Chromosomal Microarray Analysis for the Evaluation of Pregnancy Loss

Policy # 00449
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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 chromosomal microarray analysis (CMA) of fetal tissue for the evaluation of pregnancy loss in patients with indications for genetic analysis of the embryo or fetus to be eligible for coverage.

Patient Selection Criteria
Coverage eligibility for chromosomal microarray analysis (CMA) of fetal tissue will be met (if desired by parents) with the following indications:

- In cases of pregnancy loss at 20 weeks of gestation or earlier when there is a maternal history of recurrent miscarriage (defined as a history of 2 or more failed pregnancies); OR
- In all cases of pregnancy loss after 20 weeks of gestation.

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

Chromosomal microarray analysis (CMA) of fetal tissue for the evaluation of pregnancy loss when patient selection criteria are not met is considered investigational.*

Note:
This policy does not address the use of chromosomal microarray testing (CMA) for preimplantation genetic diagnosis or preimplantation genetic screening, or the evaluation of suspected chromosomal abnormalities in the postnatal period.

Background/Overview
Chromosomal microarray analysis of fetal tissue or placental tissue derived from the fetal genotype has been proposed as a technique to evaluate the cause of isolated and recurrent early pregnancy loss (miscarriages) and later pregnancy loss (intrauterine fetal demise [IUFD]). The evaluation of both recurrent and isolated miscarriages and IUFD may involve genetic testing of the products of conception (POC). Such testing has typically been carried out through cell culture and karyotyping of cells in metaphase. However, this technique is limited by the need for fresh tissue, the potential for cell culture failure, and the potential for maternal cell contamination, limitations which may be addressed with CMA.
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Pregnancy Loss – Etiology and Evaluation

Early Pregnancy Loss

Pregnancy loss is common, occurring in at least 15% to 25% of recognized pregnancies. Most pregnancy loss occurs early in the pregnancy, most often by the end of the first trimester or early second trimester. Pregnancy loss that occurs before the 20th week of gestation is referred to as a spontaneous abortion, early pregnancy loss, or miscarriage. While a wide range of factors can lead to early pregnancy loss, genetic causes are thought to be the predominant cause: when POC are examined, it is estimated that 60% of early pregnancy losses are associated with chromosomal abnormalities, particularly trisomies and monosomy X. The increasing risk of trisomies with maternal age contributes to the increased risk of early pregnancy loss with increasing maternal age.

Recurrent pregnancy loss, defined by the American Society for Reproductive Medicine (ASRM) as 2 or more failed pregnancies, is less common, occurring in approximately 5% of women. Recurrent pregnancy loss may be related to cytogenetic abnormalities, particularly balanced translocations, uterine abnormalities, thrombophilias, including antiphospholipid syndrome, and metabolic/endocrinologic disorders such as uncontrolled diabetes and thyroid disease. Estimates for the frequency of various underlying causes of recurrent pregnancy loss vary widely, with ranges from 2% to 6% for cytogenetic abnormalities, 8% to 42% for antiphospholipid antibody syndrome, and 1.8% to 37.6% for uterine abnormalities. It is likely that the risk of cytogenetic abnormalities is lower in recurrent early pregnancy loss than in isolated spontaneous early pregnancy loss.

Clinicians and patients may undertake an evaluation for the cause of a single or recurrent early pregnancy loss for several reasons. The knowledge that an early pregnancy loss is secondary to a sporadic genetic abnormality may provide parents with reassurance that there was nothing that they did or did not do that contributed to the loss, although the magnitude of this benefit is difficult to quantify. For couples with recurrent pregnancy loss and evidence of a structural genetic abnormality in one of the parents, preimplantation genetic diagnosis with transfer of unaffected embryos or the use of donor gametes might be considered for therapy. These therapies might be considered for couples with recurrent pregnancy loss without evidence of a structural genetic abnormality in one of the parents; guidelines on the management of recurrent pregnancy loss from ASRM state that “treatment options should be based on whether repeated miscarriages are euploid, aneuploid, or due to an unbalanced structural rearrangement and not exclusively on the parental carrier status.” Finally, among patients who are found to have a potential nongenetic underlying cause of recurrent pregnancy loss, such as antiphospholipid syndrome, cytogenetic analysis of pregnancy losses may provide evidence that the miscarriages were not due to treatment failure.

Genetic testing of POC, if possible, is recommended by several reproductive health organizations. A committee opinion from the ASRM recommends that the assessment of recurrent pregnancy loss include peripheral karyotyping of the parents, and states that karyotypic analysis of POC may be useful in the setting of ongoing therapy for recurrent pregnancy loss. The National Society of Genetic Counselors convened a multidisciplinary Inherited Pregnancy Loss Working Group which provided recommendations for the genetic evaluation of couples with recurrent pregnancy loss that stated that, when possible, chromosomal analysis on fetal tissue from products of conception should be pursued.
Late Pregnancy Loss
Fetal loss that occurs later in pregnancy, after 20 weeks gestation, may be referred to as IUFD, stillbirth, or intrauterine fetal death. In 2004, IUFD occurred in 6.2 of 1000 births in the United States, representing about 60% of perinatal mortality. Intrauterine fetal demise may be related to a range of disorders, including genetic disorders in the fetus, maternal infection, coexisting maternal medical disorders (eg, diabetes, antiphospholipid antibody syndrome, heritable thrombophilias) and obstetric complications, although in many cases, the precise cause is unable to be identified. Chromosomal or genetic abnormalities can be found in 8% to 13% of IUFD, most commonly aneuploidies. In 1 large series of IUFD (N=1025), cytogenic abnormalities were detected in 11.9%.

The American College of Obstetrics and Gynecology recommends that evaluation after an IUFD includes examination of the stillborn fetus, along with examination of the placenta and umbilical cord, along with genetic testing for all IUFD (after parental permission is obtained). Other evaluation should be based on maternal history and may include evaluation for thyroid disorders, systemic lupus erythematosus, and infections.

Some of the motivations for evaluation for a cause of IUFD are the same as for earlier pregnancy loss. Although both early and later pregnancy losses may cause grief for the mother and her family, IUFD can be particularly devastating. Information about the cause of the pregnancy loss may be important in counseling women about their recurrence risk. In low-risk women with an unexplained IUFD, the risk of recurrence is 7.8 to 10.5 of 1000 live births, but this increases to 21.8 per 1000 live births in women with a history of fetal growth restriction. Identification of a heritable genetic mutation in a fetus may prompt testing in the parents; if a heritable mutation is identified, parents may pursue preimplantation genetic diagnosis in future pregnancies.

Genetic Abnormalities in Miscarriage and IUFD
Genetic disorders are generally categorized into 3 main groups: single gene, chromosomal, and multifactorial. Single gene disorders (also known as monogenic disorders) result from errors in a specific gene, whereas those that are chromosomal include larger aberrations that are numerical or structural. Evidence about specific abnormalities in miscarriages and IUFD is somewhat limited. However, it is estimated that 60% of early pregnancy losses are associated with chromosomal abnormalities, particularly trisomies and monosomy X. For later pregnancy losses, aneuploidies are most common in the 8% to 13% of tested IUFD that have an identified chromosomal or genetic abnormality. Karyotypic abnormalities are identified in 6% to 12% of IUFD. Rates of single gene disorders in IUFD are less well-quantified. However, of stillborn fetuses who undergo autopsy, 25% to 35% are identified to have single or multiple malformations or deformations; of these, 25% have an abnormal karyotype, but other single gene disorders are suspected to occur in a high proportion of stillborn fetuses with malformations.

Traditionally, genetic evaluation of the POC after a miscarriage is conducted by karyotyping of metaphase cells after cells are cultured in tissue. Karyotyping can identify whole chromosome aneuploidies and large structural rearrangements. However, only visible rearrangements are likely to be identified using this method (down to a resolution of 5-10 Mb), so smaller genetic variants may not be detected. In addition, karyotype requires culturing of the target cells, which may fail or be infeasible, particularly for formalin-
preserved samples. In addition, there is the potential for maternal cell contamination, which may occur if the
POC tissue is not separated from the maternal decidua before culturing, or if there is poor growth of
noneuploid cells from the POC tissue, thereby allowing maternal cell overgrowth. The potential for maternal
cell contamination makes it impossible to know if a normal female (46 XX) karyotype testing result is due to
a normal fetal karyotype or a maternal karyotype. In 1 study that included 103 first trimester miscarriages,
culture failure occurred in 25% of cases.

CMA Testing
There has been interest in using alternative genetic testing methods, particularly array comparative
genomic hybridization (CGH), to detect chromosomal or other genetic abnormalities in the evaluation of
miscarriages and IUFD.

Types of CMA Technologies
Several types of microarray technology are in current clinical use, primarily array CGH and single nucleotide
polymorphism (SNP) microarrays. Comparative genomic hybridization CMA analysis detects copy number
variants (CNVs) by comparing a reference genomic sequence with the patient (“unknown”) sequence in
terms of binding to a microarray of cloned (from bacterial artificial chromosomes) or synthesized DNA
fragments with known sequences. The reference DNA and the unknown sample are labelled with different
fluorescent tags, and both samples are cohybridized to the fragments of DNA on the microarray. Computer
analysis is used to detect the array patterns and intensities of the hybridized samples. If the unknown
sample contains a deletion or duplication of genetic material in a region contained on the reference
microarray, the sequence imbalance is detected as a difference in fluorescence intensity.

In SNP-based CMA testing, a microarray of SNPs, which may include hundreds of thousands of SNPs, is
used for hybridization. In contrast with array CGH, a reference genomic sequence is not used. Instead, only
the “unknown” sample is hybridized to the array platform, and the presence or absence of specific known
DNA sequence variants is evaluated by signal intensity to provide information about copy numbers. In some
cases, laboratories confirm CNVs detected on CMA with an alternative technique, such as fluorescence in
situ hybridization (FISH) or flow cytometry.

Microarrays also vary in breadth of coverage of the genome that they include. Targeted CMA analysis
provides coverage of the genome with a concentration of sequences in areas with known, clinically
significant CNVs. In contrast, whole-genome CMA allows the characterization of large numbers of genes,
but with the downside that analysis may identify large numbers of CNVs of undetermined significance.

CMA Compared With Karyotyping
Chromosomal microarray analysis has several advantages over karyotyping, including improved resolution
(detection of smaller chromosomal variants that are undetectable using standard karyotyping) and therefore
can result in potentially higher rates of detection of pathogenic chromosomal abnormalities. Array CGH can
detect CNVs for larger deletions and duplications, including trisomies. However, CMA based on array CGH
cannot detect balanced translocations or diploid, triploid and tetraploid states or sequence inversions
because these are not associated with fluorescence intensity change. SNP-based CMA analysis, in addition
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to detecting deletions and duplications, can detect runs of homozygosity, which suggests consanguinity, triploidy, and uniparental disomy.

Chromosomal microarray analysis also has the advantage of not requiring successful cell culture, so may be more likely to yield a result in cases where karyotyping is technically unsuccessful due to failed culture. In the case of testing of specimens from early miscarriage, CMA may also be used to rule out maternal cell contamination, if a fetal sample is compared with a maternal sample.

Chromosomal microarray analysis has the disadvantage of higher rates of detection of variants of uncertain significance. The American College of Medical Genetics has published guidelines regarding the interpretation and reporting of CNVs in the postnatal setting that recommend that laboratories that perform array-based assessment of CNVs track their experience with CNVs and document pathogenic CNVs, CNVs of uncertain significance, and CNVs that have been determined to represent benign variation based on comparisons with internal and external databases.

Commercially Available Tests
Natera, Inc. (San Carlos, CA) offers the Anora™ miscarriage test, which uses a SNP-based array system for testing of products of conception. The test includes the company’s proprietary “Parental Support Technology,” which uses a DNA sample from 1 or both parents as a reference to the products of conception sample. This comparison can identify maternal cell contamination, uniparental disomy, and the parent of origin of a fetal chromosome abnormality. According to a description of the “Parental Support” algorithm, the algorithm uses the “SNP array data to calculate the relative amounts of each of the 2 alleles at each SNP. At heterozygous loci, disomic chromosomes are expected to have SNP ratios of approximately 50%, trisomic chromosomes are expected to have SNP ratios of approximately 33% and 66%, and monosomic chromosomes are expected to have only homozygous loci. For each chromosome, the algorithm compares the observed SNP data to each of the expected alleles for the possible ploidy states and determines which is most likely.”

According to the manufacturer’s website, the test reports the following abnormalities, including the parent of origin of any anomaly when a parental sample has been submitted:

- Any whole chromosome aneuploidy.
- Triploidy.
- Tetraploidy where 1 parent contributed 1 set of chromosomes and the other parent contributed the other 3. Tetraploidy when parental contribution is equal cannot be detected.
- Uniparental disomy.
- Interstitial deletions and duplications greater than 5 Mb.
- Any terminal deletion or duplication, as it could be an indication for a balanced translocation.
- Deletions of 1 Mb or greater and duplications of 2 Mb or greater are reviewed individually by a genetic counselor/geneticist and reported if the potential cause of a miscarriage or recurrence risk implications are identified.
- Any of the following deletions and duplications, when identified:
  - 1p36 deletion
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- 1q21.1 deletion (epilepsy)
- 2q37 deletion
- 3q29 terminal deletion
- 4p16.3 deletion (Wolf-Hirschhorn syndrome)
- 5p15.2 deletion (Cri du Chat)
- 7q11.23 deletion (Williams syndrome)
- 8q23.2-8q24.1 deletion (Langer-Giedion)
- 9q34 deletion
- 11p13-14 deletion (WAGR)
- 11q24.1 deletion (Jacobsen syndrome)
- 10p13-p14 deletion (DiGeorge 2)
- 15q11-q13 deletion (Prader-Willi/Angelman region)
- 16p11.2 deletion (epilepsy)
- 17p11.2 deletion (Smith-Magenis)
- 17p13.3 deletion (Miller-Dieker)
- 17q21.31 deletion
- 22q13 deletion (Phelan-McDermid syndrome)
- 22q11.2 deletion (DiGeorge/VCFS)
- 22q11.2 duplication
- Xq28 deletion (MECP2 deletion)
- Xq28 duplication (MECP2 duplication)

CombiMatrix (Irvine, CA) offers the CombiSNP™ Array for Pregnancy Loss, which is used for testing fresh tissue samples, formalin-fixed, paraffin-embedded tissue samples or unstained slides. According to the manufacturer’s website, the CombiSNP Array is a high resolution SNP microarray that can detect triploidy, numeric chromosome abnormalities, unbalanced structural rearrangements, microdeletion/ duplication syndromes, long stretches of homozygosity, which can indicate shared ancestry or uniparental disomy and maternal cell contamination. The company also offers maternal cell contamination studies.

GeneDx offers the Whole Genome Chromosomal Microarray for Products of Conception test, which is a SNP- and CGH-array that has whole-genome CGH-array coverage with oligonucleotide probes for the detection of CNVs and SNP probes to detect runs of homozygosity, which may indicate uniparental disomy.

Multiple laboratories offer CMA testing for prenatal samples that is not specifically designed for testing of products of conception.

FDA or Other Governmental Regulatory Approval
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). The Anora miscarriage test, the CombiSNP Array for Pregnancy Loss, the CombiBAC™ Array, and the GeneDx Whole Genome Chromosomal Microarray for Products of Conception,
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along with other chromosomal microarray analysis testing platforms currently available are LDTs available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. FDA has chosen not to require any regulatory review of this test.

Rationale/Source
The evaluation of the evidence related to the use of CMA testing of POC for the evaluation of miscarriage or IUFD can be structured around several related questions.

First, what are the test performance characteristics of CMA testing in the testing of POC, either as an alternative or in addition to standard karyotyping? Most genetic tests are evaluated based on their analytic validity (ie, technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent) and their clinical validity (ie, diagnostic performance of the test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease). Although the clinical validity of most diagnostic genetic tests are evaluated based on their ability to diagnose clinically defined disease, for the purposes of assessment of POC, the diagnosis of a known chromosomal or genetic abnormality in the setting of pregnancy loss may serve as a surrogate end point. The results of CMA can be compared directly with karyotyping, but there is not an independent reference standard that can be used to determine the performance characteristics of each test.

The clinical utility of CMA testing in miscarriage or IUFD is determined by whether results from CMA testing affect patient management and are associated with improved patient outcomes.

Analytic Validity
No studies were identified in the peer-reviewed literature that addressed the technical performance of CMA testing for POC. In general, CMA analysis has a high analytic validity for detecting CNVs, in most instances greater than 95%.

Clinical Validity
Diagnostic Accuracy of CMA
In 2014, Dhillon et al reported results from a systematic review and meta-analysis of studies that compared CMA analysis with conventional karyotyping in the evaluation of miscarriage. The authors included 9 studies that reported results from CMA on POC following miscarriage alongside conventional karyotyping and that met their inclusion criteria. There were a total of 314 miscarriage samples in the included studies. In pooled analysis, the overall agreement between karyotype and CMA results was 86.0% (95% confidence interval [CI], 77.0% to 96.0%), with high homogeneity across the studies (Cochrane Q, \( I^2 = 0.2\% \)). CMA detected 13% (95% CI, 8.0% to 21.0%) additional chromosomal abnormalities that were not detected by karyotyping (including both likely pathogenic variants and variants of uncertain significance [VOUS]). Conventional karyotyping detected 3% (95% CI, 1.0% to 10.0%) additional abnormalities that were not detected by CMA. Among 5 studies that reported VOUS, the pooled chance of having a VOUS was 2% (95% CI, 1.0% to 10.0%). This systematic review demonstrated good overall agreement between CMA and karyotype in the analysis of miscarriage specimens. However, the CI around the estimate of VOUS rate is
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large, indicating uncertainty regarding the true rate. Further research is required to determine whether CNVs found in POC are pathogenic or benign.

A number of additional studies not included in the Dhillon et al systematic review have reported results of CMA to karyotype results.

In 2014, Lathi et al reported results from a comparison of a SNP-based array with informatics assistance (“Parental Support” algorithm previously described) with conventional karyotyping in 30 first-trimester miscarriage samples. CMA was conducted using a SNP-based microarray which measures about 300,000 SNPs across the genome (≈1 every 10 Kb). The “Parental Support” technique compares results from the POC sample with parental samples to determine the number and origin of each of the chromosomes in the POC sample. On conventional karyotype, 63% of samples were chromosomally abnormal, with autosomal trisomies as the most common abnormality. All 46 XX samples on karyotype were confirmed to be from fetal tissue on microarray analysis. Four samples were discordant between CMA and karyotype, including 1 case of whole genome duplication and 1 balanced translocation, both of which would not be expected to be detected on microarray, and 2 additional discrepancies that were attributed to sampling error, tissue mosaicism, or culture artifact.

In 2009, Menten et al reported results of an evaluation of 100 pregnancy losses with conventional karyotyping, flow cytometry, and array CGH. Array CGH was performed using an investigator-developed bacterial artificial chromosome microarray with ≈1 Mb resolution. On conventional karyotyping, normal karyotypes were found in 11 male and 44 female cases. In 28 cases, karyotyping was not possible due to culture failure. Chromosomal abnormalities were found in 17 cases (9 autosomal trisomies, 2 cases of monosomy X, 3 triploidy cases, and 1 balanced and 1 unbalanced translocation). On array CGH, 23 abnormal results were found: 15 autosomal trisomies, 5 cases of monosomy X, and 3 structural abnormalities. Ten of the abnormalities on array CGH were not detected with conventional karyotype. In 1 case, balanced translocation was not detected on array CGH. In 2 additional cases, a triploidy was suspected due to aberrant ratios for the sex-chromosomes. Due to poor DNA quality, no result could be obtained for 2 samples.

In 2006, Hu et al conducted genetic analysis by both CGH and karyotyping in 38 POC from early pregnancy losses. Culture of chorionic villi and examination of metaphase chromosomes was attempted in all samples, but cytogenetic analysis was technically successful in only 31 samples. Of the 31 samples successfully karyotyped, 14 were diagnosed to be aneuploidies including 4 trisomies 21, 2 trisomies 13 and 16, 2 monosomies X, and 1 trisomy 7, 20, 18, 3, along with 2 triploidies. On CGH analysis, 17 aneuploidies were identified (14 of those found on the karyotyped samples, along with 3 cases in samples for which cell culture failed), along with 1 structural chromosomal abnormality. For the 31 samples that had both tests conducted, there was generally good concordance between the 2 approaches, with the exception that CGH did not detect the 2 cases of triploidy.
Yield of CMA in Pregnancy Loss

CMA in Early Pregnancy Loss

Several studies have evaluated the use of CMA analysis in the evaluation of early pregnancy loss when standard karyotyping is unsuccessful, or have evaluated the incremental benefit of CMA analysis in the detection of maternal cell contamination.

Viaggi et al. used a whole genome array CGH to evaluate 40 products of conception samples from first trimester miscarriages that had normal karyotypes to assess the presence and prevalence of CNVs. Frozen samples were evaluated with array CGH with a resolution of 100 Kb. Copy number variants were compared with those present in the Database of Genomic Variants (available at: http://projects.tcag.ca/variation), Decipher (http://decipher.sanger.ac.uk) and the Database of Human CNVs (http://gvariante.homelinux.net/gvariante/index.php) to differentiate between benign CNVs and CNVs that are likely to be pathogenic. Forty-five CNVs, corresponding to 22 different CNVs, were identified in 31 samples (31/40 [77.5%]). Thirty-one of the 45 CNVs identified (68%) were defined as common CNVs. When the CNVs were compared with control CNVs reported in the Database of Genomic Variants, 7 CNV frequencies were considered statistically different than the control population.

Benkhalifa et al. evaluated 26 samples from first-trimester miscarriages that failed to divide in routine cytogenetic studies with array used CMA methods with array CGH. The array CGH method used involved human genomic microarrays containing 2600 cloned areas spanning chromosome subtelomeric regions and critical areas spaced about 1 Mb along each chromosome. Of the 26 samples that failed to divide in routine cytogenetics, 15 had an abnormal genetic profile on array CGH. Abnormalities that are highly prevalent on routine karyotyping (trisomy 16, monosomy X, and triploidy, which are estimated to account for more than 55% of cytogenetically abnormal findings in routine karyotyping) were relatively uncommon among the 15 abnormal samples, with instance of monosomy 16 and 2 instances of monosomy X.

Doria et al. evaluated array CGH as part of a sequential protocol in the genetic evaluation of 232 spontaneous miscarriages or fetal deaths, 186 of which were from the first trimester, 24 from the second trimester, and 22 from the third trimester. Tissue culture and karyotype was attempted on all specimens; samples that were unable to be karyotyped were tested with array CGH, followed by additional confirmation with FISH confirmation. Culture failure occurred in 25.4% of the cases. Of the 173 (74.6%) with valid karyotypes, 66 of 173 (38.2%) were abnormal: 62 of 66 with numerical abnormalities (single, double, or triple trisomies, monosomy X, polyploidy, or mosaicism), and 5 of 66 with structural abnormalities. Array CGH was performed in 58 of 59 cases with culture failure (1 case with insufficient DNA for CGH). Fifteen of the 58 cases were abnormal, with 3 cases of monosomy X, 1 case of XY with gain for X, 7 cases of trisomy 15, 2 cases of trisomy 16, and 1 case each of trisomy 18 and 21. With the addition of FISH testing, 4 new cases of triploidy were detected. This study suggests that the use of array CGH increases the yield of testing of genetic testing of products of conception beyond that of standard karyotyping.

Barrett et al. evaluated array CGH-based CMA in 368 specimens from first- and second- trimester spontaneous abortions, of which gestational age and degree of tissue maceration was available for 276. Genetic abnormalities were detected in 206 cases, with complete or partial aneuploidy involving trisomy in
85.5%, monosomy X in 9.2%, and structural rearrangements in 5.3%. Samples were also analyzed with traditional cytogenetics, but direct comparisons between CGH and cytogenetics are not reported.

In 2014, Lathi et al reported results of a retrospective analysis of the use of CMA in detecting maternal cell contamination on conventional karyotyping in 1222 products of conception samples from first trimester miscarriages that were evaluated at the Natera laboratory from January 2010 to August 2011. The products of conception samples, along with maternal peripheral blood samples, were evaluated with a SNP-based CMA. When CMA results for the products of conception were 46 XX, a comparison with the maternal genotype fingerprint allowed investigators to determine if results were due to maternal cell contamination. On initial analysis, before comparison with the maternal genotype fingerprint, 48% of products of conception specimens were chromosomally abnormal, 37% were 46 XX, and 14% were 46 XY. Comparison with maternal bloody genotype indicated that 59% of the 46 XX results were due to maternal cell contamination. The authors suggest that the use of CMA analysis may improve the accuracy of the detection of fetal chromosomal abnormalities.

A number of studies have reported outcomes from CMA analysis of products of conception in various patient populations where karyotyping was not performed.

In the largest such study identified, Levy et al reported results of SNP microarray analysis of 2447 consecutively received POC samples, of which 2400 were fresh samples. Of the fresh samples, 2392 (99.7%) were 20 weeks of gestation or less, and 1861 (77.6%) had no or negligible maternal cell contamination. The authors used a 10-Mb cutoff to estimate the threshold of detection for routine karyotyping in POC samples. At the resolution of conventional karyotyping, 1106 (59.4%) showed classical cytogenetic abnormalities. Of the remaining 755 samples considered normal at the karyotype level, 33 (4.4%) had a CNV (microdeletion or microduplication); 12 (36.4%) were considered clinically significant and the remaining were considered VOUS.

In 2015, Maslow et al evaluated the yield of SNP-based array for determining chromosome number in paraffin-fixed POC compared with a standard evaluation for couples with recurrent first-trimester pregnancy losses. Eligible patients previously had analysis of chromosome number and screening tests recommended by the American Society for Reproductive Medicine (ASRM) for recurrent pregnancy loss, including parental karyotypes, maternal serum testing for antiphospholipid antibodies, thyrotropin, and prolactin, and a uterine cavity evaluation via sonohysterogram or hysterosalpingogram. Forty-two women with a total of 178 first-trimester losses were included, with 62 paraffin-embedded POC samples available. SNP-based microarray was able to determine a fetal chromosome number in 44 of 62 (71%) of samples, 25 (57%) of which were noneuploid. Recurrent pregnancy loss screening was normal in 35 of 42 (83%) participants. The detection rate for any cause of pregnancy loss was significantly higher with SNP microarray (0.50; 95% CI, 0.36 to 0.64) than with the ASRM-recommended recurrent pregnancy loss evaluation (0.17; 95% CI, 0.08 to 0.31, \(p=0.002\)).

Also in 2015, Romero et al reported on types of genetic abnormalities found on CMA in early pregnancy losses (<20 weeks of gestation) among 86 women. Thirteen (14.9%) of POC samples were excluded because placental villi or fetal tissue could not be identified with certainty and 9 were excluded due to
complete maternal cell contamination, leaving a sample of 64 for analysis. The overall prevalence of aneuploidy and pathogenic CNV or VOUS was 43.8% (28/64). Excluding the 2 cases with VOUS, rates of pathogenic CNV or aneuploidy differed by gestational age: 9.1%, 69.2%, and 28.0% of pre-embryonic, embryonic, and fetal samples, respectively \((p<0.01)\). Aneuploidy was the most common abnormality, occurring in 37.5% (24/64) cases.

In 2014, Mathur et al reported results from CMA testing in preserved POC samples from 58 women with 77 miscarriage specimens who were evaluated at a single recurrent pregnancy loss clinic. All women had a history of recurrent pregnancy loss, defined as 2 or more ultrasound-documented miscarriages at less than 10 weeks’ gestation. Samples were evaluated with CGH; if results were 46 XX, the genotype of the products of conception was compared with the maternal genotype at several highly polymorphic loci through microsatellite analysis (MSA) to determine if the 46 XX results were consistent with maternal cell contamination. Sixteen samples (21%) yielded uninformative results due to minimal pregnancy tissue \((n=9)\), poor quality DNA \((n=2)\), or confirmed maternal cell contamination \((n=2)\). CGH was considered informative in 66 cases (79%), with 22 noneuploid and 39 euploid. Thirty-three of the euploid specimens were 46 XX, 11 of which were not sent for reflex MSA. The author concluded that CMA testing of preserved products of conception is technically feasible, including in cases where karyotyping had failed due to cell growth failure, which had occurred in 8 samples evaluated.

Warren et al conducted a prospective case series to evaluate results from array CGH in products of conception from 35 women who had pregnancy loss between 10 and 20 weeks of gestation with either normal karyotype \((n=9)\) or no conventional cytogenetic testing \((N=26)\). Thirty-five samples were from fresh tissue obtained at the time of pregnancy loss when dilatation and curettage was performed; the remainder were from paraffin-embedded tissue. Samples were assessed with a whole genome bacterial artificial chromosome array chip. Clones that demonstrated copy number changes in the fetal tissue were compared against known copy number change regions in the Database of Genomic Variants (available at: http://projects.tcag.ca/variation), and the internal database of apparently benign copy number changes maintained by the University of Utah CGH laboratory. When CNVs were detected, parental samples were assessed with the same array chip, and CNVs present in fetal tissue but not parental DNA were defined as de novo CNVs. Samples with de novo CNVs on the bacterial artificial chromosome chip were further analyzed with an oligonucleotide microarray chip with an average resolution of 6.4 Kb for more accurate characterization. Deoxyribonucleic acid was successfully isolated in 30 cases (all from the fresh tissue samples). De novo CNVs were detected in 6 of the 30 (20%) cases using the bacterial artificial chromosome array and confirmed in 4 of 30 (13%) cases using the oligonucleotide array.

In 2007, Azmanov et al evaluated samples from 106 first- \((n=3)\) and second-trimester \((n=23)\) miscarriages with array-CGH-based CMA. Although the specific weeks of gestational age are not reported, most samples were from early miscarriages, including 8 blighted ova and 75 missed abortions, with 23 second-trimester spontaneous abortions. In the entire sample, 40 of 106 (37.7%) demonstrated chromosomal abnormalities, with 82.5% numerical abnormalities (47.5% aneuploidy, 25.0% monosomy X, 10.0% hyperdiploidy) and 17.6% structural aberrations.
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CMA in IUFD
Relatively few studies have reported the yield of CMA testing for IUFD, either in addition to or as an alternative to standard karyotyping.

In the largest study identified, Reddy et al compared CMA with karyotype in the evaluation of 532 cases of IUFD. Of the karyotypes attempted, 375 (70.5%) yielded a result. Of those, 31 of 375 (8.3%) were classified as abnormal, with trisomy 21 (n=9), trisomy 18 (n=8), trisomy 13 (n=2), and monosomy X (n=5) representing the most common abnormalities. CMA yielded results in 465 (87.4%) of samples, significantly more than were successful karyotyped (p<0.001). Of those, 32 (6.9%) were aneuploidy, 12 (2.6%) were considered a pathogenic variant, and 25 (5.4%) were considered a VOUS. Nine pathogenic variants on CMA were detected in stillbirths with normal karyotypes. Chromosomal microarray analysis detected aneuploidy in 7 cases of the 157 in which karyotyping was unsuccessful.

Sahlin et al evaluated CMA in a sample of 90 IUFD cases (after 22 weeks of gestation) with no known genetic diagnosis based on karyotype and quantitative fluorescence PCR. CMA yielded results in all cases, 77% of which yielded only benign or likely benign CNVs. Three variants were detected in genes known to be associated with IUFD or other disorders. Twenty-six VOUS were identified in 21 cases of IUFD.

Harris et al reported rates of structural abnormalities detected with array CGH-based CMA in IUFD after 22 weeks of gestation. From a cohort of 54 stillbirths, 29 were prospectively determined to be "unexplained" and determined to have a normal conventional karyotype. Of those, 24 novel CNVs were detected.

Raca et al evaluated the yield of CMA testing in a sample of stillborn fetuses from the Wisconsin Stillbirth Service Program, a statewide repository of data on IUFD cases, which includes tissue samples for 573 cases from 1994 to 2002. The authors identified 26 cases with tissue or cell samples available, in which the cause of death was thought to have been fetal, the fetal phenotype suggested that a chromosomal imbalance might be present because of the presence of multiple congenital anomalies (at least 2 abnormalities of 2 different organs or parts of the body), and cytogenetic results were either normal or were not obtained due to culture failure. In 15 cases with good-quality DNA available for analysis, array CGH detected 2 abnormalities (trisomy 21 and an unbalanced translocation between chromosomes 3 and 10).

Section Summary
The evidence related to the clinical validity of CMA comes primarily from studies that compared genetic testing results from CMA with conventional karyotype, and from several studies that evaluated the yield of CMA in patients with a normal or unsuccessful karyotype. These studies suggest that CMA has good concordance with karyotype for detection of aneuploidy and is more likely to yield results than conventional karyotyping given the need for cell culture for karyotyping. Studies on the yield of testing in early pregnancy losses suggests that aneuploidies are the most common abnormality detected, CMA may detect abnormalities not detected on karyotype. Relatively few studies have reported CMA outcomes in late pregnancy losses, but they suggest that CMA is more likely to yield a result than conventional karyotyping.
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Clinical Utility
Changes in Patient Management Following CMA Testing

Changes in management that could result from CMA testing include changes in additional testing to evaluate for causes of a pregnancy loss or changes in the management of future pregnancies, such as the decision to undertake preimplantation genetic testing. No empirical studies identified evaluated changes in management that occurred as a result of CMA testing in miscarriage or IUFD.

One argument for genetic evaluation (karyotype or CMA) in POC in cases of recurrent pregnancy loss is that an abnormal genetic evaluation would potentially forestall an evaluation for other causes of recurrent pregnancy loss, which might include assessment of the uterine cavity, thyroid function testing, and testing for antiphospholipid antibodies. In the study by Maslow et al (described above), the yield of testing using a SNP microarray in recurrent pregnancy loss was higher than the yield of other recommended testing (some of which are potentially invasive). Bernardi et al developed a decision analytic model to compare the cost of 2 strategies for recurrent pregnancy loss evaluation: (1) selective recurrent pregnancy loss evaluation, defined as an evaluation if the second miscarriage is euploid; or (2) universal recurrent pregnancy loss evaluation, defined as recurrent pregnancy loss evaluation after the second miscarriage of less than 10 weeks of size. Genetic analysis in the study's decision model in the "selected" recurrent pregnancy loss evaluation was stepwise, beginning with cytogenetic analysis. If the cytogenetic results were abnormal, no further evaluation would be needed. If the results were consistent with an unbalanced translocation, cytogenetic analysis of the parents would be indicated. If results on cytogenetics were consistent with 46 XX, microsatellite analysis would be indicated to evaluate for maternal cell contamination. If the 46 XX result was of maternal origin, CGH of stored miscarriage tissue would be indicated. Similarly, if there was no result from cytogenetic analysis, CGH of stored miscarriage tissue would be indicated. If results on CGH were consistent with unbalanced translocation, cytogenetic analysis of the parents would be indicated; if results were consistent with normal 46 XY on either karyotype or CGH or confirmed fetal normal 46 XX on karyotype or CGH, or an unbalanced translocation, further workup for recurrent pregnancy loss would be indicated.

Although this decision analysis suggests a way in which CMA of POC could be used in an algorithm to determine testing for recurrent pregnancy loss, it does not demonstrate that use of CMA improves outcomes. Further research evaluating the implementation of such a decision tool in practice is needed.

Improvement in Patient Outcomes Following CMA Testing

There are several potential health-related outcomes resulting from CMA testing products of conception in pregnancy loss. Knowledge of the cause of the loss may lead to reduced parent distress or anxiety. For couples with recurrent pregnancy loss, preimplantation genetic diagnosis with transfer of unaffected embryos or the use of donor gametes might be considered for therapy. No studies were identified that reported whether the use of CMA is associated with changes in parental mental health outcomes.

No studies were identified that addressed whether CMA of POC is associated with changes in management or future successful pregnancies.
Section Summary: Clinical Utility
Although there are several ways in which CMA of fetal tissue in pregnancy losses has potential for clinical utility, including leading to changes in diagnostic testing, reduced parental distress, or preimplantation genetic diagnosis, no studies identified directly demonstrated changes in outcomes.

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in September 2015 did not identify any ongoing or unpublished trials that would likely influence this review.

Summary of Evidence
The evidence for the use of CMA testing of fetal tissue in individuals who have pregnancy loss with indications for genetic analysis of the embryo/fetus includes prospective and retrospective cohort studies that report on the yield of CMA testing, sometimes compared with standard karyotyping. Relevant outcomes are test accuracy and validity, other test performance measures, changes in reproductive decision making, morbid events, and quality of life. The available evidence suggests that CMA has a high rate of concordance with karyotyping. For both early and late pregnancy loss, CMA is more likely to yield a result than karyotyping. Other studies have reported that CMA detects a substantial number of abnormalities in patients with normal karyotypes, although the precise yield is uncertain and likely varies based on gestational age. Rates of variants of unknown significance in CMA testing of miscarriage samples are not well characterized. Potential benefits from identifying a genetic abnormality in a miscarriage or intrauterine fetal demise include reducing emotional distress for families, altering additional testing that is undertaken to assess for other causes of pregnancy loss, and changing reproductive decision making for future pregnancies. The potential for clinical utility for CMA testing of fetal tissue in pregnancy loss is parallel to that for obtaining a karyotype of fetal tissue in pregnancy loss, which is recommended by a number of organizations. While no studies identified directly demonstrated whether or how patient management is changed based on CMA testing of POC from early or late pregnancy losses, or how patient outcomes are improved, the available evidence suggests that, for situations in which a genetic evaluation is indicated, CMA would be expected to perform as well as or better than standard karyotyping. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

Clinical Input Received From Physician Specialty Societies and Academic Medical Centers
While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

In response to requests, input was received on the policy from 3 academic medical centers, one of which provided 2 responses, and 3 physician specialty societies, one of which provided 3 responses, while this policy was under review in 2015. There was consensus that CMA testing is medically necessary in the evaluation of intrauterine fetal demise. Most reviewers noted that there are specific clinical scenarios in which the yield of CMA testing is likely to be higher, including later term losses and for fetuses with congenital anomalies. However, there was not consensus about specific criteria that should be used to limit
the use of CMA testing. While many reviewers noted that the yield of CMA testing is likely to be higher in later term losses, there was not consensus about a specific gestational age that should be used.

References
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10/02/2014 Medical Policy Committee review
01/01/2015 Coding Update
07/01/2015 Coding Update
08/03/2015 Coding update: ICD10 Diagnosis code section added; ICD9 Procedure code section removed.
10/08/2015 Medical Policy Committee review
10/21/2015 Medical Policy Implementation Committee approval. Policy updated to now include CMA testing for intrauterine fetal demise, title change and new policy statement added.
04/07/2016 Medical Policy Committee review
04/20/2016 Medical Policy Implementation Committee approval. Title change. Coverage statement revised to cover testing for loss at any stage meeting criteria.
01/01/2017 Coding update: Removing ICD-9 Diagnosis Codes and CPT coding update
04/06/2017 Medical Policy Committee review
04/19/2017 Medical Policy Implementation Committee approval. Coverage eligibility unchanged.
Next Scheduled Review Date: 04/2018

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| CPT           | 81228, 81229, 81479, 88271  
               | New codes eff 1/1/17: 81422 |
| HCPCS         | No codes   |
| ICD-10 Diagnosis | N96  
               | O26.20  
               | O26.21  
               | O26.22  
               | O26.23  
               | Z31.441 |

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A. Whether the medical treatment, procedure, drug, device, or biological product can be lawfully marketed without approval of the 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.

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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.

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