practice bulletin summary (No. 163) on screening for fetal aneuploidy. The following recommendations cell-free DNA are 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 are 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 noninvasive prenatal screening (NIPS) for fetal aneuploidy. ACMG recommends:

- “Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes).”
- “Referring patients to 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 does not recommend “NIPS to screen for autosomal aneuploidies other than those involving chromosomes 13, 18, and 21.”

**SOCIETY FOR MATERNAL AND FETAL MEDICINE**

There were several publications addressing cfDNA. The two most recent statements are summarized on a high level below. In 2015, the SMFM published a statement, recommending the following as important points to consider regarding the use of cfDNA and other tests for fetal aneuploidy testing:

- “cfDNA screening appears to be the most accurate screening test for trisomy 21, which comprises about 50% of all chromosome abnormalities, and 8-10% of all significant birth defects. Down syndrome is a relatively uncommon cause of intellectual disability in children born to young women.
- cfDNA does not screen for all chromosomal conditions. Rather, cfDNA very precisely targets the common aneuploidies and will not identify risk for the range of disorders potentially identified with traditional screening.
- cfDNA is a screening test, and both false positive and false negative results occur. This
is particularly true in lower risk women, in whom a positive test is more likely to be a false positive.

- Women who desire definitive information about chromosomal conditions in their pregnancy should be offered the option of amniocentesis or CVS.

- Diagnostic confirmation with CVS or amniocentesis is recommended for women with abnormal cfDNA results, particularly if clinical decision-making will change depending on the presence of aneuploidy. Irreversible decisions such as pregnancy termination should NOT be undertaken based solely on cfDNA results.

- A negative cfDNA result indicates a decreased risk and does not definitively rule out trisomy 21 or other chromosome conditions.

- Women with failed cfDNA tests are at increased risk for aneuploidy, and therefore need careful counseling about further testing, including the offer of diagnostic testing.

- Genetic counseling services are an important part in providing information in the care for patients. Certified genetic counselors often play a role in provision of these important services. The SMFM recommends payers provide adequate reimbursement for these services to provide ideal care for patients.

- All genetic screening is elective. Whether a woman chooses to have aneuploidy screening, prenatal diagnostic testing, or no testing is a personal decision and any of these is a reasonable option."

THE INTERNATIONAL SOCIETY FOR PRENATAL DIAGNOSIS

In 2015, the International Society for Prenatal Diagnosis (ISPD) published an updated position statement regarding prenatal diagnosis of chromosomal abnormalities.\[26\] This replaces the 2013 position statement published by the same authors.\[27\] The 2015 statement included the following screening protocol recommendations:

- cfDNA screening as a primary test offered to all pregnant women.

- cfDNA secondary to a high risk assessment based on serum and ultrasound screening protocols (options 4-9 below).

- cfDNA contingently offered to a broader group of women ascertained as having high or intermediate risks by conventional screening. Contingent provision of cfDNA, could also include a protocol in which women with very high risks are offered invasive prenatal diagnosis while those with intermediate risk are offered cfDNA.

- Ultrasound nuchal translucency at 11-13 completed weeks combined with serum markers at 9-13 weeks’ gestation.

- Extending option (4) to include other first trimester serum or sonographic markers. Ultrasound performance needs to be prospectively validated by the center where the screening is performed.

- A contingent test whereby women with borderline risks from option (4) have option (5) at a specialist center and risk is subsequently modified.
• Four maternal serum markers (quadruple test) at 15-19 weeks, for women who first attend after 13 weeks 6 days gestation.

• Combining options (4) and (7) in either a stepwise or contingent protocol - provided that all screening test data are included in the final risk assessment. Integrated screening can be offered when CVS is not available. A serum integrated test when NT measurement is unavailable.

• Contingent second trimester ultrasound to modify risks for aneuploidy for women having options (4), (7) or (8). Ultrasound performance must be prospectively validated by the center where the screening is performed."

“Except in exceptional circumstances, the following are not recommended:

1. The use of maternal age as a sole criterion for aneuploidy risk assessment.

2. First trimester measurement of NT with no additional tests.

3. Conventional screening tests for chromosome abnormalities following successful and unambiguous cfDNA screening.”

“Exceptional circumstances could include situations where tests are not applicable (e.g. triplets and higher pregnancy multiples, co-existing additional fetal or maternal conditions), test failures, and the need for urgent risk assessment.”

**SUMMARY**

There is not enough research to show an improvement in health outcomes for non-invasive screening using cell-free DNA to detect fetal sex chromosome aneuploidies or microdeletion syndromes. The current research shows mixed results for detection of abnormalities. Finally, there are no evidence-based practice guidelines that recommend testing for fetal sex chromosome aneuploidies and microdeletions. Therefore, nucleic acid sequencing-based testing of maternal plasma for fetal sex chromosome aneuploidies or microdeletion syndromes is considered investigational.

Research does not show that the use of nucleic acid sequencing-based testing for fetal sex determination is more beneficial than fetal ultrasound, which is the current clinical standard for determining fetal sex. Therefore, nucleic acid sequencing-based testing of maternal plasma for fetal sex determination is considered not medically necessary.

**REFERENCES**


5. Blue Cross Blue Shield Association Technology Evaluation Center (BCBSA TEC). Noninvasive maternal plasma sequencing-based screening for fetal aneuploidies other than trisomy 21. 2014;In Press. PMID:


28. BlueCross BlueShield Association. Noninvasive Prenatal Screening for Fetal Aneuploidies and Microdeletions Using Cell-Free Fetal DNA. MPRM 4.01.21. [cited; Available from:

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