I. POLICY

Chromosomal Microarray

In patients who are undergoing invasive diagnostic prenatal (fetal) testing, chromosome microarray (CMA) testing may be considered medically necessary, as an alternative to karyotyping (see Policy Guidelines).

Single-Gene Disorders

Invasive diagnostic prenatal (fetal) testing for molecular analysis for single-gene disorders may be considered medically necessary when a pregnancy has been identified as being at high risk:

1. For autosomal dominant conditions, at least 1 of the parents has a known pathogenic mutation.
2. For autosomal recessive conditions:
   - Both parents are suspected to be carriers or are known to be carriers, OR
   - One parent is clinically affected and the other parent is suspected to be or is a known carrier.
3. For X-linked conditions: A parent is suspected to be or is a known carrier.

AND, ALL of the following are met:

a. The natural history of the disease is well understood, and there is a reasonable likelihood that the disease is one with high morbidity in the homozygous or compound heterozygous state, AND
b. The disease has high penetrance, AND
c. The genetic test has adequate sensitivity and specificity to guide clinical decision making and residual risk is understood, AND
d. An association of the marker with the disorder has been established.
If the above criteria for molecular analysis for single-gene disorders are not met, invasive diagnostic prenatal (fetal) testing is considered **investigational**. There is insufficient evidence to support a conclusion concerning the health outcomes or benefits associated with this procedure.

### Next-Generation Sequencing

The use of next-generation sequencing in the setting of invasive prenatal testing is considered **investigational**. There is insufficient evidence to support a conclusion concerning the health outcomes or benefits associated with this procedure.

### Policy Guidelines

#### Chromosomal Microarray

The American College of Obstetricians and Gynecologists (ACOG) recommends chromosome microarray analysis (CMA) testing be performed in patients who are undergoing invasive prenatal diagnostic testing and that if:

- the fetus has one or more major structural abnormalities identified on ultrasound examination, CMA testing replaces the need for karyotyping.
- the fetus is structurally normal, either karyotyping or CMA can be performed.

#### Fetal Structural Malformations

Fetal malformations identified by ultrasound, characterized as major or minor malformations, whether isolated or multiple, may be part of a genetic syndrome, despite a normal fetal karyotype.

Major malformations are structural defects that have a significant effect on function or social acceptability. They may be lethal or associated with possible survival with severe or moderate immediate or long-term morbidity. Examples by organ system include: genitourinary: renal agenesis (unilateral or bilateral), hypoplastic/cystic kidney; cardiovascular: complex heart malformations; musculoskeletal: osteochondrodysplasia/osteogenesis imperfecta, clubfoot, craniosynostosis; central nervous system: anencephaly, hydrocephalus, myelomeningocele; facial clefts; body wall: omphalocele/gastroschisis; respiratory: cystic adenomatoid lung malformation.

#### Single-Gene Disorders

An individual may be suspected of being a carrier if there is a family history of or ethnic predilection for a disease. Carrier screening is not recommended if the carrier rate is less than 1% in the general population.

In most cases, before a prenatal diagnosis using molecular genetic testing can be offered, the family-specific mutation must be identified, either in an affected relative or carrier parent(s). Therefore, panel testing in this setting would not be considered appropriate.

In some cases, the father may not be available for testing, and the risk assessment to the fetus will need to be estimated without knowing the father’s genetic status.
Genetic Counseling
Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual’s family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Cross-reference:
MP-2.242 Genetic Testing Including Chromosomal Microarray Analysis and Next-Generation Sequencing Panels for Developmental Disorders
MP-2.324 Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders
MP-2.258 Carrier Testing for Genetic Disease
MP-1.006 Fetal Surgery for Prenatally Diagnosed Malformations
MP-7.006 First-Trimester Detection of Down Syndrome Using Fetal Ultrasound Markers Combined with Maternal Serum Assessment
MP-7.009 Preimplantation Genetic Testing

II. PRODUCT VARIATIONS

This policy is applicable to all programs and products administered by Capital BlueCross unless otherwise indicated below.

FEP PPO*

*The FEP program dictates that all drugs, devices or biological products approved by the U.S. Food and Drug Administration (FDA) may not be considered investigational. Therefore, FDA-approved drugs, devices or biological products may be assessed on the basis of medical necessity.

III. DESCRIPTION/BACKGROUND

The focus of this policy is on the use of certain invasive diagnostic testing methodologies in the prenatal (fetal) setting and to provide a framework for evaluating the clinical utility of diagnosing monogenic disorders in this setting.
Invasive fetal diagnostic testing can include obtaining fetal tissue for karyotyping, fluorescence in situ hybridization (FISH), CMA testing, quantitative polymerase chain reaction (qPCR), next-generation sequencing (NGS), and multiplex ligation-dependent probe amplification (MLPA).

This policy will only address the following:

- the diagnosis of copy number variants using CMA technology
- the diagnosis of single-gene disorders, most of which are due to point mutations or very small deletions and use molecular methods to diagnose (mainly PCR, but also MLPA)
- NGS

This policy applies only if there is not a separate medical policy that outlines specific criteria for diagnostic testing. If a separate policy does exist, then the criteria for medical necessity in that policy supersede the guidelines in this policy. Refer to cross-reference list.

Genetic disorders are generally categorized into 3 main groups: chromosomal, single gene, and multifactorial. Single-gene disorders (also known as monogenic) result from errors in a specific gene, whereas those that are chromosomal include larger aberrations that are numerical or structural.

Invasive prenatal testing refers to the direct testing of fetal tissue, typically by chorionic villus sampling (CVS) or amniocentesis. Invasive prenatal procedures are typically performed in pregnancies of women who have been identified as having a fetus at increased risk for a chromosomal abnormality, or if there is a family history of a single-gene disorder.

**Chromosomal Microarray**

Chromosomal microarray (CMA) technology 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. However, there are disadvantages to CMA, including the detection of variants of unknown clinical significance and the fact that it cannot detect certain types of chromosomal abnormalities, including balanced rearrangements.

CMA analyzes abnormalities at the level of the chromosome and measures gains and losses of DNA (known as copy number variants [CNVs]) throughout the genome.

CMA analysis detects CNVs by comparing a reference genomic sequence (“normal”) with the corresponding patient sequence. Each sample has a different fluorescent label so that they can be distinguished, and both are cohybridized to a sample of a specific reference (also normal) DNA fragment of known genomic locus. If the patient sequence is missing part of the normal sequence (deletion) or has the normal sequence plus additional genomic material within that genomic location (e.g., a duplication of the same sequence), the sequence imbalance is detected as a difference in fluorescence intensity. For this reason, standard CMA (non-SNPs, see the following) cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (same sequence is present in reverse base pair order) because the fluorescence intensity would not change.
CMA analysis uses thousands of cloned or synthesized DNA fragments of known genomic locus immobilized on a glass slide (microarray) to conduct thousands of comparative reactions at the same time. The prepared sample and control DNA are hybridized to the fragments on the slide, and CNVs are determined by computer analysis of the array patterns and intensities of the hybridization signals. Array resolution is limited only by the average size of the fragment used and by the chromosomal distance between loci represented by the reference DNA fragments on the slide. High resolution oligonucleotide arrays are capable of detecting changes at a resolution of up to 50 to 100 Kb.

**Types of CMA Technologies**

There are some differences in CMA technology, most notably in the various types of microarrays. They can differ first by construction; earliest versions were used of DNA fragments cloned from BAC. These have been largely replaced by oligonucleotide (oligos; short, synthesized DNA) arrays, which offer better reproducibility. Finally, arrays that detect hundreds of thousands of SNPs across the genome have some advantages as well. A SNP is a DNA variation in which a single nucleotide in the genomic sequence is altered. This variation can occur between 2 different individuals or between paired chromosomes from the same individual and may or may not cause disease. Oligo/SNP hybrid arrays have been constructed to merge the advantages of each.

The 2 types of microarrays both detect CNVs, but they identify different types of genetic variation. The oligo arrays detect CNVs for relatively large deletions or duplications, including whole chromosome duplications (trisomies), but cannot detect triploidy. SNP arrays provide a genome-wide copy number analysis, and can detect consanguinity, as well as triploidy and uniparental disomy.

Microarrays may be prepared by the laboratory using the technology, or, more commonly by commercial manufacturers, and sold to laboratories that must qualify and validate the product for use in their assay, in conjunction with computerized software for interpretation. The proliferation of in-house developed and commercially available platforms prompted the American College of Medical Genetics (ACMG) to publish guidelines for the design and performance expectations for clinical microarrays and associated software in the postnatal setting.

At this time, no guidelines exist as to whether targeted or genome-wide arrays should be used, or what regions of the genome should be covered. Both targeted and genome-wide arrays search the entire genome for CNVs, however, targeted arrays are designed to cover only clinically significant areas of the genome. The ACMG guideline for designing microarrays recommends probe enrichment in clinically significant areas of the genome to maximize detection of known abnormalities. Depending on the laboratory that develops a targeted array, it can include as many or as few micro deletions and micro duplication syndromes as thought to be needed. The advantage, and purpose, of targeted arrays is to minimize the number of variants of unknown significance (VOUS).

Whole genome CMA analysis has allowed the characterization of several new genetic syndromes, with other potential candidates currently under study. However, the whole genome
arrays also have the disadvantage of potentially high numbers of apparent false positive results, because benign CNVs are also found in phenotypically normal populations; both benign and pathogenic CNVs are continuously cataloged and, to some extent, made available in public reference databases to aid in clinical interpretation relevance.

Clinical Relevance of CMA Findings and Variants of Unknown Significance

CNVs are generally classified as pathogenic (known to be disease-causing), benign or a VOUS. A VOUS is defined as a CNV that:

- has not been previously identified in a laboratory’s patient population, or
- has not been reported in the medical literature, or
- is not found in publicly available databases, or
- does not involve any known disease-causing genes.

To determine clinical relevance (consistent association with a disease) of CNV findings, the following actions are taken:

- CNVs are confirmed by another method (e.g., FISH, MLPA, polymerase chain reaction [PCR]).
- CNVs detected are checked against public databases and, if available, against private databases maintained by the laboratory. Known pathogenic CNVs associated with the same or similar phenotype as the patient are assumed to explain the etiology of the case; known benign CNVs are assumed to be nonpathogenic.
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (micro deletion) or overexpressed (micro duplication).
- The laboratory may establish a size cutoff; potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs; cutoffs for CNVs not previously reported typically range from 300 kb to 1 Mb.
- Parental studies are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign polymorphisms whereas those appearing de novo are likely pathogenic; etiology may become more certain as other similar cases accrue.

In 2008, the International Standards for Cytogenomic Arrays (ISCA) Consortium was organized (available online at: https://www.iscaconsortium.org/index.php); it has established a public database containing de-identified whole genome microarray data from a subset of the ISCA Consortium member clinical diagnostic laboratories. Array analysis was carried out on subjects with phenotypes including intellectual disability, autism, and developmental delay. As of November 2011, there were over 28,500 total cases in the database. Additional members are planning to contribute data; participating members use an opt-out, rather than an opt-in approach that was approved by the National Institutes of Health (NIH) and participating center.
institutional review boards. The database is held at NCBI/NIH (National Center for Biotechnology Information) and curated by a committee of clinical genetics laboratory experts. A 2012 update from ISCA summarizes their experience as a model for ongoing efforts to incorporate phenotypic data with genotypic data to improve the quality of research and clinical care in genetics.

Use of the database includes an intralaboratory curation process, whereby laboratories are alerted to any inconsistencies among their own reported CNVs or other mutations, as well as any not consistent with the ISCA “known” pathogenic and “known” benign lists. The intralaboratory conflict rate was initially about 3% overall; following release of the first ISCA curated track, the intralaboratory conflict rate decreased to about 1.5%. A planned interlaboratory curation process, whereby a group of expert’s curates reported CNVs/mutations across laboratories, is currently in progress.

The consortium recently proposed “an evidence-based approach to guide the development of content on chromosomal microarrays and to support interpretation of clinically significant copy number variation.” The proposal defines levels of evidence (from the literature and/or ISCA and other public databases) that describe how well or how poorly detected mutations or CNVs are correlated with phenotype. The consortium will apparently coordinate a volunteer effort to describe the evidence for targeted regions across the genome.

ISCA is also developing vendor-neutral recommendations for standards for the design, resolution, and content of cytogenomic arrays using an evidence-based process and an international panel of experts in clinical genetics, clinical laboratory genetics, genomics, and bioinformatics.

**Single-Gene (Monogenic) Disorders**

Monogenic or single-gene disorders include those with an inheritance mode of autosomal dominant or recessive, X-linked dominant or recessive. Women may be identified as being at increased risk for having a fetus with an inherited genetic condition because of previously affected pregnancies, a family history in a suggestive pattern of inheritance, or being a member of a subpopulation with elevated frequencies of certain autosomal recessive conditions.

Most Mendelian disorders are caused by a point mutation or very small deletions or duplications. Monogenic mutations are diagnosed by molecular methods, mainly PCR for point mutations, but also other methods like MLPA for very small deletions and duplications. There are approximately 5000 known disorders that are inherited in this fashion. Diagnostic tests are currently available for most of the common monogenic disorders, as well as for a number of the more rare disorders. For most single-gene disorders, testing in the prenatal setting requires knowledge of the family-specific mutation.

**Next-Generation Sequencing**

Next-generation sequencing (NGS) has been used to identify causative genes in many Mendelian disorders. Approximately 85% of known disease-causing mutations occur within the 1% of the genome that encodes for proteins (exome). Therefore, whole exome sequencing could rapidly and cost-effectively capture the majority of protein-coding regions. However, although whole
exome and whole genome sequencing have significant potential, there remain concerns of technical complexity, difficulties with bioinformatic interpretation and variants of unknown significance, as well as ethical issues.¹

### Commercially Available Tests

Many academic and commercial laboratories offer CMA testing and testing for single-gene disorders. The following is not inclusive and is only an example of some laboratories that offer CMA testing. The test should be cleared or approved by FDA, or performed in a Clinical Laboratory Improvement Amendment (CLIA)–certified laboratory.

GeneDx offers prenatal CMA for copy number abnormalities in fetuses with ultrasound abnormalities. The targeted CMA includes oligonucleotide probes placed throughout the genome and within 100 common or novel microdeletion and microduplication syndromes, as well as those involving subtelomeric regions and any other intrachromosomal region greater than 1.5 Mb. This array also contains SNP probes covering chromosomes known to contain uniparental disomy. Exon-level probe coverage is added to some genes associated with some monogenic disorders.

GeneDx offers a whole genome that contains oligonucleotide probes placed throughout the genome and within more than 220 targeted regions. This array detects CNVs of greater than 200 kb across the entire genome and between 500 bp to 15 kb in targeted regions. Approximately 65 genes associated with neurodevelopmental disorders are targeted at the exon level. This array also contains SNP probes throughout the genome to detect some types of uniparental disomy (UPD).

ARUP laboratory provides former Signature Genomics clients with prenatal tests, including targeted CMA with SNP coverage.

Many laboratories offer reflex testing, which may be performed with microarray testing added if karyotyping is normal or unable to be performed (due to no growth of cells).

### Regulatory Status

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

### IV. Rationale

This evidence review is based on the published literature, the general principles of prenatal (fetal) testing, and accepted practice guidelines from major medical societies (see Appendix Table 1 for genetic testing categories).

There are many ethical considerations in testing a fetus for a condition that is of adult-onset. In general, there is consensus in the medical and bioethical communities that prenatal testing should
not include testing for late-onset/adult-onset conditions, or for diseases for which there is a
known intervention that would lead to improved health outcomes, but would only need to be
started after the onset of adulthood.

Ideally, peer-reviewed literature on the performance and indications for the test should be
available. The evaluation of a genetic test focuses on 3 main principles: (1) analytic validity
(technical accuracy of the test in detecting a mutation that is present or in excluding a mutation
that is absent); (2) clinical validity (diagnostic performance of the test [sensitivity, specificity,
positive and negative predictive values] in detecting clinical disease); and (3) clinical utility
(how the results of the diagnostic test will be used to change management of the patient and
whether these changes in management lead to clinically important improvements in health
outcomes).

Genetic Counseling
According to the American College of Obstetricians and Gynecologists, comprehensive patient
pretest and posttest genetic counseling from qualified personnel such as a genetic counselor or
geneticist regarding the benefits, limitations, and results of chromosomal microarray analysis
(CMA) is essential. CMA should not be ordered without informed consent, which should be
documented in the medical record and include discussion of the potential to identify findings of
uncertain significance, nonpaternity, consanguinity, and adult-onset disease.

CMA Testing for Copy Number Variants
Most of the literature on the use of CMA in the prenatal (fetal) setting consists of prospective
and retrospective analyses of CMA findings compared with conventional karyotyping, either in
patients with known karyotype results or in patients with concurrently performed karyotyping
and CMA. CMA has been proposed as being used as either a first tier test (in place of or in
conjunction with karyotype) or as a second tier test (after a negative karyotype).

Jansen et al conducted a systematic review and meta-analysis of the additional diagnostic gain of
array comparative genomic hybridization (CGH) compared with standard karyotyping and 22q11
by fluorescence in situ hybridization (FISH) in prenatally diagnosed cardiac malformations.2
Thirteen studies with 1131 cases of congenital heart disease (CHD) were included, with a
literature search through September 2014. A meta-analysis identified an incremental yield of
7.0% (95% confidence interval [CI], 5.3% to 8.6%) for the detection of copy number variants
using aCGH, excluding aneuploidy and 22q11 microdeletion cases. A subgroup analysis showed
a 3.4% (95% CI, 0.3% to 6.6%) incremental yield in isolated CHD cases, and 9.3%
(95% CI, 6.6% to 12%) when extracardiac malformations were present. Overall, an incremental
yield of 12% (95% CI, 7.6% to 16%) was found when 22q11 deletion cases were included. The
rate of variants of unknown significance (VOUS) was 3.4% (95% CI, 2.1% to 4.6%).

Armengol et al conducted a comparative study of available technologies, including karyotyping
and CMA, for detection of chromosomal abnormalities after invasive prenatal sampling.3 The
multiple techniques were performed on the same sample. The study included 900 women and the
main indications for testing were abnormal ultrasound findings, altered biochemical screening,
family history of a chromosomal disorder or other genetic condition, and advanced maternal age
(AMA). A total of 57 clinically relevant chromosomal aberrations were found (6.3%), with CMA testing having the highest detection rate, 32% above other methods. Most VOUS could be classified as likely benign after proving they were inherited. Cross-validation was provided by the simultaneous use of multiple techniques, and additional molecular techniques were performed in the follow-up of some of the alterations identified by CMA.

The reported diagnostic accuracy for karyotyping and CMA was as follows:

- **Karyotyping**: sensitivity of 76.4% (95% CI, 63.0 to 87.0); specificity of 99.9% (99.2 to 99.9); positive predictive value (PPV) of 97.7 (87.7 to 99.9); negative predictive value (NPV) of 98.3 (97.1 to 99.1); and diagnostic accuracy of 98.2 (97.1 to 99.0).
- **CMA**: sensitivity of 98.2 (90.4 to 99.9); specificity of 99.7 (99.1 to 99.9); PPV of 96.5 (87.9 to 99.5); NPV of 99.9 (99.3 to 100); and diagnostic accuracy of 99.7 (99.0 to 99.9).

Shaffer et al reported the results of microarray testing for prenatal diagnosis in over 5000 prospectively collected prenatal samples received from 2004 to 2011 for a variety of indications. They used CGH microarrays targeted to known chromosomal syndromes with later versions providing backbone coverage of the entire genome. Cases were stratified according to the test result (normal, VOUS, abnormal) and indication for the study, and compared with karyotyping results. Of 5003 prenatal specimens, 56% were referred with normal karyotypes, 13% had known abnormal karyotypes, 16% had karyotypes performed concurrently with microarray testing, and 15% had unknown karyotype status. Indications for microarray testing included a known abnormal karyotype (n=648), family history of a parent known to carry a chromosomal rearrangement or imbalance (n=62), fetal demise (n=417), abnormal ultrasound (n=2858) [further detailed in the next study], abnormal first- or second-trimester screen (n=77), other family history of a genetic condition (n=487), AMA (n=346), parental anxiety (n=95), or other/not specified (n=13). The overall detection rate of clinically significant results with microarray testing was 5.3%. The detection rate of clinically significant CNVs was 5.5% among cases with known normal karyotypes. After excluding the cases of fetal demise, the VOUS rate was 4.2%, but if only de novo CNVs were considered (the rate was 0.39%).

Shaffer et al performed a retrospective analysis of 2858 pregnancies, with abnormal ultrasound findings (as stratified by organ system). Most cases had previously normal karyotypes (n=2052 [72%]). The remaining had karyotyping performed concurrently with microarray testing (n=465 [16%]) or had unknown or failed karyotypes (n=341 [12%]). Ultrasound anomalies were categorized in several ways: multiple structural anomalies, structural anomalies involving a single-organ system, isolated abnormalities of growth, isolated abnormal amniotic fluid volume, single or multiple soft marker(s), or multiple nonstructural anomalies (e.g., IUGR). Soft markers included choroid plexus cysts, echogenic foci in the heart or bowel, isolated short long bones, absent nasal bones, sandal gap between the first and second toes, fifth finger clinodactyly, single umbilical artery, and persistent right umbilical vein. The average maternal age at the time of testing was 31.8 years. Most tests were whole genome, oligoarrays (n=2161 [76%]), and the remaining were bacterial artificial chromosome–based arrays, either with coverage of the whole genome (n=506 [18%]) or targeted coverage (n=191 [7%]). Overall, with microarray testing, 6.5% showed clinically significant results, and 4.8% had VOUS. For the cases with a previously

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normal karyotype, the detection rate for significant CNVs was similar (6.2%). Clinically significant genomic alterations were identified in cases with a single ultrasound anomaly (n=99/1773 [5.6%]), anomalies in 2 or more organ systems (n=77/808 [9.5%]), isolated growth abnormalities (n=2/76 [2.6%]), and soft markers (n=2/77 [2.6%]). Certain anomalies, either in isolation or with additional anomalies, had higher detection rates: holoprosencephaly (n=9/85 [10.6%]), posterior fossa defects (n=21/144 [14.6%]), skeletal anomalies (n=15/140 [10.7%]), ventricular septal defect (n=14/132 [10.6%]), hypoplastic left heart (n=11/68 [16.2%]), and cleft lip/palate (n=14/136 [10.3%]).

Hillman et al conducted a prospective cohort study and systematic review and meta-analysis. The cohort study involved 243 women undergoing CMA and karyotyping for a structural abnormality detected on prenatal ultrasound. There was an excess detection rate of abnormalities by CMA of 4.1% over conventional karyotyping, with a VOUS rate of 2.1% (95% CI, 1.3% to 3.3%). The meta-analysis included studies through December 2012 that reported on prenatal microarray testing performed for any indication and was not limited to cases referred for abnormal fetal ultrasound findings. Twenty-five studies were included, with a collective number of 18,113 samples analyzed. The detection rate in the meta-analysis was 10% (95% CI, 8% to 13%). The VOUS rate was 1.4% (95% CI, 0.5% to 3.7%) when any indication for prenatal CMA was meta-analyzed and 2.1% (95% CI, 1.3 to 3.3) when the indication for the CMA was an abnormal ultrasound finding.

Wapner et al conducted a prospective study to evaluate the accuracy, efficacy, and incremental yield of CMA compared with karyotyping for routine prenatal diagnosis. A total of 4406 women undergoing routine prenatal diagnosis in 1 of 29 diagnostic centers by either chorionic villus sampling (CVS) or amniocentesis had a sample split in 2 for standard karyotyping and CMA. Indications for prenatal diagnosis included AMA (46.6%), a positive aneuploidy screening result (18.8%), structural anomalies detected by ultrasound (25.2%), and other indications (9.4%). CMA analysis was successful in 98.8% of the fetal samples. The primary analysis classified microarray results as being true positive, true negative, false positive, or false negative relative to the findings by karyotyping. Secondary outcomes included the occurrence and classification of CNVs identified by microarray in the presence of a normal karyotype and the ability of CMA to identify uncommon cytogenetic abnormalities found on karyotyping. Two array platforms were used, one covering targeted regions of known disease association, and one genome-wide single-nucleotide polymorphism (SNP) assay. The data for the second platform were masked by the analysis software to emulate the same resolution and coverage as the first platform; therefore, review of the SNPs was not performed. Microarray analysis of DNA from maternal and paternal blood samples was used to determine whether CNVs detected in fetal samples were inherited. All de novo array findings seen in samples with a normal karyotype were confirmed by a second method, preferentially FISH. Deletions and duplications identified exclusively by means of microarray analysis were classified as “pathogenic” when they encompassed a region implicated in a well-described abnormal phenotype, and all other deletions and duplications were classified as being of “uncertain clinical significance.” A total of 4282 samples were included in the primary analysis. Of these, common autosomal aneuploidies were identified in 317 (7.4%) and sex-chromosome aneuploidies were identified in 57 (1.3%) by
standard karyotyping. CMA identified all of these aneuploidies. None of the balanced rearrangements identified on karyotyping were identified with CMA, nor did CMA identify any of the triploid samples (0.4%).

Of the 3822 cases with a normal karyotype, on microarray, 1399 samples were identified as having CNVs; of these, 88.2% were classified as common benign; and 0.9% were on the predetermined list of pathogenic CNVs. The cases of uncertain clinical significance were adjudicated by a clinical advisory committee, which reclassified them as likely to be benign (1.8% of all 1399 samples) or of potential clinical significance (1.6% of all 1399 samples). Overall, a total of 96 of the 3822 fetal samples with normal karyotypes (2.5%; 95% CI, 2.1 to 3.1) had a microdeletion or duplication of clinical significance.

In subgroup analysis (n=755) of women with normal karyotypes and fetuses with suspected growth or structural anomalies, 45 (6.0%; 95% CI, 4.5 to 7.9) had clinically relevant findings on microarray. These included CNVs that were predetermined as known pathogenic, as well as those classified by the clinical advisory committee as clinically relevant. In this population with structural abnormalities identified on ultrasound, CNVs of uncertain clinical significance, but likely benign, were found in 16 patients (2.1%). Of the women tested for AMA, 1.7% (95% CI, 1.2 to 2.4) had a clinically relevant finding on microarray, as did 1.6% (95% CI, 0.9 to 2.9) of women who tested positive on Down syndrome screening. Recurrent CNVs associated with autism and neurocognitive alterations were detected in 1.3% of karyotypically normal pregnancies: 3.6% with and 0.8% without structural anomalies.

In summary, the study included 3822 patients with normal karyotype and the following indications for prenatal diagnosis: AMA (n=1966), positive Down syndrome screen (n=729), anomaly on ultrasound (n=755), and other (n=372). CMA provided additional clinically relevant CNVs (95% CI) and VOUS rate of:

- AMA: 1.7% (95% CI, 1.2 to 2.4) and 1.9%
- Positive Down screen: 1.6% (95% CI, 0.9 to 2.9) and 1.8%
- Ultrasound anomaly: 6.0% (95% CI, 4.5 to 7.9) and 2.1%

Breman et al evaluated the prenatal CMA results on more than 1000 fetal samples sent for testing at Baylor College of Medicine Medical Genetics Laboratories between 2005 and 2011.8 A total of 1124 specimens were received, of which reportable results were obtained in 1115. Maternal blood samples were required with every fetal sample (and paternal if possible) to exclude maternal cell contamination and to assist with interpretation of CNVs. The CMA was performed on DNA extracted from amniotic fluid, CVS, or cultured cells (amniocytes/CVS) in most of the cases. The gestational ages for direct amniotic fluid samples ranged from 14 to 36 weeks; samples from pregnancies that were more than 16 weeks of gestation provided the most optimal DNA yield. Samples were submitted for either standard cytogenetic studies (karyotype with or without aneuploidy FISH) plus CMA, or for CMA only with a karyotype analysis having been performed elsewhere. For those samples, unless only DNA was submitted, a culture was established in the Baylor laboratory so that any CMA findings could be confirmed by an independent method (FISH, karyotype, or other).
The most common clinical indications were abnormal ultrasound findings (n=410) and AMA (n=394). Other indications included a previous child with or a family history of a genetic disorder or chromosome abnormality (n=137), further workup of a known chromosomal abnormality detected by karyotype or FISH (n=61), parental concern (n=61), an abnormal maternal serum screen (n=37), and other or unclassified (n=4). Twelve cases had no indication provided. The cases spanned 5 years, over which time different types of targeted clinical arrays were used, with progressively increasing complexity and sensitivity. Targeted BACs were used for 282 samples, and all others with targeted oligonucleotide arrays.

In 881 (79%) of the 1115 samples, no deletions or duplications were observed using prenatal CMA analysis. Copy number changes were detected in 234 (21%) cases. Of these, 131 (11.7%) were classified as likely benign. Eighty-five cases (7.6%) were found to have clinically significant genomic imbalances. Twenty-seven microdeletion or microduplication findings (2.4% of total cases; 32% of abnormal cases) were small gains or losses below the resolution of prenatal karyotype analysis, and would not have been detected by conventional chromosome studies alone. Of these, family history was the indication for testing in 8 cases, an abnormal FISH result was the indication for 1 case, and the remaining 18 abnormal findings were unanticipated. Eighteen specimens of total 1115 (1.6%) had results of uncertain clinical significance. An additional 17 cases were found to have multiple inherited CNVs interpreted as likely benign familial variants. The indications yielding the greatest number of clinically significant findings by microarray analysis were abnormal karyotype/FISH (42.6%), a family history of chromosomal abnormality (9.5%), all abnormal prenatal ultrasound findings (9.3%), abnormal serum screening (5.4%), and AMA (1.3%).

In summary, the overall detection rate for clinically significant CNVs was 7.6%; the detection rate was 4.2% when the abnormal cases that had a previously identified chromosome abnormality or a known familial genomic imbalance were excluded. In 1.7% of the cases, abnormal results were obtained that were neither anticipated before microarray analysis nor detectable by conventional prenatal chromosome analysis. The clinical significance of the microarray results could not be determined in 1.7% of cases.

Section Summary: CMA Testing for CNVs
CMA testing has been shown to have a higher rate of detection of pathogenic chromosomal abnormalities than karyotyping. CMA testing is associated with a certain percentage of results that have unknown clinical significance, however, this can be minimalized by the use of targeted arrays, testing phenotypically normal parents for the CNV, and the continued accumulation of pathogenic variants in international databases. A 2014 review by Wapner et al summarized the existing literature of the largest studies that reported the estimates of detectable pathogenic CNVs according to indication for CMA testing. For studies that included all high-risk pregnancies (which were primarily because of abnormal ultrasound abnormalities), the range of pathogenic CNV detection was 2.6% to 7.8%, with a combination of all studies (n=1800) being 5.0%. For pregnancies in which CMA was performed for other indications (advanced maternal age, abnormal Down syndrome screening test, parental anxiety), the range of pathogenic CNV detection was 0.5% to 1.6%, with a combination of all studies (n=10,099) being 0.9%.
Single-Gene Disorders
The analytic validity in the diagnosis of single-gene disorders depends on the individual mutation being tested. In general, it is necessary to identify the particular mutation(s) in the affected parent(s) so that the particular mutation(s) can be sought for prenatal diagnosis.

When a family-specific mutation is known, the analytic validity of testing for this mutation is expected to be high, approaching 100% accuracy.

For clinical validity, when there is a known pathogenic family-specific mutation, the sensitivity and specificity for testing for the mutation in other family members is expected to be very high.

Next-Generation Sequencing
Next-generation sequencing (NGS) is a newer technology for DNA sequencing and can include multigene panel testing, as well as whole exome and whole genome sequencing. While the use of NGS has been accepted in certain noninvasive prenatal testing settings, its use in the setting of invasive prenatal testing for detecting CNVs and single-gene mutations is still considered to be investigational, and includes concerns about interpretation of the data generated and the data’s clinical relevance.

Whole exome sequencing has not yet been well standardized for the clinical laboratory and has not been fully characterized in publicly available documents with regard to the analytic validity for the various types of relevant mutations. The few existing professional guidelines give only high-level direction.

Technical limitations include error rates due to uneven sequencing coverage and gaps in exon capture prior to sequencing, and the variability contributed by the different platforms and procedures used by different clinical laboratories offering exome sequencing as a clinical service is unknown.

Published literature on the use of NGS in the invasive prenatal setting is lacking.

Clinical Utility of Invasive Prenatal (Fetal) Diagnostic Testing
The clinical utility of invasive prenatal (fetal) diagnostic testing is in how the results of the test would have an impact on management decisions and health outcomes. Prenatal (fetal) testing may be performed for the purpose of anticipatory guidance and management, either during the pregnancy or at the time of delivery.

Clinical management decisions may include the following:

- continuation of the pregnancy,
- enabling for timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth,
- birthing decisions.

Ongoing and Unpublished Clinical Trials
A search of ClinicalTrials.gov in October 2015 did not identify any ongoing or unpublished trials that would likely influence this review.
Summary of Evidence
The evidence for chromosomal microarray analysis (CMA) testing in patients who are undergoing invasive diagnostic prenatal (fetal) testing includes a systematic review and meta-analysis and prospective cohort and retrospective analyses of the diagnostic yield compared with karyotyping. Relevant outcomes are test accuracy and validity and changes in reproductive decision making. CMA testing has been shown to have a higher rate of detection of pathogenic chromosomal abnormalities than karyotyping. CMA testing is associated with a certain percentage of results that have unknown clinical significance; however, this can be minimalized by the use of targeted arrays, testing phenotypically normal parents for the copy number variant and the continued accumulation of pathogenic variants in international databases. The highest yield of pathogenic copy number variants by CMA testing has been found in fetuses with malformations identified by ultrasound. Changes in reproductive decision making could include decisions regarding continuation of the pregnancy, enabling for timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth and birthing decisions. The American College of Obstetricians and Gynecologists recommends CMA testing in women who are undergoing an invasive diagnostic procedure. Therefore, the evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for testing for single-gene disorders in patients who are undergoing invasive diagnostic prenatal (fetal) testing includes rare case series that generally report which disorders are detected. Relevant outcomes are test accuracy and validity and changes in reproductive decision making. The analytic validity in the diagnosis of single-gene disorders depends on the individual mutation tested. In general, it is necessary to identify the particular mutation(s) in the affected parent(s) so that the particular mutation(s) can be sought for prenatal diagnosis. When a family-specific mutation is known, the analytic validity of testing for this mutation is expected to be high, approaching 100% accuracy. For clinical validity, when there is a known pathogenic family-specific mutation, the sensitivity and specificity for testing for the mutation in other family members is expected to be very high. Changes in reproductive decision making could include decisions regarding continuation of the pregnancy, enabling for timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth and birthing decisions. Therefore, the evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

The evidence for next-generation sequencing (NGS) in patients who are undergoing invasive diagnostic prenatal (fetal) testing is lacking. Relevant outcomes are test accuracy and validity and changes in reproductive decision making. There are concerns about interpretation of data generated by NGS and the data’s clinical relevance. Analytic and clinical validity of NGS in the prenatal setting are unknown. The evidence is insufficient to determine the effects of the technology on health outcomes.
Practice Guidelines and Position Statements
The American College of Obstetricians and Gynecologists Committee on Genetics and the Society for Maternal Fetal Medicine Committee Opinion 581 (December 2013)\(^\text{10}\) offers the following recommendations for the use of CMA in prenatal diagnosis:

- “In patients with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who are undergoing invasive prenatal diagnosis, chromosomal microarray analysis is recommended. This test replaces the need for fetal karyotype.”
- “In patients with a structurally normal fetus undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed.”
- “Most genetic mutations identified by chromosomal microarray analysis are not associated with increasing maternal age; therefore, the use of this test for prenatal diagnosis should not be restricted to women aged 35 years and older.”
- “Comprehensive patient pretest and posttest genetic counseling from qualified personnel such as a genetic counselor or geneticist regarding the benefits, limitations, and results of chromosomal microarray analysis is essential. Chromosomal microarray analysis should not be ordered without informed consent, which should be documented in the medical record and include discussion of the potential to identify findings of uncertain significance, nonpaternity, consanguinity, and adult-onset disease.”

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

Medicare National Coverage
There is no national coverage determination (NCD).

V. DEFINITIONS

**Amniocentesis** - A test that removes a small amount of fluid that surrounds the fetus and can be used for genetic testing of the fetus or the measurement of certain biochemical markers. Traditional amniocentesis is usually performed between weeks 15 and 20 of gestation.

**Aneuploidy** - A chromosomal abnormality in which the number of chromosomes is abnormal, either having more or less than the normal 46 chromosomes (44 autosomal, 2 sex chromosomes).

**Autosomal** - Any chromosome other than the sex-chromosomes (X and Y).

**Chorionic Villus Sampling** - CVS is generally performed after 9 weeks of gestation. It involves obtaining chorionic villi through transcervical or transabdominal access to the placenta. (Chorionic villi are of fetal origin, and are vascular processes that emerge from the outer sac that
surrounds the developing fetus and provide for exchange between the fetal and maternal circulation).

**Chromosomal Inversion** - A chromosome inversion occurs when 2 breaks occur in the same chromosome and the intervening genetic material is inverted before the breaks are repaired. Even though no genetic material is lost or duplicated, and the person may not show abnormalities at the phenotypic level, gene function may be altered by the rearrangement, and carriers of inversions may have children with abnormalities.

**Chromosomal Translocation/Rearrangement** - A chromosomal translocation refers to an abnormal rearrangement of chromosomes. There are 2 main types: a reciprocal translocation, which occurs when 2 fragments break off from 2 different chromosomes, and they change places; and a Robertsonian translocation, in which 1 chromosome becomes attached to another. Approximately 1 in 500 people have a translocation. In reciprocal and Robertsonian translocations, no chromosome material is gained or lost (which is called a balanced translocation). Most people who carry a balanced translocation are phenotypically normal, but they are at risk of having a child with an unbalanced translocation. With an unbalanced translocation, there is either an extra piece of 1 chromosome and/or a missing piece of another chromosome, which can lead to a child with learning disabilities, developmental delay, and health problems.

**Cytogenetics** - The study of chromosomes.

**Imprinted Genes** - Usually, both copies of each gene (1 copy of each gene inherited from each parent) are active. Sometimes, only 1 copy is active, which depends on parent of origin; this is what is referred to as genomic imprinting. In genes that undergo genomic imprinting, certain segments of DNA undergo methylation. Imprinted genes tend to cluster in the same regions of chromosomes. Two major clusters of imprinted genes have been identified on chromosomes 11 and 15. Prader-Willi and Angelman syndrome are caused by UPD or other errors in imprinting involving genes on chromosome 15. Beckwith-Wiedemann syndrome is associated with abnormalities of imprinted genes on chromosome 11.

**Karyotyping** - A test that examines chromosomes in a sample of cells (i.e., from amniotic fluid and CVS), and can count the number of chromosomes and look for large structural changes in chromosomes. A regular human cell has 46 chromosomes, 44 autosomes, and 2 sex chromosomes which specify gender (XX=female, XY=male).

**Structural Chromosome Abnormality** - There is a normal number of chromosomes (46), however, a segment(s) of chromosome(s) are missing (deleted), extra (inserted), or rearranged (trans located or inverted).
Subtelomeric Rearrangements - Subtelomeric regions (present on most chromosomes) are prone to rearrangements that have been suggested to represent a high proportion of abnormalities in individuals with idiopathic intellectual disability.

Triploidy - A chromosome number of 69 (3 copies of each chromosome).

Trisomy - The presence of an extra chromosome (e.g., trisomies 13, 18, 21 [Down syndrome]).

Uniparental Disomy - Normally, for each of the 23 pairs of chromosomes, 1 is inherited from the mother and the other from the father. UPD is an abnormal situation in which both chromosomes in a pair are inherited from 1 parent, and the other parent’s chromosome from that pair is missing. UPD for most chromosomes is without consequence, but for some chromosomes, it can result in a genetic disorder. The most well-known conditions that result from UPD include Prader-Willi syndrome and Angelman syndrome.

VI. BENEFIT VARIATIONS

The existence of this medical policy does not mean that this service is a covered benefit under the member’s contract. Benefit determinations should be based in all cases on the applicable contract language. Medical policies do not constitute a description of benefits. A member’s individual or group customer benefits govern which services are covered, which are excluded, and which are subject to benefit limits and which require preauthorization. Members and providers should consult the member’s benefit information or contact Capital for benefit information.

VII. DISCLAIMER

Capital’s medical policies are developed to assist in administering a member’s benefits, do not constitute medical advice and are subject to change. Treating providers are solely responsible for medical advice and treatment of members. Members should discuss any medical policy related to their coverage or condition with their provider and consult their benefit information to determine if the service is covered. If there is a discrepancy between this medical policy and a member’s benefit information, the benefit information will govern. Capital considers the information contained in this medical policy to be proprietary and it may only be disseminated as permitted by law.

VIII. CODING INFORMATION

Note: This list of codes may not be all-inclusive, and codes are subject to change at any time. The identification of a code in this section does not denote coverage as coverage is determined by the terms of member benefit information. In addition, not all covered services are eligible for separate reimbursement.
When used to bill for next-generation sequencing in the setting of invasive prenatal testing it is considered Investigational; therefore not covered:

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<tr>
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Covered when medically necessary:

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<table>
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<tr>
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<td>Supervision of other high risk pregnancies, first trimester</td>
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<tr>
<td>O09.892</td>
<td>Supervision of other high risk pregnancies, second trimester</td>
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<tr>
<td>O09.893</td>
<td>Supervision of other high risk pregnancies, third trimester</td>
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<tr>
<td>O28.5</td>
<td>Abnormal chromosomal and genetic finding on antenatal screening of mother</td>
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<td>O35.1XX1</td>
<td>Maternal care for (suspected) chromosomal abnormality in fetus, fetus 1</td>
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<td>O35.1XX2</td>
<td>Maternal care for (suspected) chromosomal abnormality in fetus, fetus 2</td>
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<td>Maternal care for (suspected) chromosomal abnormality in fetus, fetus 3</td>
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<td>O35.1XX9</td>
<td>Maternal care for (suspected) chromosomal abnormality in fetus, other fetus</td>
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<td>Maternal care for (suspected) hereditary disease in fetus, fetus 1</td>
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<td>Maternal care for (suspected) hereditary disease in fetus, other fetus</td>
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*If applicable, please see Medicare LCD or NCD for additional covered diagnoses.

IX. REFERENCES

<table>
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<tr>
<td><strong>MP-2.278</strong></td>
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### Appendix Table 1. Categories of Genetic Testing Addressed in MP-2.278

<table>
<thead>
<tr>
<th>Category</th>
<th>Addressed</th>
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<tbody>
<tr>
<td>1. Testing an affected individual’s germline to benefit the individual</td>
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<tr>
<td>1a. Diagnostic</td>
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</tr>
<tr>
<td>1b. Prognostic</td>
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<tr>
<td>1c. Therapeutic</td>
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<tr>
<td>2. Testing cancer cells from an affected individual to benefit the individual</td>
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</tr>
<tr>
<td>2a. Diagnostic</td>
<td></td>
</tr>
<tr>
<td>2b. Prognostic</td>
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</tr>
<tr>
<td>2c. Therapeutic</td>
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<tr>
<td>3. Testing an asymptomatic individual to determine future risk of disease</td>
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<tr>
<td>4. Testing of an affected individual’s germline to benefit family members</td>
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<tr>
<td>5. Reproductive testing</td>
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<td>5a. Carrier testing: preconception</td>
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<tr>
<td>5b. Carrier testing: prenatal</td>
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<tr>
<td>5c. In utero testing: aneuploidy</td>
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<tr>
<td>5d. In utero testing: mutations</td>
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<tr>
<td>5f. Preimplantation testing with in vitro fertilization</td>
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