Invasive Prenatal (Fetal) Diagnostic Testing

Preauthorization is required.

The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient’s contract at the time the services are rendered.

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Description

Invasive prenatal (fetal) diagnostic testing may be used to confirm the presence of a pathogenic abnormality after it has been determined by prenatal screening that the fetus is at increased risk for one of these conditions. This protocol will only address the use of chromosomal microarray testing, molecular diagnosis of single-gene disorders, and next-generation sequencing.

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 minimized by the use of targeted arrays, testing phenotypically normal parents for the copy number variant and the continued accumu-
lation 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.

**Policy**

**Chromosomal Microarray**

In patients who are undergoing invasive diagnostic prenatal (fetal) testing, chromosome microarray analysis 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 one 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**.

**Next-Generation Sequencing**

The use of next-generation sequencing in the setting of invasive prenatal testing is considered **investigational**.

**Policy Guidelines**

**Chromosomal Microarray**

According to ACOG recommendations,

- CMA is recommended to replace karyotyping for patients who are undergoing invasive prenatal diagnosis testing when the fetus has one or more major structural abnormalities identified on ultrasonographic examination.
- Either CMA or karyotyping can be performed for patients who are undergoing invasive prenatal diagnosis testing when the fetus is structurally normal.

**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.

**Background**

The focus of this protocol 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 protocol 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 protocol applies only if there is not a separate Corporate Medical Protocol (CMP) that outlines specific criteria for diagnostic testing. If a separate CMP does exist, then the criteria in it supersede the guidelines herein. This protocol does NOT cover the use of:

- prenatal carrier testing (Carrier Testing for Genetic Diseases Protocol)
- preimplantation genetic diagnosis or screening (Preimplantation Genetic Testing Protocol)
- noninvasive prenatal testing (Genetic Testing for Noninvasive Prenatal Testing Protocol)

Genetic disorders are generally categorized into three 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**

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–single nucleotide polymorphisms [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 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 bacterial artificial chromosome. They 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 two 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 two 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 indicate 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 microdeletions and microduplication 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 VOUS

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, 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 (microdeletion) or overexpressed (microduplication).
- 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 one 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; it established a public database containing deidentified 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 and participating center institutional review boards. The database is held at the National Center for Biotechnology Information/ National Institutes of Health 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 experts 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 correlate 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**

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 VOUS, as well as ethical issues.¹

**Commercially Available Tests**

Many academic and commercial laboratories offer CMA testing and single-gene disorder testing. The following is not inclusive; it is only an example of some laboratories that offer CMA testing. The test should be cleared or approved by the United States Food and Drug Administration (FDA), or performed in a Clinical Laboratory Improvement Amendment–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 greater than 200 kb across the entire genome and between 500 bp and 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).

**Definitions**

**Amniocentesis**
A test that removes a small amount of fluid surrounding the fetus and that can be used for genetic testing of the fetus or measuring 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 more or less than the normal number (46) of chromosomes (44 autosomal, two sex chromosomes).

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

**Chorionic Villus Sampling**
CVS is generally performed after nine 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 two 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 two main types: a reciprocal translocation, which occurs when two fragments break off from two different chromosomes, and they change places; and a Robertsonian translocation, in which one chromosome becomes attached to another. Approximately one in 500 people have a translocation. In reciprocal and Robertsonian translocations, no chromosome material is gained or lost (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 one 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 (one copy of each gene inherited from each parent) are active. Sometimes, only one 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 typical human cell has 46 chromosomes (44 autosomes, two sex chromosomes) that 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 (translocated 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 (three 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, one 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 one 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.

**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, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**Related Protocols**

Carrier Testing for Genetic Diseases

Chromosomal Microarray Analysis for the Evaluation of Pregnancy Loss

Genetic Testing for Developmental Delay and Autism Spectrum Disorder

Genetic Testing for Noninvasive Prenatal Testing

Preimplantation Genetic Testing
Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. **Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.**

**References**

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.