Regence Oregon and Utah | Regence Idaho and select counties of Washington

Medical Policy Manual Genetic Testing, Policy No. 44

Noninvasive Prenatal Testing to Determine Fetal Aneuploidies, Microdeletions, and Twin Zygosity

Effective: October 1, 2022

Next Review: January 2023
Last Review: September 2022

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 and fetal cells 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, twin zygosity, 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. For *member contracts subject to Washington's State Board of Health Rule* (WAC 246-680), genetic testing of maternal plasma for fetal sex chromosome aneuploidies (e.g., sex chromosome aneuploidy (SCAs) or sex chromosome aneuploidy panel (SCAP) testing) may be considered **medically necessary**.

II. For *all other member contracts*, genetic 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.**

III. Genetic testing of maternal plasma for fetal sex determination is considered **not medically necessary**.

IV. Genetic testing of maternal plasma for fetal microdeletion syndromes is considered **investigational**.

V. Genetic testing of maternal plasma for twin zygosity is considered **investigational.**

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

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

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

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

LIST OF INFORMATION NEEDED FOR REVIEW

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

- Name of the genetic test(s) or panel test
- Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
- The analyses included in the test (e.g., trisomies, sex chromosome aneuploidies, etc.)
- Relevant billing codes
- Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
- Medical records related to this genetic 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. Genetic Testing for the Evaluation of Products of Conception and Pregnancy Loss, Genetic Testing, Policy No. 79
5. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81
6. Maternal Serum Analysis for Risk of Adverse Obstetric Outcomes, Laboratory, Policy No. 75

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) or fetal cells 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, twin zygosity, 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). 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.[1]

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

SEX CHROMOSOME ANEUPLOIDY

Some of the NIPT prenatal tests also include testing for sex chromosome aneuploidies (SCAs) or sex chromosome aneuploidy panel (SCAP) testing. Abnormalities in the number of X or Y chromosomes result in the following syndromes:

- Turner syndrome (Monosomy X or 45, X)
- Klinefelter syndrome (47, XXY)
- Triple X syndrome (47, XXX)
- Jacob syndrome (47, XYY)

- XXYY syndrome (48, XXYY)

Sex chromosome aneuploidies occur in approximately 1 in 400 live births. These aneuploidies are typically diagnosed postnatally, sometimes not until adulthood, such as during an evaluation of diminished fertility. Alternatively, sex chromosome aneuploidies may be diagnosed incidentally during invasive karyotype testing of pregnant women at high risk for Down syndrome. 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)

TWIN ZYGOSITY TESTING

Twin gestations occur in approximately 1 in 30 live births in the United States and have a 4- to 10-fold increased risk of perinatal complications.[2] Dizygotic or "fraternal" twins occur from ovulation and fertilization of two oocytes, which results in dichorionic placentation and two separate placentas. In contrast to dichorionic twins, monochorionic twin pregnancies share their blood supply. Monochorionic twins account for about 20% of twin gestations and are at higher risk of structural defects, miscarriage, preterm delivery, and selective fetal growth restriction compared to dichorionic twins.[2] Up to 15% of monochorionic twin pregnancies are affected by twin-to-twin transfusion syndrome (TTTS), a condition characterized by relative

hypovolemia of one twin and hypervolemia of the other.[3] According to estimates from live births, TTTS occurs in up to 15% of monochorionic twin pregnancies. In these twin pregnancies, serial fetal ultrasound examinations are necessary to monitor for development of TTTS as well as selective intrauterine growth restriction because these disorders have high morbidity and mortality and are amenable to interventions that can improve outcomes.[3] NIPT using cell-free fetal DNA to determine zygosity in twin pregnancies could potentially inform decisions about early surveillance for TTTS and other monochorionic twin-related abnormalities. In particular, determining zygosity with NIPT could potentially assist in the assessment of chorionicity when ultrasound findings are not clear.

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, now Roche).

  Tests for fetal trisomies.

  Additional options for testing fetal sex chromosome aneuploidies, fetal sex, monosomy X, and 22q11.2 microdeletion.

- **InformaSeq**[SM] **Prenatal Test** (Integrated Genetics, a division of LabCorp)

  Tests for fetal trisomies.

  Optional testing includes fetal sex chromosome and fetal sex.

- **MaterniT Genome** (Sequenom Laboratories, now LabCorp)

  Tests for genome wide aneuploidies

- **MaterniT21™ Plus** (Sequenom Laboratories, now LabCorp).

  Tests for fetal trisomies and fetal sex.

  Additional items to include microdeletions, other chromosomes (T16, T22), and sex chromosomes aneuploidies.

- **Panorama** (Natera).

  Tests for fetal trisomies, fetal sex chromosome aneuploidies, triploidy, microdeletions, and fetal sex.

- **Prequel™ Prenatal Screen** (Myriad)

  Tests for fetal trisomies, with options for sex chromosome and microdeletion testing.

- **Progenity Innatal® Prenatal Screen** (Progenity)

  Tests for fetal trisomies, may include fetal sex chromosome aneuploidies and fetal sex.

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

There are two options for these tests which may include fetal trisomies, fetal sex chromosomes aneuploidies, microdeletions, and fetal sex.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[4] 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 clinical validity and utility of these tests.

The evidence regarding these three questions was addressed in the 2012 and 2014 BlueCross BlueShield Association Technology Evaluation Center (TEC) Assessments.[5, 6] 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.

CLINICAL VALIDITY

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% confidence interval [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 and 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 2,101). 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 ($p<0.0001$).

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

A Cochrane review by Badeau (2017) evaluated diagnostic accuracy of NIPS for sex chromosome anomalies.[10] Twelve studies were identified on the 45,X chromosome with sensitivities of 91.7% to 92.4% and specificities of 99.6% to 99.8%. Reviewers calculated that of 100,000 pregnancies, 1,039 would be affected by 45,X. Of these, 953 tested with massively parallel shotgun sequencing and 960 tested with targeted massively parallel sequencing would be detected and 86 and 79 cases, respectively, would be missed. Of the 98,961 unaffected women, 396 and 198 pregnant women would undergo an unnecessary invasive test. The authors were unable to perform meta-analyses of NIPS for chromosomes 47,XXX, 47,XXY, and 47,XYY due to insufficient evidence.

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.[11] 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 to 94.2%), and the false-positive rate was 0.23% (95% CI 0.14 to 0.34%). The remaining 12 studies reported on the performance of sex chromosome abnormalities other than monosomy X (i.e., 47XXX, 47XXY, 47XYY), 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 to 97.8% and the false-positive rate was 0.14% (95% CI 0.06 to 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.[6] 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.[12] 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 study by Petersen (2017) evaluated patient samples sent to a single diagnostic genetic laboratory to confirm NIPT results.[13] 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%.

A larger study by Guy (2019) reported results for NIPT testing from a large laboratory testing company.[14] Of the 75,658 samples received (from 72,176 women), 69,794 had successful testing. Approximately 87% represented “high risk” pregnancies. The reported PPV was 69% for SCAs and 75% for microdeletions.

A study by Yang (2021) evaluated the performance of NIPT for SCA detection in a cohort of 47,800 patients in Southern China.[15] Of the 238 high-risk cases that were detected by NIPT, 170 patients had available information on subsequent prenatal diagnostic testing, such as karyotyping and CMA. These included 137 cases of 45X, 27 cases of 47XXX, and 74 cases of 47XYY/47XXY. The PPV of the NIPT testing was reported to be 30.00% for 45X, 70.58% for 47XXX, and 81.13% for 47XYY/47XXY.

Bevilacqua (2017) reported on patient choice and performance of cfDNA testing for SCA.[16] 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

Familiari (2021) conducted a systematic review of the literature on screening for fetal microdeletions and microduplications using cfDNA.[17] A total of seven studies met inclusion criteria, representing 210 cases of microdeletions or microduplications. The overall pooled PPV was 44.1% (95% CI 31.49 to 63.07, range 28.9% to 90.6%). Limitations in the individual studies included retrospective design, low number of cases for each condition, lack of a standardized confirmation of the disease, low detail regarding the presence of absence of ultrasound anomalies and sonographic protocol used, different gestational ages at the time of the test, and variation in background risk. The authors noted that confirmatory testing was seldom reported in studies, under the assumption that all anomalies would have been identified in the newborn by physical exam. However, because many newborns with microdeletion and microduplication syndromes will not demonstrate phenotypical anomalies, standard neonatal examination cannot be considered a reliable ascertainment method and the detection rate and negative predictive value could not be determined from this body of evidence.

Additional non-randomized studies from companies offering microdeletion testing have been published evaluating data from clinical samples submitted for screening. Soster (2021) conducted a retrospective analysis of 55,517 samples submitted for genome-wide cfDNA screening at a commercial laboratory between 2015 and 2018.[18] Diagnostic testing results were available in 42.5% (n = 1,142) of screen-positive samples, and 0.82% of screen-negative samples, with overall 2.98% of samples with diagnostic outcomes. Microdeletion syndromes included 1p36 deletion, Wolf–Hirschhorn, Cri-du-chat, Langer–Giedion, Jacobsen, Prader–Willi, Angelman, and DiGeorge syndrome. Test performance characteristics were based on the 1,569 patients who had diagnostic testing performed, and an overall PPV of 72.6% was reported.

Gross (2016) published a study evaluating clinical validity of the Natera cfDNA test to identify 22q11.2 deletion syndrome.[19] The study was a retrospective analysis of 21,949 samples submitted for screening. After 1,172 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%) 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.

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.

In the study by Petersen (2017) described earlier,[13] 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.

Liang (2019) reported on the performance of an expanded cfDNA test for the detection of microdeletion/microduplication syndromes.[20] Of the 94,085 women with a singleton pregnancy that were enrolled in the study, 163 (0.174%) had positive screening results for a microdeletion/microduplication syndrome. After follow-up tests were performed to confirm the results, the PPVs were 93% for DiGeorge syndrome, 68% for 22q11.22 microduplication, 75% for Prader-Willi/Angelman syndromes, and 50% for cri du chat syndrome. The combined PPVs for the remaining duplication/deletions detected were 32% for variants ≥10 Mb and 19% for variants <10 Mb.

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.[21] From 57 selected studies, 80 data sets (representing 3524 male-bearing pregnancies and 3,017 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 RT-qPCR 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.[22] The authors reviewed 90 studies, incorporating 9,965 pregnancies and 10,587 fetal sex results. Overall mean sensitivity was 96.6% (95% 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.[23] 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.

Twin Zygosity

Norwitz (2019) conducted a validation study of a single-nucleotide polymorphism-based NIPT in twin pregnancies.[2] The study included 95 samples with confirmed zygosity: 30 monozygotic and 65 dizygotic. Two of the 95 samples did not receive results due to low fetal fraction. Among the 93 pregnancies that yielded results, monozygotic sensitivity was 100% (29/29) and monozygotic specificity was 100% (64/64). A major limitation of this study was a lack of information on timing of the index test and the use of different methods to confirm zygosity.

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.[6] Finally, fetal sex aneuploidies are generally diagnosed postnatally in association with specific health problems, such as 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 specific microdeletion or any panel of microdeletions is uncertain.

There is a potential that prenatal identification of individuals with microdeletion syndromes could improve health outcomes due to the ability to allow for informed reproductive decision making, and/or 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.

Twin Zygosity

No studies were identified that evaluated whether cfDNA testing for twin zygosity improves outcomes compared with standard care.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS AND SOCIETY FOR MATERNAL-FETAL MEDICINE (ACOG/SMFM)

In 2020, ACOG and SMFM released a practice bulletin summary (No. 226) on screening for fetal aneuploidy.[24] The following recommendations are based on "good and consistent" scientific evidence:

- "Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing."
- "Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results."
- "Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected. The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings become evident such as fetal anomalies identified on ultrasound examination."
- "Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing."

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

- "The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test. However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities." "No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies; this information should be incorporated into pretest counseling for patients with multiple gestations."

- "Cell-free DNA screening can be performed in twin pregnancies. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13."

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

- "In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based aneuploidy screening or cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.
- "Patients with unusual or multiple aneuploidies detected by cell-free DNA should be referred for genetic counseling and maternal–fetal medicine consultation."

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.[25] Relevant recommendations are as follows. 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."

SUMMARY

FOR MEMBER CONTRACTS SUBJECT TO WASHINGTON'S STATE BOARD OF HEALTH RULE (WAC 246-680)

For member contracts subject to Washington's State Board of Health Rule, criteria for sex chromosome aneuploidy testing are based on the Rule. Therefore, for member contracts subject to Washington's State Board of Health Rule (WAC 246-680), sex chromosome aneuploidy testing using cell-free DNA may be considered medically necessary.

FOR MEMBER CONTRACTS *NOT* SUBJECT TO WASHINGTON'S STATE BOARD OF HEALTH RULE (WAC 246-680)

There is not enough research to show an improvement in health outcomes for non-invasive screening using fetal DNA to detect fetal sex chromosome aneuploidies or microdeletion syndromes. The current research shows mixed results for detection of abnormalities. In addition, there are no evidence-based practice guidelines that recommend testing for fetal sex chromosome aneuploidies and microdeletions. Therefore, non-invasive prenatal testing (NIPT) for fetal sex chromosome aneuploidies or microdeletion syndromes is considered investigational.

There is not enough research to show that non-invasive screening using fetal DNA to detect twin zygosity leads to improvements in health outcomes. The current research for this type of testing is very limited. In addition, there are no evidence-based practice guidelines that recommend this testing. Therefore, non-invasive prenatal testing (NIPT) for twin zygosity 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, non-invasive prenatal testing (NIPT) for fetal sex determination is considered not medically necessary.

REFERENCES

1. 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. *Prenatal diagnosis.* 2013;33(6):575-9. PMID: 23613152
2. Norwitz ER, McNeill G, Kalyan A, et al. Validation of a Single-Nucleotide Polymorphism-Based Non-Invasive Prenatal Test in Twin Gestations: Determination of Zygosity, Individual Fetal Sex, and Fetal Aneuploidy. *J Clin Med.* 2019;8(7). PMID: 31261782
3. UptoDate (2022).Twin-twin transfusion syndrome and twin anemia polycythemia sequence: Screening, prevalence, pathophysiology, and diagnosis. [cited 8/15/2022]. 'Available from:' https://www.uptodate.com/contents/twin-twin-transfusion-syndrome-and-twin-anemia-polycythemia-sequence-screening-prevalence-pathophysiology-and-diagnosis.
4. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human mutation.* 2016;37(6):564-9. PMID: 26931183
5. TEC Assessment 2012. "Sequencing-based Tests to Determine Fetal Down Syndrome (Trisomy 21) from Maternal Plasma DNA " BlueCross BlueShield Association Technology Evaluation Center, Vol. 27 No. 10.
6. TEC Assessment 2014. "Noninvasive maternal plasma sequencing-based screening for fetal aneuploides other than trisomy 21." Blue Cross Blue Shield Association Technology Evaluation Center, In Press
7. Gil MM, Accurti V, Santacruz B, et al. Analysis of Cell-Free DNA in Maternal Blood in Screening For Aneuploidies: Updated Meta-Analysis. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology.* 2017. PMID: 28397325

8. Norton ME, Baer RJ, Wapner RJ, et al. Cell-free DNA vs sequential screening for the detection of fetal chromosomal abnormalities. *American journal of obstetrics and gynecology.* 2016;214(6):727 e1-6. PMID: 26709085
9. Mackie FL, Hemming K, Allen S, et al. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *BJOG : an international journal of obstetrics and gynaecology.* 2017;124(1):32-46. PMID: 27245374
10. Badeau M, Lindsay C, Blais J, et al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. *The Cochrane database of systematic reviews.* 2017;11:CD011767. PMID: 29125628
11. 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 in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology.* 2015;45(3):249-66. PMID: 25639627
12. Wang JC, Sahoo T, Schonberg S, et al. Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2015;17:234-6. PMID: 25101914
13. Petersen AK, Cheung SW, Smith JL, et al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. *American journal of obstetrics and gynecology.* 2017;217(6):691 e1-91 e6. PMID: 29032050
14. Guy C, Haji-Sheikhi F, Rowland CM, et al. Prenatal cell-free DNA screening for fetal aneuploidy in pregnant women at average or high risk: Results from a large US clinical laboratory. *Molecular genetics & genomic medicine.* 2019:e545. PMID: 30706702
15. Yang J, Hou Y, Guo F, et al. Noninvasive prenatal detection of fetal sex chromosome abnormalities using the semiconductor sequencing platform (SSP) in Southern China. *Journal of assisted reproduction and genetics.* 2021. PMID: 33564935
16. Bevilacqua E, Ordonez E, Hurtado I, et al. Screening for Sex Chromosome Aneuploidy by Cell-Free DNA Testing: Patient Choice and Performance. *Fetal diagnosis and therapy.* 2017. PMID: 28848190
17. Familiari A, Boito S, Rembouskos G, et al. Cell-free DNA analysis of maternal blood in prenatal screening for chromosomal microdeletions and microduplications: a systematic review. *Prenatal diagnosis.* 2021;41(10):1324-31. PMID: 33710639
18. Soster E, Boomer T, Hicks S, et al. Three years of clinical experience with a genome-wide cfDNA screening test for aneuploidies and copy-number variants. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2021;23(7):1349-55. PMID: 33731879
19. Gross SJ, Stosic M, McDonald-McGinn DM, et al. Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology.* 2016;47(2):177-83. PMID: 26396068
20. Liang D, Cram DS, Tan H, et al. Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2019;21(9):1998-2006. PMID: 30828085
21. Devaney SA, Palomaki GE, Scott JA, et al. Noninvasive fetal sex determination using cell-free fetal DNA: a systematic review and meta-analysis. *JAMA.* 2011;306:627-36. PMID: 21828326

22. Wright CF, Wei Y, Higgins JP, et al. Non-invasive prenatal diagnostic test accuracy for fetal sex using cell-free DNA a review and meta-analysis. *BMC Res Notes.* 2012;5:476. PMID: 22937795
23. Colmant C, Morin-Surroca M, Fuchs F, et al. Non-invasive prenatal testing for fetal sex determination: is ultrasound still relevant? *European journal of obstetrics, gynecology, and reproductive biology.* 2013;171(2):197-204. PMID: 24094458
24. Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin Summary, Number 226. *Obstetrics and gynecology.* 2020;136(4):859-67. PMID: 32976375
25. 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. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2016;18(10):1056-65. PMID: 27467454

CODES

NOTE: There are specific CPT codes for trisomy testing and for microdeletion testing. It is inappropriate to use nonspecific molecular pathology codes (e.g., 81404) for this testing.

Codes	Number	Description
CPT	0060U	Twin zygosity, genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood
	0341U	Fetal aneuploidy DNA sequencing comparative analysis, fetal DNA from products of conception, reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplication, mosaicism, and segmental aneuploid
	81422	Fetal chromosomal microdeletion(s) genomic sequence analysis (eg, DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood
	81479	Unlisted molecular pathology procedure
	81599	Unlisted multianalyte assay with algorithmic analysis
HCPCS	None	

Date of Origin: *January 2013*