CHROMOSOME, BLOOD, ROUTINE
(CHROMOSOME ANALYSIS, BLOOD (CONSTITUTIONAL))

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Table of Contents                                      Page
GUIDELINES                                          1
BACKGROUND                                         2
CLINICAL EVIDENCE                                   3
Guidelines and Recommendations                      4
US FOOD AND DRUG ADMINISTRATION (US FDA)            6
CENTERS FOR MEDICARE AND MEDICAID SERVICES (CMS)    6
APPLICABLE CODING                                   7
REFERENCES                                         8
POLICY HISTORY/REVISION HISTORY                     9

INSTRUCTIONS FOR USE
Physician Decision Support (PDS) is a lab ordering tool operated by BeaconLBS. This Clinical Guideline supports the Questions and Answers that appear in PDS for tests referenced in this document. UnitedHealthcare reserves the right, in its sole discretion, to modify its Clinical Guidelines as necessary. This Clinical Guideline is provided for informational purposes. It does not constitute a Medical Policy or medical advice.

GUIDELINES

BeaconLBS recommends routine chromosome analysis, blood (constitutional) for first-tier clinical genetics evaluation in the following settings:

- Patients with clinical features of autosomal trisomy, such as Down syndrome (trisomy 21)
- Patients with clinical features of sex chromosome aneusomy, such as Turner syndrome (45,X)
- Patients with family history of chromosome aberration
- Couples with recurrent pregnancy loss
- Men with non-obstructive azoosperma or severe oligosperma prior to fertility treatments including ICSI

BeaconLBS recommends fluorescence in situ hybridization (FISH) for the above patients if routine chromosome analysis does not yield a clear diagnosis. FISH may be targeted to detect subtelomeric rearrangements or aberrations in specific loci, depending on the results of routine chromosome analysis and clinical history, family history, physical examination, patient choice, and clinician choice.
BeaconLBS considers both routine and high resolution chromosome analysis to be diagnostic tests, not screening tests. For example, all neonates are screened for the presence of clinical features of Down syndrome and other chromosome anomalies, but only those with positive findings are offered routine chromosome analysis to confirm the diagnosis.

These recommendations are based upon guidelines from the American College of Medical Genetics (ACMG), the American Conference of Obstetricians and Gynecologists (ACOG), the International Standard Cytogenomic Array Consortium (ISCA Consortium) and the American Society for Reproductive Medicine (ASRM) and American Urological Association (AUA).

Note regarding Chromosome, SNP MicroArray analysis:

For first-tier clinical genetics evaluation in children with unexplained congenital anomalies, developmental delay (DD), mental retardation (MR), neurologic abnormality, or autism spectrum disorder (ASD), Beacon LBS recommends a Chromosome Microarray analysis (CMA). These recommendations are based upon the American College of Medical Genetics (ACMG) and other clinical practice guidelines for use of CMA.

BACKGROUND

Chromosome aberrations are major causes of congenital anomalies, developmental delay (DD), mental retardation (MR), neurologic abnormality, or autism spectrum disorder (ASD), abnormal sexual development, recurrent pregnancy loss, and infertility.¹,²

Although microscopic chromosome aberrations are present in only 0.014% of neonates,² they are present in 50 - 70% of products of conception (POC) after spontaneous, first-trimester pregnancy loss,³ in at least one partner in 2 - 6% of couples with infertility,²,⁴ in 7% of men with infertility, and in 3 - 12% of children evaluated for DD or MR.⁵-⁷ Down syndrome is the most commonly diagnosed cause of MR.⁸ Aneusomy is almost always lethal;² the few aneusomies that are compatible with life are associated with high spontaneous abortion rates.² For example, the rate of spontaneous abortion is 99% when the fetus has Turner syndrome and 21% when the fetus has 47,XXX or 47,XY.² In men with infertility, Klinefelter syndrome accounts for the majority of chromosome aberrations.⁷

Clinically significant microscopic chromosome aberrations usually involve aneusomy or trisomy, but may also involve structural rearrangements such as deletions, duplications, translocations, inversions, and other abnormalities.¹,² Chromosome rearrangements are termed balanced when the normal complement of genetic material is present, and unbalanced when it is not.² Unbalanced chromosome rearrangements usually cause symptoms of a genetic disorder, such as MR associated with submicroscopic telomeric rearrangements.² In contrast, balanced chromosome rearrangements usually cause no symptoms in the carrier, but may result in unbalanced rearrangements in the carrier’s sperm or eggs, causing recurrent pregnancy or newborn loss, or infertility.² Some chromosome rearrangements appear to have no clinical significance.²

For the purposes of this policy:

- Developmental delay (DD) describes children aged <5 years with significant delay in development of motor, language, cognitive, social, and self-care skills, compared to their same-aged peers.⁸
- Infertility is diagnosed when a couple is unable to conceive after ≤ 1 year of unprotected
sexual intercourse; the cause may be male factors, female factors, or both.⁷

- Mental retardation (MR) is diagnosed in individuals aged ≥ 5 years who exhibit deficits in intelligence, adaptation, and self-care.⁸

- Recurrent pregnancy loss is diagnosed after a couple experiences ≥ 2 pregnancy losses prior to fetal viability (< 24 weeks of gestation).³

### CLINICAL EVIDENCE

Routine chromosome analysis, also known as conventional chromosome analysis, karyotyping or standard cytogenetic analysis, has played a central role in clinical genetics evaluations since the 1970s, and is used to diagnose genetic disorders characterized by abnormal chromosome number or other microscopic chromosome aberrations.⁹ Diagnosis improves outcomes for individuals with genetic disorders and their families, helps patients and their families receive appropriate therapeutic and psychosocial support, and assists clinicians in identifying appropriate treatment, support, prognosis, and risk of transmission.⁵

G-banding, the most common DNA staining method for chromosome analysis,² produces characteristic patterns of dark and light bands on condensed chromosomes from mitotic cells. Usually, G-banding is performed on mitogen-stimulated lymphocytes from peripheral blood that are chemically arrested in mitosis. The chromosomes are stained with Giemsa, treated with trypsin, and examined microscopically, usually at 1,000X magnification.¹,² The International System for Human Cytogenetic Nomenclature (ISCN) provides a standardized format for reporting the results of G-banding, and of other banding techniques (see list below).²,¹⁰,¹¹ Routine chromosome analysis with G-banding visualizes a total of 400 to 550 light and dark bands in each set of chromosomes (haploid karyotype), which allows detection of chromosome aberrations involving > 5,000,000 contiguous base pairs (> 5 Mb) in any of the chromosomes.¹ Enhancement of G-banding techniques makes it possible to do high resolution chromosome analysis (> 550 bands), which allows detection of chromosome aberrations involving DNA segments > 2-3 Mb.² High resolution chromosome analysis is more sensitive, but is more time-consuming and costly than routine chromosome analysis.¹,²,⁶,⁹

To further evaluate chromosome aberrations observed with G-banding, laboratories may use other banding methods, such as Q-banding, C-banding, and nucleolar organizing region (NOR) staining of chromosomes.² Q-banding, C-banding, and NOR staining may be used to evaluate morphological differences in chromosomes caused by variations in satellite DNA.² These techniques are recommended less often since the advent of chromosome microarray analysis (CMA).

In most patients, any tissue with dividing cells is a potential source of genetic material for chromosome analysis, because the vast majority of all chromosome aberrations are constitutional.¹ Peripheral blood is a convenient and appropriate source of cells in neonates and older individuals, but tissues other than blood are evaluated in some cases.² For example, a chromosome aberration occasionally arises in one embryonic cell after conception and results in a mixed population of body cells, which is called mosaicism.¹ Routine chromosome analysis may detect mosaicism only 14% of the time, so diagnosis of this condition may require analysis of several tissues.¹ The source of fetal cells for prenatal chromosome analysis may be amniotic fluid or chorionic villi. Chromosome analysis may also be performed on the POC after spontaneous abortion or stillbirth, if viable cells are available.¹
Some chromosome aberrations are acquired, not constitutional.\textsuperscript{1} For example, chromosome aberrations are present in most cancers. Thus, chromosome analysis of malignant cells may be used to identify microscopic chromosome aberrations for diagnosis, or as an indicator of prognosis.\textsuperscript{2}

Chromosome studies have also been indicated in the diagnostic testing workup for fertility problems.\textsuperscript{1, 2} Chromosomal causes rank high among the causes of infertility. It has also been noted that men with idiopathic infertility should be karyotyped and have Y chromosome molecular testing performed prior to the initiation of fertility treatments particularly in azoospermic and severe oligozoospermic men.\textsuperscript{12} The European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have described the occurrence of microdeletions in infertile patients and the molecular diagnosis of deletions has become an important test in the diagnostic workup of male infertility.\textsuperscript{12}

Since the 1990s, clinicians have used fluorescence in situ hybridization (FISH) to evaluate specific loci for submicroscopic aberrations (usually > 100 kb).\textsuperscript{1, 10} FISH can reveal information not available with chromosome analysis, but can only evaluate region(s) of chromosomes that are targeted by fluorescently-labeled nucleotide probe(s), which are chosen by the clinician.\textsuperscript{9} The resolution obtained with FISH depends on the choice of probe(s).\textsuperscript{1, 9} FISH is performed on cells immobilized on glass slides; the nucleotide probes are hybridized to complementary sequences in the cells’ denatured chromosomal DNA.\textsuperscript{9} FISH can provide information about microscopic or submicroscopic aberrations in specific loci, repetitive DNA, satellite DNA, or subtelomeric regions of chromosomes.\textsuperscript{9} In particular, FISH is used to detect certain submicroscopic, subtelomeric rearrangements associated with DD or MR; these are present in many patients with apparently normal results from chromosome analyses.\textsuperscript{9} FISH is also used to identify marker chromosomes, monitor or identify clones in patients with leukemia or other cancers, and investigate mosaicism by examining tissues other than blood. Targeted FISH targeted is also used to study familial correlations after CMA identifies a chromosome aberration in a family member.\textsuperscript{10}

CMA is also known as molecular karyotyping or molecular cytogenetic testing, and was introduced in the late 1990s.\textsuperscript{13, 14} It is an evolving technology with the potential to detect and identify chromosome aberrations throughout the genome at much higher resolution than is possible with routine or high resolution chromosome analysis, and with more flexibility than FISH.\textsuperscript{2, 8} A SNP Microarray is an oligonucleotide-based microarray and is discussed further in Clinical Policy number PDS-012, CHROMOSOME, SNP MICROARRAY.

**Guidelines and Recommendations**

This section summarizes recent, evidence-based guidelines for clinical use of routine chromosome analysis (constitutional). Recommendations for individuals with congenital anomalies, DD, MR, or ASD have changed recently. Earlier guidelines recommended routine or high resolution chromosome analysis for first-tier clinical genetics evaluation of these patients,\textsuperscript{5, 8, 9} but more recent guidelines recommend CMA for this step in most patients.\textsuperscript{10, 15, 16} These guidelines recommend use of routine chromosome analysis as a diagnostic test, not as a screening test.

*American College of Medical Genetics (ACMG)*\textsuperscript{5, 15, 16}

ACMG published practice guidelines for detecting chromosome aberrations in patients with DD or MR in 2005,\textsuperscript{9} 2007,\textsuperscript{16} and 2010.\textsuperscript{15} The 2007 and 2010 ASMG guidelines were updates to the 2005 ACMG guideline, and provided
recommendations for including CMA in the clinical genetics evaluation of this population; the 2005 ACMG guideline did not discuss this new technique.

The 2010 ACMG guideline stated that a combination of conventional chromosome analysis and FISH may be appropriate as a first-tier genetic test in the following settings:

- Confirmation of diagnosis with a previously described chromosome aberration
- Family history of chromosome aberration
- Recurrent pregnancy loss
- Patients who may have low-level mosaicism or polyploidy

The 2010 ACMG practice guideline recommended CMA as the first-tier genetic test in patients with unexplained congenital anomalies, DD, MR, or ASD. The exceptions were children with unexplained congenital anomalies, DD, or ASD, who also had unbalanced rearrangements identified with first-tier CMA; and parents of these children. For these 2 groups, the 2010 ACMG practice guidelines recommended high resolution chromosome analysis and ancillary FISH.

American Congress of Obstetricians and Gynecologists (ACOG)4

The ACOG Committee on Practice Bulletins recommended that both partners in all couples with recurrent pregnancy be offered routine chromosome analysis, and cited level C evidence (consensus and expert opinion) supporting use of this analysis to detect balanced chromosome rearrangements. ACOG also stated that many clinicians recommend chromosome analysis of POC after spontaneous pregnancy loss, if possible.

International Standard Cytogenomic Array Consortium (ISCA Consortium)10

The ISCA Consortium, a group of 19 independent experts in clinical genetics, including clinical geneticists, genetic counselors, genome scientists, and bioinformatics specialists, collaborated to compile a consensus statement regarding the clinical use of CMA in individuals with unexplained congenital anomalies, DD, or ASD. As part of this process, the group compared the clinical utility of chromosome analysis with that of CMA in this population. 10

The ISCA Consortium recommended chromosome analysis with G-banding in patients with unexplained congenital anomalies, DD, MR, or ASD in the following settings only:

- Family history of balanced chromosome rearrangements
- Recurrent pregnancy loss

The ISCA Consortium recommended either chromosome analysis with G-banding or CMA in patients who may have low-level mosaicism (< 10% of cells). This group recommended that CMA be the first-tier genetic test in patients with unexplained congenital anomalies, DD, or ASD.

American Society for Reproductive Medicine (ASRM) and Society for Assisted Reproductive Technology (SART)17-19

In 2008, a combined ASRM and SART Practice Committee presented guidelines for genetic testing of gamete and embryo donors based on recent information available regarding genetic diseases, among other information. 17 The Practice Committee recommended that routine chromosome analysis of donors be considered optional, but
recommended this test be performed in donors suspected to be carriers of a genetic disorder. The Practice Committee stated that most individuals with a genetic disorder or known unbalanced chromosome rearrangement should not be gamete or embryo donors. Similarly, the Practice Committee recommended that couples including a partner with abnormal results from routine chromosome analysis should receive genetic counseling before undergoing ICSI.

In January 2013, a revised committee opinion document was published. The 2013 version includes information from the American Association of Tissue Banks, the US Food and Drug Administration, and the US Centers for Disease Control and should replace the previous version. However, the 2013 revisions did not alter the chromosome analysis recommendations or information provided in the 2008 version.

National Society of Genetic Counselors (NSGC)

According to the Inherited Pregnancy Loss Working Group of the NSGC, class III evidence (published literature, clinical experience, and consensus of experts) supports performance of routine chromosome analysis of each partner in a couple experiencing recurrent pregnancy loss, this practice is standard, and this analysis should be performed on POC, if possible.

American Society for Reproductive Medicine (ASRM) and American Urological Association (AUA)

A joint report from the Male Infertility Best Practice Policy Committee (AUA) and Practice Committee (ASRM) recommended routine chromosome analysis and Y chromosome analysis in men with low sperm counts prior to intracytoplasmic sperm injection. The goal of the report was to provide clinicians with a guideline based on published literature, clinical experience, and consensus among experts. This guideline states that:

- Karyotyping and Y-chromosome analysis should be offered to the male who has nonobstructive azoospermia or severe oligospermia prior to performing ICSI. Genetic counseling may be offered whenever a genetic abnormality is suspected in either the male or female partner and should be provided whenever a genetic abnormality is detected.

US FOOD AND DRUG ADMINISTRATION (US FDA)

The FDA does approve some devices for chromosome analysis, however some tests may be laboratory developed and performed by various CLIA licensed laboratories. Platforms, assay protocols, and analysis systems may vary.

CENTERS FOR MEDICARE AND MEDICAID SERVICES (CMS)

For Medicare populations only, CMS often does not cover screening tests.

CMS reimbursement may be limited and providers should refer to their state’s specific guidelines.
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<td>88262</td>
<td>Chromosome analysis; count 15-20 cells, 2 karyotypes, with banding</td>
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<tr>
<td>88291</td>
<td>Molecular cytogenetics, interpretation and report</td>
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REFERENCES


14. Shaikh TH, Oligonucleotide arrays for high-resolution analysis of copy number alteration in mental retardation/multiple congenital anomalies. Genetics in medicine: official journal of the American College


**POLICY HISTORY/REVISION HISTORY**

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<td>10/23/2014</td>
<td>Reference added in reference section: Recommendations for gamete and embryo donation: a committee opinion. Fertil Steril. 2013 Jan;99(1):47-62. Within the body of the policy, in the &quot;Guidelines and Recommendations&quot; section, the following was added: “In January 2013, a revised committee opinion document was published. The 2013 version includes information from the American Association of Tissue Banks, the US Food and Drug Administration, and the US Centers for Disease Control and should replace the previous version. However, the 2013 revisions did not alter the chromosome analysis recommendations or information provided in the 2008 version.”</td>
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