Policy Statement

Chromosomal microarray testing of fetal tissue may be considered medically necessary for the evaluation of pregnancy loss in patients with indications for genetic analysis of the embryo or fetus (see Policy Guidelines section).

Policy Guidelines

Clinical guidelines and recommendations to address the management of cases of miscarriage or intrauterine fetal demise (IUFD) where genetic analysis of the embryo, fetus, or stillborn infant is indicated. These guidelines, which specifically address the use of karyotyping and/or microarray testing in miscarriage or intrauterine fetal demise, were developed by several reproductive health associations, including the American Society for Reproductive Medicine (ASRM [2013]; ASRM [2012]), the National Society of Genetic Counselors (Laurino et al [2005]), and the American College of Obstetrics and Gynecology (ACOG [2009]). According to such guidelines, genetic testing may be indicated (if desired by parents):

• 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 two or more failed pregnancies)
• In all cases of pregnancy loss after 20 weeks of gestation

The decision to obtain genetic testing should be made jointly by the mother or parents and the treating clinician.

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

Genetics Nomenclature Update

The Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the Human Genome Organization (HUGO), and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—“pathogenic,” "likely pathogenic," "uncertain significance," "likely benign," and “benign”—to describe variants identified that cause Mendelian disorders.

Table PG1. Nomenclature to Report on Variants Found in DNA

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Family variant</td>
<td>Disease-associated variant</td>
<td>Change in the DNA sequence</td>
</tr>
<tr>
<td>Familial variant</td>
<td></td>
<td>Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives</td>
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Chromosomal Microarray Testing for the Evaluation of Pregnancy Loss

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

Genetic Counseling
Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Definitions
Fetal tissue may consist of fetal tissue, a formed fetus, or placental tissue derived from the fetal genotype, depending on the stage of pregnancy at the time of the fetal loss.

Early pregnancy loss or miscarriage is considered to be a pregnancy loss that occurs at or before 20 weeks of gestational age.

Intrauterine fetal demise is defined as delivery of a non-live-born fetus after 20 weeks of gestational age.

Description
Chromosomal microarray (CMA) testing 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. Such testing has typically been carried out through cell culture and karyotyping of cells in metaphase. However, the analysis of fetal or placental tissue has been inhibited by the following limitations: the need for fresh tissue, the potential for cell culture failure, and the potential for maternal cell contamination.

Related Policies
- Carrier Screening for Genetic Diseases
- Invasive Prenatal (Fetal) Diagnostic Testing
- Preimplantation Genetic Testing

Benefit Application
Benefit determinations should be based on all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.
Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

**Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Act for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

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

**Rationale**

**Background**

**Pregnancy Loss: Biology and Evaluation**

**Early Pregnancy Loss**

Pregnancy loss is common, occurring in at least 15% to 25% of recognized pregnancies. Pregnancy loss primarily 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 abnormalities are thought to be the predominant cause: when products of conception are examined, it has been estimated that 60% of early pregnancy losses are associated with chromosomal abnormalities, particularly trisomies and monosomy X.1,2 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 as 2 or more failed pregnancies, is less common, occurring in approximately 5% of women.3 Recurrent pregnancy loss may be related to cytogenetic abnormalities, particularly balanced translocations, uterine abnormalities, thrombophilias, including antiphospholipid syndrome, and metabolic or 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.1 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 evaluate 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 the reassurance there was nothing 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 the 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; American Society for Reproductive Medicine (2012) guidelines on the management of recurrent pregnancy loss have indicated that “treatment options should be based on whether repeated miscarriages are euploid,”
aneuploidy, or due to an unbalanced structural rearrangement and not exclusively on the parental carrier status. Finally, among patients found to have a potential non-genetic underlying cause of recurrent pregnancy loss, such as antiphospholipid syndrome, cytogenetic analysis of pregnancy losses could provide evidence that the miscarriages were not due to treatment failure.

Late Pregnancy Loss

Fetal loss that occurs later in pregnancy, after 20 weeks of gestation, may be referred to as intrauterine fetal demise (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. In many cases, the precise cause of IUFD is unidentifiable; however, it may be related to a range of disorders, including genetic disorders in the fetus, maternal infection, coexisting maternal medical disorders (e.g., diabetes, antiphospholipid antibody syndrome, heritable thrombophilias), and obstetric complications. Chromosomal or genetic abnormalities can be found in 8% to 13% of IUFD-most commonly aneuploidies. In a large 2012 series of IUFD (N=1025), Korteweg et al (2012) reported a cytogenic abnormality rate of 11.9%.

Reasons to evaluate for a cause of IUFD are similar to those 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 variant in a fetus may prompt testing in the parents; if a heritable variant is identified, parents may pursue preimplantation genetic diagnosis in future pregnancies.

Chromosomal Microarray Testing

There is interest in using alternative genetic testing methods, particularly array comparative genomic hybridization, to detect chromosomal or other genetic abnormalities in the evaluation of miscarriages and IUFD.

Literature Review

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Chromosomal Microarray Testing of Fetal Tissue

Clinical Context and Test Purpose

The purpose of chromosomal microarray (CMA) testing in patients who have early spontaneous pregnancy loss or intrauterine fetal demise (IUFD) is to inform decisions regarding risk for subsequent pregnancies and whether to implement relevant clinical evaluation and management.

The question addressed in this evidence review is: Does CMA testing in patients who have early spontaneous pregnancy loss or IUFD result in improvement in the net health outcome? 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.

The following PICO was used to select literature to inform this review.
Patients
The relevant populations of interest are women who have experienced single or recurrent early spontaneous pregnancy loss or an IUFD. Evidence on 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 stillbirth fetuses who undergo an 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 stillbirth fetuses with malformations.

Interventions
The test being considered is CMA testing. Several types of microarray technology are in current clinical use, primarily array comparative genomic hybridization (aCGH) and single nucleotide variant (SNV) microarrays. CGH CMA testing 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. In SNV-based CMA testing, a microarray of SNVs, which may include hundreds of thousands of SNVs, is used for hybridization. In contrast with aCGH, a reference genomic sequence is not used. Instead, only the “unknown” sample is hybridized to the array platform, and the presence or absence of specifically 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 or flow cytometry.

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

CMA testing would be performed in any of the trimesters of pregnancy when there is an indication for genetic evaluation of a spontaneous pregnancy loss or IUFD. CMA testing would be provided in an obstetrics or perinatal care setting. Genetic counseling may also be provided.

Comparators
The following tools are currently being used to make decisions about the presence of genetic abnormalities as the cause of early pregnancy loss or IUFD. Traditionally, genetic evaluation of the products of conception (POC) after a miscarriage is conducted by karyotyping of metaphase cells after the 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 megabases [Mb]), so smaller genetic variants may not be detected. In addition, karyotyping requires culturing the target cells, which may fail or be infeasible, particularly for formalin-preserved samples. Further still, 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 a 2009 study that included 103 first-trimester miscarriages, Robberecht et al (2009) reported a culture failure rate in 25% of cases. The results of CMA testing can be compared directly with karyotyping, but there is no independent reference standard that can be used to determine the performance characteristics of each test.
Outcomes
The general outcomes of interest are test accuracy and validity, other test performance measures, changes in reproductive decision making, morbid events, and quality of life.

CMA testing 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 detection rates of detection of pathogenic chromosomal abnormalities. Array CGH can detect CNVs for larger deletions and duplications, including trisomies. However, CMA based on aCGH cannot detect balanced translocations or diploid, triploid, and tetraploid states, or sequence inversions because they are not associated with fluorescence intensity change. SNV-based CMA, in addition to detecting deletions and duplications, can detect runs of homozygosity, which suggests consanguinity, triploidy, and uniparental disomy.

Another advantage of CMA is that it does not require successful cell culture, so it may be more likely to yield a result in cases where karyotyping is technically unsuccessful due to failed culture. In the case of testing 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.

One distinct disadvantage of CMA is its higher rates of detection of variants of uncertain significance (VUS). The American College of Medical Genetics (2011) published guidelines on the interpretation and reporting of CNVs in the postnatal setting. The American College of Medical Genetics recommended that laboratories performing an array-based assessment of CNVs track their experience with CNVs and document pathogenic CNVs, CNVs of uncertain significance, and CNVs determined to represent benign variations based on comparisons with internal and external databases.

Study Selection Criteria
For the evaluation of clinical validity of CMA testing, studies that meet the following eligibility criteria were considered:
- Patient/sample clinical characteristics were described and
- Patient/sample selection criteria were described.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Systematic Reviews
Dhillon et al (2014) reported on the results of a systematic review and meta-analysis of studies that compared CMA testing with conventional karyotyping in the evaluation of miscarriage. Reviewers included 9 studies that reported results from CMA on POC following miscarriage alongside conventional karyotyping. There were 314 miscarriage samples in the included studies. In the 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, I²=0.2%). CMA detected 13% (95% CI, 8.0% to 21.0%) additional chromosomal abnormalities not detected by karyotyping (including both likely pathogenic variants and VUS). Conventional karyotyping detected 3% (95% CI, 1.0% to 10.0%) additional abnormalities not detected by CMA. Among 5 studies that reported VUS, the pooled chance of having a VUS was 2% (95% CI, 1.0% to 10.0%). This systematic review demonstrated good overall agreement.
between CMA and karyotype testing in the analysis of miscarriage specimens. However, the confidence interval around the estimate of the VUS rate was large, indicating uncertainty in the true rate. Further research is required to determine whether CNVs found in POC are pathogenic or benign.

Retrospective Studies
A number of additional studies not included in the Dhillon systematic review have compared CMA with karyotyping. For example, CMA testing was conducted using an SNV-based microarray, which measures about 300,000 SNVs across the genome (≈1 every 10 kilobase pairs). A "Parental Support" technique was used to compare results from the POC sample with parental samples to determine the number and origin of each chromosome 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 karyotyping were confirmed to be from fetal tissue on microarray analysis. Four samples were discordant between CMA and karyotype, including a case of whole-genome duplication and a balanced translocation, both of which would not be expected to be detected on the microarray, and 2 additional discrepancies were attributed to sampling error, tissue mosaicism, or culture artifact.

Menten et al (2009) reported on the results of an evaluation of 100 pregnancy losses with conventional karyotyping, flow cytometry, and aCGH. Array CGH was performed using an investigator-developed bacterial artificial CMA at a resolution of approximately 1 Mb. 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, 1 balanced and 1 unbalanced translocation). On aCGH, 23 abnormal results were found: 15 autosomal trisomies, 5 cases of monosomy X, and 3 structural abnormalities. Ten of the abnormalities on aCGH were not detected with conventional karyotyping. In 1 case, balanced translocation was not detected on aCGH. 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.

Hu et al (2006) conducted a genetic analysis by both CGH and karyotyping in 38 POC from early pregnancy losses. The culture of chorionic villi and examination of metaphase chromosomes were attempted in all samples, but the cytogenic analysis was technically successful in only 31 samples. Of the 31 samples successfully karyotyped, 14 were diagnosed to be aneuploidies, including 4 with trisomy 21, 2 each with trisomies 13 and 16, 2 with monosomy X, and 1 each with trisomies 3, 7, 18, and 20. An additional 2 cases of triploidy were detected. 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 approaches, with the exception that CGH did not detect the 2 cases of triploidy.

Yield of CMA Testing in Pregnancy Loss
Early Pregnancy Loss
Several studies have assessed the use of CMA in the evaluation of early pregnancy loss when standard karyotyping was unsuccessful, or have evaluated the incremental benefit of CMA testing in the detection of maternal cell contamination.

Viaggi et al (2013) used a whole-genome aCGH to evaluate 40 POC samples from first-trimester miscarriages that had normal karyotypes to assess for the presence and prevalence of CNVs. Frozen samples were evaluated with aCGH at a resolution of 100 kilobases. CNVs were compared with those present in the Database of Genomic Variants, Decipher, and the Database of Human CNVs to differentiate between benign CNVs and possibly pathogenic CNVs. Forty-five CNVs, corresponding to 22 different CNVs, were identified in 31 samples (31/40 [77.5%]). Thirty-one (68%) of the 45 CNVs identified were defined as common CNVs.
CNVs were compared with control CNVs reported in the Database of Genomic Variants. 7 CNV frequencies were considered statistically different from the control population.

Benkhalifa et al (2005) evaluated 26 samples from first-trimester miscarriages that failed to divide in routine cytogenetic studies with array an used that CMA methods with aCGH. The aCGH 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 aCGH. Abnormalities that are highly prevalent on routine karyotyping (trisomy 16, monosomy X, triploidy, which are estimated to account for >55% of cytogenetically abnormal findings in routine karyotyping) were relatively uncommon among the 15 abnormal samples, with an instance of monosomy 16 and 2 instances of monosomy X.

Doria et al (2009) evaluated aCGH 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 karyotyping were attempted on all specimens; samples that could not be karyotyped were tested with aCGH, followed by additional confirmation with fluorescence in situ hybridization. Culture failure occurred in 25.4% of the cases. Of the 173 (74.6%) with valid karyotypes, 66 (38.2%) of 173 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 had 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 trisomies 18 and 21. With the addition of fluorescence in situ hybridization testing, 4 new cases of triploidy were detected. This study suggested that the use of aCGH increases the yield of testing of genetic testing of POC beyond that of standard karyotyping.

Barrett et al (2001) evaluated aCGH-based CMA testing in 368 specimens from first- and second-trimester spontaneous abortions, of which gestational age and degree of tissue maceration were 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 were not reported.

Lathi et al (2014) reported on the results of a retrospective analysis of CMA testing to detect maternal cell contamination of conventional karyotyping in 1222 POC samples from first-trimester miscarriages evaluated at a Natera laboratory from January 2010 to August 2011. The POC samples, along with maternal peripheral blood samples, were evaluated with a SNV-based CMA. When CMA results for the POC were 46 XX, a comparison with the maternal genotype fingerprint allowed investigators to determine whether results were due to maternal cell contamination. On initial analysis, before comparison with the maternal genotype fingerprint, 48% of POC 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 suggested that the use of CMA testing might improve accurate detection of fetal chromosomal abnormalities.

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

In the largest such study identified, Levy et al (2014) reported on the results of SNV 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 a 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 VUS.

Maslow et al (2015) evaluated the yield of the SNV-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 had been previously analyzed for chromosome number and screening tests recommended by the American Society for Reproductive Medicine 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. SNV-based microarray testing determined a fetal chromosome number in 44 (71%) of 62 of samples, 25 (57%) of which were noneuploid. Recurrent pregnancy loss screening was normal in 35 (83%) of 42 participants. The detection rate for any cause of pregnancy loss was significantly higher with SNV microarray (0.50; 95% CI, 0.36 to 0.64) than with the American Society for Reproductive Medicine-recommended recurrent pregnancy loss evaluation (0.17; 95% CI, 0.08 to 0.31, p=0.002).

Romero et al (2015) reported on types of genetic abnormalities found on CMA testing 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 VUS was 43.8% (28/64). Excluding the 2 cases with VUS, 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.

Mathur et al (2014) reported on 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 of gestation. Samples were evaluated with CGH; if results were 46 XX, the genotype of the POC was compared with the maternal genotype at several highly polymorphic loci through microsatellite analysis to determine whether the 46 XX results were consistent with maternal cell contamination. Sixteen (21%) samples 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 61 (79%) cases, with 22 noneuploid and 39 euploid. Thirty-three of the euploid specimens were 46 XX, 11 of which were not sent for reflex microsatellite analysis. The authors concluded that CMA testing of preserved POC is technically feasible, including cases where karyotyping has failed due to cell growth failure, which had occurred in 8 samples evaluated.

Warren et al (2009) conducted a prospective case series to evaluate results from aCGH in POC 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 was 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 with known copy number change regions in the Database of Genomic Variants 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 kilobases for more accurate characterization. DNA was successfully isolated in 30 cases (all from the fresh tissue samples). De novo CNVs were detected...
Azmanov et al (2007) evaluated samples from 106 first- (n=83) and second-trimester (n=23) miscarriages with aCGH-based CMA testing. Although the specific weeks of gestational age were 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 (37.7%) of 106 demonstrated chromosomal abnormalities, with 82.5% numerical abnormalities (47.5% aneuploidy, 25.0% monosomy X, 10.0% hyperdiploidy) and 17.6% structural aberrations.

Intrauterine Fetal Demise
Relatively few studies have reported on the yield of CMA testing for IUFD, either in addition to or as an alternative to standard karyotyping. Sahlin et al (2014) evaluated CMA testing in a sample of 90 IUFD cases (after 22 weeks of gestation) with no known genetic diagnosis based on karyotype and quantitative fluorescence polymerase chain reaction. CMA testing yielded results in all cases, 77% of which were benign or likely benign CNVs. Three variants were detected in genes known to be associated with IUFD or other disorders. Twenty-six VUS were identified in 21 cases of IUFD.

In the largest study identified, Reddy et al (2012) compared CMA testing with karyotyping in the evaluation of 532 cases of IUFD. Of the karyotypes attempted, 375 (70.5%) yielded a result. Of those, 31 (8.3%) of 375 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 testing yielded results in 465 (87.4%) of samples, significantly more than were successfully 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 VUS. Nine pathogenic variants on CMA testing were detected in stillbirths with normal karyotypes. CMA testing detected aneuploidy in 7 cases of the 157 in which karyotyping was unsuccessful.

Harris et al (2011) reported on rates of structural abnormalities detected with aCGH-based CMA testing in IUFD after 22 weeks of gestation. From a cohort of 54 stillbirths, 29 were prospectively determined to be "unexplained" or to have a normal conventional karyotype. Of those, 24 novel CNVs were detected.

Raca et al (2009) evaluated the yield of CMA testing in a sample of stillborn fetuses from a statewide repository of data on IUFD cases, which included tissue samples for 573 cases from 1994 to 2002. The authors identified 26 cases with tissue or cell samples available that met the following criteria: (1) the cause of death was thought to have been fetal; (2) 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 (3) cytogenetic results were either normal or were not obtained due to culture failure. In 15 cases with good-quality DNA available for analysis, aCGH detected 2 abnormalities (trisomy 21, an unbalanced translocation between chromosomes 3 and 10).

Section Summary: Clinically Valid
The evidence on the clinical validity of CMA testing comes primarily from studies that have compared genetic testing results from CMA with conventional karyotype, and from several studies that have evaluated the yield of CMA in patients with a normal or unsuccessful karyotype. These studies have suggested 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 testing yield in early pregnancy losses have suggested 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 do suggest that CMA testing is more likely to yield a result than conventional karyotyping.
Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

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.

In addition, no studies identified addressed whether CMA testing of POC is associated with changes in management or future successful pregnancies.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Changes in Patient Management Following CMA Testing
One argument for genetic evaluation (karyotype or CMA) in POC in cases of recurrent pregnancy loss is that an abnormal genetic evaluation could 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. As described above in Maslow et al (2015), the testing yield using an SNV microarray in recurrent pregnancy loss was higher than the yield of other recommended testing (some of which are potentially invasive). Bernardi et al (2012) 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 fewer 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 testing 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 the cytogenetic analysis, CGH of stored miscarriage tissue would be indicated. If results on CGH were consistent with an 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 would suggest a way in which CMA testing of POC could be used in an algorithm to determine testing for recurrent pregnancy loss, it does not demonstrate that use of CMA testing improves outcomes. Further research evaluating the implementation of such a decision tool in practice is needed.

Improvement in Patient Outcomes Following CMA Testing
Several potential health-related outcomes could result from CMA testing of POC in pregnancy loss. Knowledge of the cause of the loss might lead to reduced parent distress or anxiety. For
couples with recurrent pregnancy loss, preimplantation genetic diagnosis with the transfer of unaffected embryos or the use of donor gametes might be considered for therapy. No studies identified reported whether the use of CMA is associated with changes in parental mental health outcomes.

**Section Summary: Clinically Useful**

No studies identified have directly demonstrated how CMA testing would change management outcomes, however, based on a change of evidence, there are several ways in which CMA testing of fetal tissue in pregnancy losses could have clinical utility, including leading to changes in diagnostic testing, reduced parental distress, or preimplantation genetic diagnosis.

**Summary of Evidence**

For individuals who have pregnancy loss with indications for genetic analysis of the embryo or fetus who receive CMA testing of fetal tissue, the evidence includes prospective and retrospective cohort studies that report on the yield of CMA testing. 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 has suggested that CMA testing has a high rate of concordance with standard karyotyping. For both early and late pregnancy loss, CMA is more likely to yield a result than karyotyping. Other studies have reported that CMA testing 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 uncertain significance in CMA testing of miscarriage samples are not well characterized. Potential benefits from identifying a genetic abnormality in a miscarriage or IUFD include reducing emotional distress for families, altering additional testing undertaken to assess for other causes of pregnancy loss, and changing reproductive decision making for future pregnancies. The potential for clinical utility with 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. None of the studies identified directly demonstrated whether (or how) patient management would change based on CMA testing of the products of conception from early or late pregnancy losses, nor did they demonstrate how patient outcomes would improve. However, the available evidence suggests that, for situations in which a genetic evaluation is indicated, CMA testing would be expected to perform as well as (or better) than standard karyotyping. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

**Supplemental Information**

**Clinical Input 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 from Blue Cross Blue Shield Association, input was received from 3 academic medical centers, one of which provided 2 responses, and 3 physician specialty societies, one of which provided 3 responses, in 2015. There was a consensus that chromosomal microarray (CMA) testing is medically necessary for 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 no consensus about specific criteria that should be used to limit the use of CMA testing. While many reviewers noted that the CMA testing yield is likely to be higher in later term losses, there was no consensus about a specific gestational age that should be used.

**Practice Guidelines and Position Statements**

**American College of Obstetrics and Gynecologists**

The American College of Obstetrics and Gynecologists and the Society for Maternal-Fetal Medicine (2013) published a joint opinion on the use of chromosomal microarray testing in
prenatal diagnosis. The guidelines made the following recommendations about the evaluation of fetal losses:

- "In cases of intrauterine fetal demise or stillbirth when further cytogenetic analysis is desired, chromosomal microarray analysis on fetal tissue (i.e., amniotic fluid, placenta, or products of conception) is recommended because of its increased likelihood of obtaining results and improved detection of causative abnormalities."
- "Limited data are available on the clinical utility of chromosomal microarray analysis to evaluate first-trimester and second-trimester pregnancy losses; therefore, this is not recommended at this time."

**American Society for Reproductive Medicine**
The American Society for Reproductive Medicine (2012) issued an opinion on the evaluation and treatment of recurrent pregnancy loss. The statement drew the following conclusions:

- "Evaluation of recurrent pregnancy loss can proceed after 2 consecutive clinical pregnancy losses."
- "Assessment of recurrent pregnancy loss focuses on screening for genetic factors and antiphospholipid syndrome, assessment of uterine anatomy, hormonal and metabolic factors, and lifestyle variables. These may include:
  - Peripheral karyotype of the parents.
  - Screening for lupus anticoagulant, anticardiolipin antibodies, and anti-β2 glycoprotein I.
  - Sonohysterogram, hysterosalpingogram, and/or hysteroscopy.
  - Screening for thyroid and prolactin abnormalities."
- "Karyotypic analysis of products of conception may be useful in the setting of ongoing therapy for recurrent pregnancy loss."

**U.S. Preventive Services Task Force Recommendations**
Not applicable.

**Medicare National Coverage**
There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

**Ongoing and Unpublished Clinical Trials**
A search of ClinicalTrials.gov in July 2018 did not identify any ongoing or unpublished trials that would likely influence this review.

**References**


**Documentation for Clinical Review**

Please provide the following documentation (if/when requested):
- History and physical and/or consultation notes including:
  - History of pregnancies
    - Isolated and recurrent early pregnancy loss (miscarriages)
    - Later pregnancy loss (intrauterine fetal demise [IUDF])
  - Previous treatment plan(s) and response(s)
  - Current treatment plan
  - Clinical justification for Chromosomal Microarray Analysis (CMA)
- Genetic Counseling Reports (if available)

**Post Service**
- CMA of fetal tissue, if applicable
- Results/reports of tests performed

**Coding**

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

**MN/NMN**

The following services may be considered medically necessary when policy criteria are met. Services may be considered not medically necessary when policy criteria are not met.

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<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>Description</th>
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<tr>
<td>CPT®</td>
<td>81228</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (e.g., bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)</td>
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<td></td>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
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<tr>
<td></td>
<td>88261</td>
<td>Chromosome analysis; count 5 cells, 1 karyotype, with banding</td>
</tr>
<tr>
<td></td>
<td>88262</td>
<td>Chromosome analysis; count 15-20 cells, 2 karyotypes, with banding</td>
</tr>
<tr>
<td></td>
<td>88263</td>
<td>Chromosome analysis; count 45 cells for mosaicism, 2 karyotypes, with banding</td>
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Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

<table>
<thead>
<tr>
<th>Effective Date</th>
<th>Action</th>
<th>Reason</th>
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<tr>
<td>04/30/2015</td>
<td>BCBSA Medical Policy Adoption</td>
<td>Medical Policy Committee</td>
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<tr>
<td>03/01/2016</td>
<td>Policy title change from Chromosomal Microarray Testing for the Evaluation of Early Pregnancy Loss and Intrauterine Fetal Demise Policy revision without position change</td>
<td>Medical Policy Committee</td>
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<tr>
<td>12/01/2016</td>
<td>Administrative Update (Coding clarification)</td>
<td>Administrative Review</td>
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<td>Policy title change from Chromosomal Microarray Analysis for the Evaluation of Pregnancy Loss Policy revision without position change</td>
<td>Medical Policy Committee</td>
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<tr>
<td>10/01/2019</td>
<td>Policy revision without position change</td>
<td>Medical Policy Committee</td>
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</table>

Definitions of Decision Determinations

**Medically Necessary:** A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

**Investigational/Experimental:** A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

**Split Evaluation:** Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.
Disclaimer: This medical policy is a guide in evaluating the medical necessity of a particular service or treatment. Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.