Technical Brief

Chromosomal Microarray OS (oligo-based)

Background Information

Evidence-based studies with the aid of technical developments in molecular cytogenetics are rapidly changing the way the human genome is being analyzed and interpreted. Until recently, cytogenetics evaluation has relied to a great extent on chromosome banding techniques for global genome analysis of chromosomal abnormalities at a resolution of five to 10 megabases (Mb). The advent of chromosomal microarray analysis (CMA), also known as array comparative genomic hybridization (aCGH), has changed the field of cytogenetics dramatically. Microarray CGH has been developed to identify large regions of DNA losses (deletions) or gains (amplifications). Such alterations in the DNA are often involved in both constitutional (germline) and acquired (somatic) conditions or disease. Microarray CGH is based on the use of differentially labeled test and reference genomic DNA samples that are simultaneously hybridized to DNA targets arrayed on a glass slide or other solid platform.

The NimbleGen CGX array contains 720,000 oligonucleotides that cover every region known to be involved in cytogenetic abnormalities, including over 200 recognized genetic syndromes, over 980 gene regions of functional significance in human development covering with an average resolution of > 50 kb within targeted regions, the pericentromeric regions, and the subtelomeres. This array platform contains DNA sequences representing specific regions of the human genome designed to detect copy-number variation (loss or gain of DNA). This platform offers excellent performance and exceeds current guidelines for specificity, sensitivity, and resolution across the genome.

Fluorescence in situ hybridization (FISH) or routine chromosome studies on parental blood specimens may be recommended in order to identify familial rearrangements or variants detected by microarray.

Clinical Indications

In the pediatric population many abnormal phenotypes are associated with chromosomal imbalances that can be identified using microarray analysis to detect copy number changes (CNC). Thus, whole-genome CMA has become the first-tier diagnostic test for the evaluation of children with unexplained developmental disabilities, intellectual disabilities, dysmorphic features, congenital anomalies and autism. Based on numerous published studies, the yield of pathogenic or clinically significant CNC by CMA is approximately 15-20% in a pediatric population, compared with a yield of 3-5% by standard cytogenetic analysis in the same population. Variants of uncertain clinical significance (VOUS), or clinical significance unknown, are found in less than 10% and could play an important role in the clinical diagnosis. To a great extent, parental and family studies can be helpful in the clinical interpretation of these unknowns, as de novo occurrence of the CNC is more likely related to a pathogenic event. CMA testing for CNC is recommended as a first-line test in the initial postnatal evaluation of individuals with the following:

- Multiple anomalies not specific to a well-delineated genetic syndrome
- Apparently nonsyndromic DD/ID
- Autism spectrum disorders.

Further prospective studies and aftermarket analyses are needed to evaluate the use of CMA testing in the diagnosis of children with growth retardation, speech delay and other less well-studied indications.

Appropriate follow-up is recommended in cases of chromosome imbalance identified by CMA to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetics consultation. [Please note: To avoid confusion, the term CNC has been used in this article rather than CNV, as copy number variant (CNV) or “Variants” are generally considered alterations or uncommon forms of no clinical significance.]
Interpretation

A written summary and interpretation of the microarray findings are provided in the Test Overview. Gains and losses are reported based on genomic content; duplications smaller than 700 kb and deletions smaller than 500 kb may not be investigated or reported. Copy number variations (CNV) devoid of relevant gene content or reported as common findings in the general population may not be reported. A copy-number change of uncertain clinical significance may be detected.

The NimbleGen CGX Cytogenetic arrays design (via Signature) was built from the analysis of more than 40,000 cytogenetic samples. The NimbleGen CGX array has average probe spacing of one probe every 35 kb throughout the genome and one probe every 10 kb in regions of clinical relevance. Probes along the X and Y chromosomes detect sex chromosome numerical abnormalities. A complete list of targets is available upon request. This microarray will detect aneuploidy, deletions and duplications of the loci represented on the microarray. It will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions and balanced insertions), point mutations or imbalances of regions not represented on the microarray; it may not detect low levels of mosaicism. The laboratory can assist the clinician in determining whether other testing is appropriate. This discussion should be considered in the context of the clinical phenotype. The failure to detect an alteration at any locus does not exclude the diagnosis of any disorders represented on the microarray.

Array CGH Data: Tetrasomy 18p

Karyotype: 47,XY,+i(18)(pter—p10::p10—pter)

Extra chromosome 18 composed of two copies of the short arm of chromosome 18

Clinical indication: Developmental delay

Result: Abnormal (gain) for the short arm of chromosome 18 and the findings verified by chromosome analysis.
Methodology

Comparative genomic hybridization-oligo based on the NimbleGen CGX-3 array platform is utilized. Copy number changes are calculated based on hybridization signal intensity data from the gender matched control DNA. Data is analyzed using Genoglyphix software from Signature Genomics.

In aCGH, two genomic DNAs, a test and a reference, are fluorescently labeled with different dyes and competitively hybridized to the array probes. The relative fluorescence intensities are quantified and the ratio is calculated of the test and reference hybridization signals. Probes for the aCGH are oligonucleotides that represent areas of interest across the genome.

Figure 2.
Array CGH Data: Trisomy 21 (Down Syndrome)
Karyotype: 47,XX,+21

Clinical indication: Rule out Down Syndrome
Result: Abnormal (gain) detected by CMA depicted in figure 2, verified by chromosome analysis and found to be a pure trisomy 21.

Metaphase FISH or chromosome analysis will be performed to verify the changes detected by CMA whenever possible.

Chromosomal Microarray Analysis (CMA) being offered has accurately identified known cytogenetic abnormalities involving whole chromosomes, isochromosomes and, interstitial duplications and deletions in 100% of the specimens examined. In paired-samples, it has correctly identified all the CNCs associated with clinical abnormalities that have been documented for 100% of the specimens examined at the Signature Genomics (SG). The sensitivity of the NimbleGen assay to detect mosaicism is —30% with Genoglyphix default settings (it can be — 20% by visual examination of whole genome plot/individual chromosome plot). The assay has a high degree of precision (coefficient variation (CV) for log2 ratio of specific CNV. CMA is offered on the basis of extensive study involving analytical performance metrics.

References


# Test Overview

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<thead>
<tr>
<th>Test Name</th>
<th>Chromosomal Microarray OS (oligo-based)</th>
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<tbody>
<tr>
<td>Specimen Requirements</td>
<td>4 mL (minimum 1 mL in newborns) whole blood EDTA (lavender top) and 4 mL (minimum 1 mL in newborns) whole blood in sodium heparin (green-top) tube, shipped at room temperature. High-quality DNA from blood may also be accepted. A minimum of 1 μg of DNA (concentration between 100 ng/μl and 400 ng/μl) will be required, with an OD260/280 ratio of &gt;1.8 and an OD260/230 ratio of &gt;1.8. Also, send 4 mL (minimum 1 mL in newborns) whole blood in sodium heparin (green-top) tube, shipped at room temperature. All samples must have a clinical indication for testing. Please indicate this is for chromosomal microarray analysis (CMA) oligo-based testing. Additional test information can be found in the test directory at <a href="http://portals.ccf.org/SearchDetails/tabid/4476/Default.aspx?ID=4546">http://portals.ccf.org/SearchDetails/tabid/4476/Default.aspx?ID=4546</a></td>
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<td>Testing Information</td>
<td>Turnaround time (TAT) 8-9 days; results requiring follow-up studies for the completion of microarray testing may exceed the standard TAT.</td>
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<td>CPT Codes</td>
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