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Sequencing-based Tests to Determine Trisomy 21 from Maternal Plasma DNA

Policy # 00345
Original Effective Date: 02/20/2013
Current Effective Date: 02/19/2014

Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc.(collectively referred to as the "Company"), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.

When Services May Be Eligible for Coverage
Coverage for eligible medical treatments or procedures, drugs, devices or biological products may be provided only if:

- *Benefits are available in the member's contract/certificate, and*
- *Medical necessity criteria and guidelines are met.*

Based on review of available data, the Company may consider nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with high-risk singleton pregnancies undergoing screening for trisomy 21 to be **eligible for coverage.** (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.)

Patient Selection Criteria
Coverage eligibility will be considered when high-risk singleton pregnancies, as defined by the American College of Obstetricians and Gynecologists (ACOG) Committee Opinion, Number 454, December 2012 include women who meet at least ONE of the following criteria:.

- Maternal age 35 years or older at delivery; OR
- Fetal ultrasonographic findings indicating increased risk of aneuploidy; OR
- History of previous pregnancy with a trisomy; OR
- Standard serum screening test positive for aneuploidy; OR
- Parental balanced robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21.

When Services Are Considered Not Medically Necessary
Based on review on available data, the Company considers the use of nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with average-risk singleton pregnancies to be **not medically necessary.****

When Services Are Considered Investigational
Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.

The use of nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with high-risk singleton pregnancies undergoing screening for trisomy 21 when patient selection criteria are not met is considered to be **investigational.***

Based on review of available data, the Company considers nucleic acid sequencing-based testing of maternal plasma for trisomy 21 in women with twin or multiple pregnancies to be **investigational.***

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Background/Overview

This policy focuses on detection of trisomy 21, as it is the most common cause of human birth defects and provides the impetus for current maternal serum screening programs. Detection of trisomy 21 by deoxyribonucleic acid (DNA)-based sequencing methods would likely be representative of the testing technology and interpretation for autosomal trisomy detection such as trisomy 18 and 13 (but not for aneuploidies of sex chromosomes). However, screening for these other trisomy syndromes is not currently the main intent of prenatal screening programs. The prevalence of other trisomy syndromes is much lower than the prevalence of trisomy 21. Also, the clinical implications of identifying trisomy 18 and 13 are unclear, as most fetuses with trisomy 18 and 13 do not survive to term.

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. In the decision model conducted for the 2012 Technology Evaluation Centers (TEC) Assessment, using an overall estimate for predictive value calculations, even in a high risk population, the predictive value of a positive result was only 83%. Thus, in the absence of substantial data to confidently characterize the false positive rate, a karyotyping test would be necessary to confirm a positive result.

In some cases, tissue samples from chorionic villus sampling (CVS) or amniocentesis may be insufficient for karyotyping; confirmation by specific fluorescent in situ hybridization (FISH) assay is acceptable for these samples.

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). The trisomy syndromes are aneuploidies involving 3 copies of 1 chromosome. Trisomies 21, 18, and 13 are the most common forms of fetal aneuploidy that survive to birth. There are numerous limitations to standard screening for these disorders using maternal serum and fetal ultrasound. Commercial noninvasive, sequencing-based testing of maternal serum for fetal trisomy 21, 18, and 13 has recently become available and has the potential to substantially alter the current approach to screening.

Fetal chromosomal abnormalities occur in approximately 1 in 160 live births. The majority of fetal chromosomal abnormalities are aneuploidies, defined as an abnormal number of chromosomes. The trisomy syndromes are aneuploidies involving 3 copies of 1 chromosome. Trisomy 21 (Down syndrome, T21), trisomy 18 (Edwards syndrome, T18), and trisomy 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.

Current national guidelines recommend that all pregnant women be offered screening for fetal aneuploidy (referring specifically to trisomy 21, 18, and 13) before 20 weeks of gestation, regardless of age. Combinations of maternal serum markers and fetal ultrasound done at various stages of pregnancy are used, but there is not a standardized approach. 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 trisomy 21 or another trisomy is present. Both amniocentesis and CVS are invasive procedures and have an associated risk of miscarriage. A new screening strategy that reduces unnecessary amniocentesis and CVS procedures and increases detection of trisomy 21, 18, and 13 has the potential to improve outcomes.

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

Sequencing-based tests use 1 of 2 general approaches to analyzing cell-free DNA. The first category of tests uses quantitative or counting methods. The most widely used technique to date uses massively parallel shotgun sequencing (MPS; also known as next generation or “next-gen” sequencing). Deoxyribonucleic acid fragments are amplified by polymerase chain reaction; during the sequencing process, the amplified fragments are spatially segregated and sequenced simultaneously in a massively parallel fashion. Sequenced fragments can be mapped to the reference human genome in order to obtain numbers of fragment counts per chromosome. The sequencing-derived percent of fragments from the chromosome of interest reflects the chromosomal representation of the maternal and fetal DNA fragments in the original maternal plasma sample. Another technique is direct DNA analysis, which analyzes specific cell-free DNA fragments across samples and requires approximately a tenth the number of cell-free DNA fragments as MPS. The digital analysis of selected regions (DANSR™)‡ is an assay that uses direct DNA analysis.

The second general approach is single-nucleotide polymorphism (SNP)-based methods. These use targeted amplification and analysis of approximately 20,000 SNPs on selected chromosomes (eg, 21, 18 and 13) in a single reaction. A statistical algorithm is used to determine the number of each type of chromosome.

In order to be clinically useful, the technology must be sensitive enough to detect a slight shift in DNA fragment counts among the small fetal fragment representation of a genome with a trisomic chromosome against a large euploid maternal background. Whether sequencing-based assays require confirmation by invasive procedures and karyotyping depends on assay performance. However, discrepancies between sequencing and invasive test results that may occur for biological reasons could make confirmation by invasive testing necessary at least in some cases, regardless of sequencing test performance characteristics.

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FDA or Other Governmental Regulatory Approval

U.S. Food and Drug Administration (FDA)
None of the commercially available sequencing assays for detection of trisomy 21, 18 and 13 or other chromosomal abnormalities has been submitted to or reviewed by the FDA. Clinical laboratories may develop and validate tests in-house (laboratory-developed tests or LDTs; previously called "home-brew") and market them as a laboratory service; 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. Information on commercially available tests is as follows:

- In October 2011, Sequenom (San Diego, CA) introduced its MaterniT21™‡ test to test for trisomy 21, 18 and 13. The test is offered through the company's CLIA laboratory, the Sequenom Center for Molecular Medicine. (Uses MPS; reports results as positive or negative.)
- In March 2012, Verinata Health (Redwood, CA) launched its Verifi®‡ prenatal test for trisomy 21, 18, and 13. (Uses MPS and calculates a normalized chromosomal value [NPS]; reports results as 1 of 3 categories: No Aneuploidy Detected, Aneuploidy Detected, or Aneuploidy Suspected.)
- In May 2012, Ariosa Diagnostics (San Jose, CA) (formerly Aria) launched its Harmony™‡ test for trisomy 21 and 18, which is available from Integrated Genetics, a division of LabCorp. (Uses directed DNA analysis, results reported as risk score.)
- In March 2013, Natera (San Carlos, CA) introduced its Panorama™‡ prenatal test for detecting trisomy 21, 18 and 13, as well as for detecting select sex chromosome abnormalities. The test is available at ARUP Laboratories. (Uses SNP technology; results reported as risk score.)

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

Rationale/Source

Literature Review
The policy is based on a 2012 TEC Assessment and a search of the literature. The TEC Assessment focused on detection of trisomy 21/Down syndrome because a relatively large number of cases were available, and it also reviewed the available data for detection of trisomy 18 and 13. Both the TEC Assessment and the policy limit their scope to the evaluation of tests that are available in the United States.

Assessment of a diagnostic technology such as maternal plasma DNA sequencing tests typically focuses on 3 parameters: (1) analytic validity; (2) clinical validity (ie, sensitivity and specificity) in appropriate populations of patients; and (3) demonstration that the diagnostic information can be used to improve patient health outcomes (clinical utility). The evidence on these 3 questions is described below.

What is the analytic validity of the available maternal plasma DNA sequencing-based tests?
No studies were identified that provided direct evidence on analytic validity. Each of the commercially available tests uses MPS (also called next generation sequencing [NGS]), a relatively new technology but not an entirely new concept for the clinical laboratory. Currently, there are no recognized standards for conducting clinical sequencing by MPS. On June 23, 2011, the FDA held an exploratory, public meeting on the topic of MPS, in preparation for an eventual goal of developing "a transparent evidence-based

regulatory pathway for evaluating medical devices/products based on next generation sequencing, 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 result." A consistent discussion trend was that validation be application-specific. Thus, technical performance may need to be more closed 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 for fetal trisomy 21 has developed a specific procedure for its private, CLIA-licensed laboratory where all testing takes place.

Section Summary
Although all currently available commercially available tests use MPS, actual performance and interpretive procedures vary considerably. Clinical sequencing in general is not standardized or regulated by 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.

What is the clinical validity of the available maternal plasma DNA sequencing-based tests for trisomy 21 compared to the gold standard of karyotype analysis?
High-risk pregnancies
Studies evaluating sequencing-based tests for detecting trisomy 21 in high-risk singleton pregnancies are summarized in Table 1 in the Appendix. Sensitivity and specificity of the tests, as shown in Table 1, were uniformly high. Sensitivity ranged from 99.1% to 100%, and specificity from 99.7% to 100%.

Tests from 4 commercial sources were identified: 2 studies used the Sequenom test, 2 studies used the Verinata test, 4 studies used the Ariosa Diagnostics test, and 1 study used the Natera test. All but 2 studies were prospective and all but 2 were industry funded; in the non-industry-funded study, testing was provided by the company without charge. The enrolled study populations included women at increased risk due to older age and/or positive standard screening results or because they were already scheduled for amniocentesis or CVS. Studies generally included women at a wide range of gestational ages (eg, 8-36 weeks or 11-20 weeks) spanning first and second trimesters.

The approach to analysis varied. Some studies analyzed samples from all enrolled women and others analyzed samples from all women with pregnancies known to have a trisomy syndrome and selected controls (ie, nested case-control analysis within a cohort). The studies evaluated the results of maternal fetal DNA testing in comparison to the gold standards of karyotyping or, in individual cases when a sample did not allow karyotyping, FISH for specific trisomies. All studies included testing for trisomy 21 (T21) and some additionally tested for trisomy 18 and/or trisomy 13. There were fewer cases of T18 and T13 per study compared to T21. Four studies had 50 or more cases of T21, and 1 study, Palomaki et al, had 212 cases.

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Section Summary
Data from the available published studies consistently reported a very high sensitivity and specificity of maternal plasma DNA sequencing-based tests for detecting trisomy 21 in high-risk women with singleton pregnancies. Thus, there is sufficient evidence that the tests are accurate when used in this population.

Average-risk pregnancies
Two studies have evaluated sequencing-based tests available in the U.S. for detecting trisomy 21 in average-risk singleton pregnancies. The studies were conducted by the same research group in the U.K. and both used the Ariosa (Harmony) test, which provides risk scores rather than a positive versus negative result. The first study, by Nicolaides et al did a preliminary analysis of the accuracy of cell-free DNA testing in a general population sample. The authors evaluated archived samples from 2,049 women attending their routine first pregnancy visit at 11 to 14 weeks' gestation. Karyotyping results were available for only a small percentage of women in the study; for the rest of the enrollees, ploidy was imputed by phenotype at birth obtained from medical records. This study was judged to have a high risk of bias due to a high number of exclusions from analysis. Twenty-eight pregnancies ending in stillbirth or miscarriage were excluded for lack of karyotype; while unavoidable, these exclusions likely affect the case detection rate. Cases were primarily verified by phenotype at birth from medical records. Results were available for 1949 of 2049 cases (95%). In the remaining 5%, either the fetal fraction was too low or the assay failed. Overall, using the risk cutoff for the Harmony test, the trisomy detection rate was 100% (ie, 10 of 10 cases identified), and there was a false-positive rate of 0.1%. The risk score was over 99% in all of the 8 cases of trisomy and both cases of trisomy 18. In the 1939 known or presumed euploid cases, risk scores for trisomy 21 and trisomy 18 were less than 0.01% in 1939 (99.9%).

Gill et al prospectively studied 1005 pregnant women. They evaluated a testing strategy that included analysis of serum markers (ie, pregnancy-associated plasma protein-A [PAPP-A] and free beta-human chorionic gonadotropin) and cell-free DNA at 10 weeks and ultrasound markers (ie, nuchal translucency and presence or absence of fetal nasal bone) at 12 weeks. Parents were counseled primarily on the finding of the Harmony test if it indicated either a high or low risk of trisomy. If no results were available on the Harmony tests, parents were counseled based on combined first-trimester serum marker and ultrasound findings. Risk scores from cell-free DNA testing were available for 984 cases (98%); 27 of these required a second round of sampling. Risk scores were greater than 99% for trisomy 21 in 11 cases and for trisomy 18 in 5 cases. In 1 case, the risk score for trisomy 13 was 34%. Sixteen of the 17 women with a high risk score for aneuploidy underwent CVS and the suspected abnormality was confirmed in 15 of the 16 cases. There was 1 case with a high risk score for trisomy 21 and a negative CVS; at the time the article was written, the woman was still pregnant so the presence or absence of trisomy 21 could not be confirmed.

Section Summary
There are fewer data on the diagnostic accuracy of cell-free DNA testing of women with average-risk singleton pregnancies. Two studies have been published—both are from the same research group in the U.K. and use the same sequencing-based test. The studies identified a small number of trisomies and did not confirm negative or positive findings in all cases. Thus, the evidence on accuracy of sequencing-based tests is less definitive for women with average-risk pregnancies as it is for women with high-risk pregnancies.

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Twin and multiple pregnancies
Detection of trisomy 21 in twin pregnancies was systematically evaluated in only 1 study, published in 2012 by Canick et al; the study used the Sequenom test. All 7 cases of twin pregnancies with Down syndrome were correctly classified. Five of these were discordant, where 1 twin had T21 aneuploidy and the other did not; 2 were concordant where both twins had T21 aneuploidy.

Section Summary
For women with multiple pregnancies, there is insufficient evidence to draw conclusions about the diagnostic accuracy of these tests for detecting trisomy 21.

What is the clinical utility of the available maternal plasma DNA sequencing-based tests for aneuploidy?
No comparative studies were evaluated that compared health outcomes in patients managed using the maternal plasma DNA tests compared to standard screening tests.

As part of the 2012 TEC Assessment, a decision model was constructed to model health outcomes of sequencing-based testing for trisomy 21 compared to standard testing. The primary health outcomes of interest included the number of cases of aneuploidy correctly identified, the number of cases missed, the number of invasive procedures potentially avoided (ie, with a more sensitive test), and the number of miscarriages potentially avoided as a result of fewer invasive procedures. The results were calculated for a high-risk population of women age 35 years or older (estimated antenatal prevalence of T21, 0.95%), and an average risk population including women of all ages electing an initial screen (estimated antenatal prevalence of T21, 0.25%). For women testing positive on initial screen and offered an invasive, confirmatory procedure, it was assumed that 60% would accept amniocentesis or CVS. Sensitivities and specificities for both standard and sequencing-based screening tests were varied to represent the range of possible values; estimates were taken from published studies whenever possible.

According to the model results, sequencing-based testing improved outcomes for both high-risk and average risk women. As an example, assuming there are 4.25 million births in the U.S. per year and two-thirds of the population of average risk pregnant women (2.8 million) accepted screening, the following outcomes would occur for the 3 screening strategies under consideration:

- Standard screening. Of the 2.8 million screened with the stepwise sequential screen, 87,780 would have an invasive procedure (assuming 60% uptake after a positive screening test and a recommendation for confirmation), 448 would have a miscarriage, and 3976 of 4200 (94.7%) trisomy 21/Down syndrome cases would be detected.
- Sequencing as an alternative to standard screening. If sequencing-based testing were used instead of standard screening, the number of invasive procedures would be reduced to 7504 and the number of miscarriages reduced to 28, while the cases of Down syndrome detected would increase to 4144 of 4200 (97.6% of total), using conservative estimates.
- Sequencing following standard screening. Another testing strategy would be to add sequencing-based testing only after a positive standard screen. In this scenario, invasive procedures would be further decreased to 4116, miscarriages would remain at 28, but fewer Down syndrome cases

would be detected (3948 of 4200, 94.0% of total).. Thus, while this strategy has the lowest rate of miscarriages and invasive procedures, it detects fewer cases than sequencing-based testing alone.

At least two decision models have been presented in industry-funded publications, each using a different commercially available test and published estimates of sensitivity and specificity. Findings of both these models are similar to the TEC Assessment model in that detection of T21 is increased and miscarriage rates are decreased using sequencing-based testing compared to standard screening. Both of the studies specifically model use of sequencing-based tests offered to women who have had a positive standard screening test.

Garfield and Armstrong published a study modeling use of the Verinata test. In the model, women were eligible for screening following a positive first-trimester or second-trimester screening test or following a second-trimester ultrasound. The model assumed that 71% of women at average risk and 80% of women at high risk would choose the test. In a theoretical population of 100,000 pregnancies, the detection rate of T21 increased from 148 with standard testing to 170 with Verifi testing. In addition, the number of miscarriages associated with invasive testing (assumed to be 0.5% for amniocentesis and 1% with CVS) was reduced from 60 to 20.

Palomaki et al modeled use of the Sequenom sequencing-based test offered to women after a positive screening test, with invasive testing offered only in the case of a positive sequencing-based test. As in the TEC Assessment, they assumed 4.25 million births in the U.S. per year, with two-thirds of these receiving standard screening. The model assumed a 99% detection rate, 0.5% false-positive rate, and 0.9% failure rate for sequencing-based testing. Compared to the highest performing standard screening test, the addition of sequencing-based screening would increase the Down syndrome detection rate from 4450 to 4702 and decrease the number of miscarriages associated with invasive testing from 350 to 34.

It is important to note that all of the above models include confirmatory invasive testing for positive screening tests. Sequencing-based testing without confirmatory testing carries the risk of misidentifying normal pregnancies as positive for trisomy. Due to the small but finite false-positive rate, together with the low baseline prevalence of trisomy in all populations, a substantial percent of positive results on sequencing tests could be false-positive results.

In 2013, Ohno and Caughey published a decision model comparing use of sequencing-based tests in high-risk women with confirmatory testing (ie, as a screening test) and without confirmatory testing (ie, as a diagnostic test). Results of the model concluded that using sequencing-based tests with a confirmatory test results in fewer losses of normal pregnancies compared to sequencing-based tests used without a confirmatory test. The model made their estimates using the total population of 520,000 high-risk women presenting for first-trimester care each year in the U.S. Sequencing-based tests used with confirmatory testing resulted in 1441 elective terminations (all with Down syndrome). Without confirmatory testing, sequencing-based tests resulted in 3873 elective terminations, 1449 with Down syndrome and 2424 without Down syndrome. There were 29 procedure-related pregnancies losses when confirmatory tests were used.

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Section Summary
There is no published direct evidence that managing patients using sequencing-based testing improves health outcomes compared to standard screening. Modeling studies using published estimates of diagnostic accuracy and other parameters predict that sequencing-based testing as an alternative to standard screening will lead to an increase in the number of Down syndrome cases detected and a large decrease in the number of invasive tests and associated miscarriages.

Ongoing Clinical Trials
Prenatal Non-invasive Aneuploidy Test Utilizing SNPs [single nucleotide polymorphism] Trial (PreNATUS) (NCT01545674): This is a prospective, blinded study evaluating the diagnostic accuracy of the Natera test for diagnosing aneuploidies (chromosomes 13, 18, 21) and sex aneuploidy (X and Y). It includes women with singleton pregnancies at high or moderate risk for trisomy who were planning on undergoing invasive testing. Gestational age of the fetus is between 8 weeks 0 days and 23 weeks 6 days. The estimated enrollment is 1,000 participants and the expected final date of data collection is December 2013.
Non-invasive Chromosomal Examination of Trisomy study (NEXT) (NCT01511458): This is a prospective blinded case-control study comparing the Aria test for trisomy 21 with standard first-trimester prenatal screening (maternal serum testing and nuchal translucency). Cases will consist of patients with trisomy 21 pregnancies confirmed by genetic testing, and controls will consist of patients without trisomy 21 pregnancies, as confirmed by genetic testing or live birth. The study is sponsored by Aria Diagnostics. The estimated enrollment is 25,000 individuals. The expected date of study completion is January 2014.
Clinical Evaluation of the SEQureDx T21 Test in Low-Risk Pregnancies (NCT01597063): This is a prospective study and includes pregnant women between 10 to 22 weeks' gestation who are at low risk for trisomy 21 aneuploidy (ie, no positive prenatal screening tests, and no personal or family history of Down syndrome). Blood samples will be collected at a scheduled prenatal care visit and analyzed with the SEQureDX T21 test; pregnancies will be followed until the birth outcome is recorded. The study is sponsored by Sequenom; estimated enrollment is 3000. The expected final date of data collection is December 2013.

Clinical Input Received through Physician Specialty Societies and Academic Medical Centers
In response to requests, input was received through 3 physician specialty societies and 4 academic medical centers while this policy was under review in 2012. While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

There was consensus that sequencing-based tests to determine trisomy 21 from maternal plasma DNA may be considered medically necessary in women with high-risk singleton pregnancies undergoing screening for trisomy 21. Input was mixed on whether sequencing-based tests to determine trisomy 21 from maternal plasma DNA may be considered medically necessary in women with average-risk singleton pregnancies. An ACOG Genetics Committee Opinion, included as part of the specialty society's input, does not recommend the new tests at this time for women with singleton pregnancies who are not at high risk of aneuploidy. There was consensus that sequencing-based tests to determine trisomy 21 from maternal plasma DNA are investigational for women with multiple pregnancies. In terms of an appropriate protocol for

using sequencing-based testing, there was consensus that testing should not be used as a single-screening test without confirmation of results by karyotyping. There was mixed input on use of the test as a replacement for standard screening tests with karyotyping confirmation and use as a secondary screen in women with screen positive on standard screening tests with karyotyping confirmation. Among the 5 reviewers who responded to the following questions (which did not include ACOG), there was consensus that the modeling approach is sufficient to determine the clinical utility of the new tests and near-consensus there is a not a need for clinical trials comparing a screening protocol using the new tests to a screening protocol using standard serum screening prior to initiation of clinical use of the tests.

Summary

Published studies from all commercially available tests have consistently demonstrated very high sensitivity and specificity for detecting Down syndrome (trisomy 21) in singleton pregnancies. Nearly all of the studies included only women at high risk of trisomy 21. Direct evidence of clinical utility is not available. A 2012 TEC Assessment modeled comparative outcomes based on the published data on test performance, published estimates of standard screening performance, patient uptake of confirmatory testing, and miscarriage rates associated with invasive procedures. For each comparison and in each risk population, sequencing-based testing improved outcomes, ie, increased the rate of Down syndrome detection and reduced the number of invasive procedures and procedure-related miscarriages. In the modeling, the negative predictive value of testing approached 100% across the range of aneuploidy risk, while the positive predictive value varied widely according to baseline risk. The variable positive predictive value highlights the possibility of a false-positive finding and thus testing using karyotyping is necessary to confirm a positive result.

Based on the available evidence, including modeling in the TEC Assessment, as well as input from clinical vetting and recommendations from national organizations, nucleic acid sequencing-based testing for trisomy 21 may be considered medically necessary in women with high-risk singleton pregnancies who meet criteria and not medically necessary in women with average-risk singleton pregnancies. Testing is considered investigational in women with twin or multiple pregnancies.

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22. Sponsored by Sequenom Inc. Clinical Evaluation of the SEQureDx T21 Test in Low Risk Pregnancies (NCT01597063). Available online at: www.clinicaltrials.gov.

Coding

The five character codes included in the Blue Cross Blue Shield of Louisiana Medical Policy Coverage Guidelines are obtained from Current Procedural Terminology (CPT®)‡, copyright 2013 by the American Medical Association (AMA). CPT is developed by the AMA as a listing of descriptive terms and five character identifying codes and modifiers for reporting medical services and procedures performed by physician.

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CPT is a registered trademark of the American Medical Association.

Sequencing-based Tests to Determine Trisomy 21 from Maternal Plasma DNA

Policy # 00345
Original Effective Date: 02/20/2013
Current Effective Date: 02/19/2014

Codes used to identify services associated with this policy may include (but may not be limited to) the following:

Code Type	Code
CPT	81479, 81507 (code 81507 is a new code for 1/1/2014; it replaced code 0005M which was deleted for 2014)
HCPCS	No codes
ICD-9 Diagnosis	V23.81, V26.33, V28.89
ICD-9 Procedure	No codes

Policy History

Original Effective Date: 02/20/2013
Current Effective Date: 02/19/2014
02/07/2013 Medical Policy Committee review
02/20/2013 Medical Policy Implementation Committee approval. New policy.
02/06/2014 Medical Policy Committee review
02/19/2014 Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
Next Scheduled Review Date: 02/2015

*****Investigational – A medical treatment, procedure, drug, device, or biological product is Investigational if the effectiveness has not been clearly tested and it has not been incorporated into standard medical practice. Any determination we make that a medical treatment, procedure, drug, device, or biological product is Investigational will be based on a consideration of the following:

A. whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the U.S. Food and Drug Administration (FDA) and whether such approval has been granted at the time the medical treatment, procedure, drug, device, or biological product is sought to be furnished; or
B. whether the medical treatment, procedure, drug, device, or biological product requires further studies or clinical trials to determine its maximum tolerated dose, toxicity, safety, effectiveness, or effectiveness as compared with the standard means of treatment or diagnosis, must improve health outcomes, according to the consensus of opinion among experts as shown by reliable evidence, including:
    1. Consultation with the Blue Cross and Blue Shield Association technology assessment program (TEC) or other nonaffiliated technology evaluation center(s);
    2. credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community; or
    3. reference to federal regulations.

**Medically Necessary (or "Medical Necessity") - Health care services, treatment, procedures, equipment, drugs, devices, items or supplies that a Provider, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury, disease or its symptoms, and that are:

A. in accordance with nationally accepted standards of medical practice;
B. clinically appropriate, in terms of type, frequency, extent, level of care, site and duration, and considered effective for the patient's illness, injury or disease; and
C. not primarily for the personal comfort or convenience of the patient, physician or other health care provider, and not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.

For these purposes, "nationally accepted standards of medical practice" means standards that are based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community, Physician Specialty Society recommendations and the views of Physicians practicing in relevant clinical areas and any other relevant factors.

‡ Indicated trademarks are the registered trademarks of their respective owners.

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NOTICE: Medical Policies are scientific based opinions, provided solely for coverage and informational purposes. Medical Policies should not be construed to suggest that the Company recommends, advocates, requires, encourages, or discourages any particular treatment, procedure, or service, or any particular course of treatment, procedure, or service.

Appendix

Table 1: Aneuploidy detection by sequencing in singleton pregnancies: test performance

Study[a]	**N in final analysis (after indeterminate samples removed)**	**Indeterminate samples**	**Sensitivity[b](%) (95% CI)**			**Specificity[b](%) (95% CI)**		
			T21	T13	T18	T21	T13	T18
Sequenom (MaterniT21™)								
	Total N = 1971							
Palomaki 2012[b]	Trisomy 21: N = 212	17/1988 (0.9%)	99.1	91.7	100	99.9	99.1	99.7
3rd-party[c]	Trisomy 18: N = 59	Test failure including fetal fraction QC	(96.6–99.9)	(61.5-99.8)	(93.9-100)	(99.7-99.9)	(98.5-99.5)	(99.3-99.9)
	Trisomy 13: N = 12							
Ehrich 2011	Total N = 449	18/467 (3.8%)	100			99.7		
In-house	Trisomy 21: N = 39	Failed test QC, including fetal fraction	(91.0-100)			(98.6-99.9)		
Verinata (verifi®)								
	Total N = 516[d]							
Bianchi 2012	Trisomy 21: N = 89	16/532 (3%)	100	78.6	97.2	100	100	100
3rd-party[c]	Trisomy 18: N = 36	Low fetal DNA	(95.9-100)	(49.2-95.3)	(85.5-99.9)	(99.1-100)	(99.2-100)	(99.2-100)
	Trisomy 13: N = 14							
Sehnert 2011	Total test set = 46	1/47 (2%)	100		100	100		100
In-house	Trisomy 21: N = 13	T13 classified as "no call"	(75.3-100)		(63.1-100)	(89.7-100)		(91.0-100)
	Trisomy 18: N = 8							

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	Trisomy 13: N = 1					
Ariosa (Harmony™)						
	Total N = 2049	N=46/2049 (2.2%)				
Nicolaides 2012	Trisomy 21: N = 8	Low fetal DNA	100	100	99.9	99.9
	Trisomy 18: N = 3 (2)	54/2049 (2.6%)	(63.1-100)	(15.8-100)	(99.6-99.9)	(99.6-99.9)
3rd-party [c]	(1 T18 sample was a test failure)	Test failure				
		Total (4.9%)				
	Total N = 3,080	N=57/3228 (1.8%)				
Norton 2012	Trisomy 21: N = 81	Low fetal DNA	100	97.4	99.97	99.93
	Trisomy 18: N = 38	91/3228 (2.8%)	(95.5-100)	(86.2-99.9)	(99.8-99.9)	(99.7-99.9)
3rd-party [c]	[73 = 'other' based on invasive testing]	Test failure				
		Total (4.6%)				
	Total N = 397					
Ashoor 2012	Trisomy 21: N = 50	3/400 (0.75%)	100	98	100	100
3rd-party [c]	Trisomy 18: N = 50	Test failure	(92.9-100)	(89.4-99.9)	(98.8-100)	(98.8-100)
	Validation set					
Sparks 2012	Total N = 167	N=0	100	100	100	100
In-house	Trisomy 21: N = 36	No failures in test set	(90.3-100)	(63.1-100)	(97.0-100)	(97.0-100)
	Trisomy 18: N = 8					
Natera (Panorama™)						
	Total N = 242					
Nicholaides (2013)	Trisomy 21: n = 25	13/242 (5.4%)	100		100	
	Trisomy 18: n = 3	Failed internal quality control	(86.3-100)		(98.2-100)	
	Trisomy 13: n = 1					

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Abbreviations: T13, trisomy 13; T18, Trisomy 18; T21, Trisomy 21; N, number of patients

[a]Other than Ashoor 2012, all studies had industry-funding and additionally, at least some authors were company employees and/or shareholders. 'In-house' indicates that all study authors were employees of the company at the time of the study. '3rd-party' indicates that the first author and at least some of the other authors were not employees of the company.

[b]All 95% confidence intervals were calculated by exact methods, see Methods, Data Abstraction, Calculations.

[c]Results for T21 were abstracted from Palomaki 2012, rather than Palomaki 2011, because of data corrections for GC content and use of repeat masking, part of the current test procedure.

[d]Patients with complex karyotypes were censored from the total population for the analysis of each trisomy; the exact number was dependent on the trisomy being analyzed.