4 Cataraqui Street, Suite 310, Kingston, ON K7K 1Z7 |
info@ccmg-ccgm.org | www.ccmg-ccgm.org |
613-507-8345

CCMG Guidelines for Genomic Microarray Testing

Submitted by:
CCMG Cytogenetics Committee

Approved by:
CCMG Board of Directors

Prepared by:
James Stavropoulos and Mary Shago
Hospital for Sick Children
with contributions from the Canadian microarray user group:

Bruyere H; Chan M; Chernos J; Chun K; Cote G; Craddock K; Dawson A; Duncan A; Eydoux P; Feuk L; Finzel R; Freeman V; George A; Gillan T; Halbgewachs J; Harrison K; Hrynchak M; Kolomietz E; Lavoie J; Lemyre E; Lu C; Marshall C; McCready E; McGowan-Jordan J; Morash B; Mueller R; Parslow M; Pinto D; Scherer SW; Shetty S; Soucy J-F; Speevak M; Wang, J; Winsor E; Xu J.

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CCMG Laboratory Practice Committee

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1. Genomic Microarray - Capabilities

- Array comparative genomic hybridization using platforms with bacterial artificial chromosomes (BAC) or non-polymorphic oligonucleotide probes can detect:
  - Copy number gains and losses across the genome i.e. unbalanced microscopic and submicroscopic chromosome rearrangements.
- Microarray platforms containing single nucleotide polymorphism (SNP) probes can detect:
  - Copy number gains and losses across the genome i.e. unbalanced microscopic and submicroscopic chromosome rearrangements.
  - Triploidy.
  - Long contiguous stretches of homozygosity also known as Absence of Heterozygosity (AOH) (Kearney HM et al. 2011; Papenhausen et al. 2011).
  - AOH affecting multiple regions of the genome may be indicative of parental consanguinity.
  - AOH confined to a single chromosome in the absence of parental consanguinity may be suggestive of UPD for that chromosome. However, this result may also be observed in the absence of UPD and should therefore be followed up with standard molecular testing (e.g. methylation MLPA) to confirm whether there is UPD, if clinical features are consistent with an imprinting disorder.
  - SNP microarray testing is not a suitable replacement for UPD testing by standard molecular methods since it will not detect all forms of UPD.

- Resolution:
  - Depends on probe size, number, and the placement of probes across the genome.
  - Is determined by the software algorithm and settings selected by the user to detect copy number alterations.

2. Genomic Microarray Limitations

- Array comparative genomic hybridization using platforms with BAC or non-polymorphic oligonucleotide probes cannot detect:
  - Balanced rearrangements, long contiguous stretches of homozygosity, low level mosaicism of unbalanced rearrangements/aneuploidy, and polyploidy.
- Microarray platforms containing SNP probes cannot detect:
  - Balanced rearrangements, low level mosaicism of unbalanced rearrangements/aneuploidy, tetraploidy .

3. Indications

- For Constitutional Postnatal Indications – Practice guidelines should refer to the Canadian College of Medical Geneticists (CCMG) Position Statement on the use of array genomic hybridization, developed by Clinical Practice, Cytogenetics, and Prenatal Diagnosis committees.
- Clinical indications for constitutional postnatal microarray include:

  - Idiopathic intellectual disability/developmental delay/autism/multiple congenital abnormalities.
  - Apparently balanced inherited or *de novo* rearrangements in a phenotypically abnormal individual.

- Malignancies:
  The utility of microarrays designed for use in the analysis of bone marrow, soft and solid tumors, as well as paraffin embedded tissue is currently being studied by the Cancer Genomics Consortium (CGC) Clinical trial group. For general guidelines regarding implementation of genomic microarray for oncology specimens, the CCMG endorses the *American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders* (Cooley et al. 2013).

4. Requisition Requirements

1. Patient name and address
2. Patient date of birth
3. Patient sex
4. Unique identifying number
5. Name of physician or other authorized person requesting test
6. Specimen source
7. Specimen collection date
8. Test requested
9. Clinical indications for the test(s)
10. Ethnicity – polymorphic CNV frequencies may differ between populations

5. Specimen Requirements

DNA extracted from each tissue type must be validated at the discretion of the laboratory director, since performance characteristics and sensitivity may vary between DNA samples extracted from different tissues.

- Peripheral blood:
  - Two specimens in appropriate anticoagulants – one for DNA extraction and when required, one for cytogenetic preparation to confirm or further characterize positive findings by FISH/G-banding .
- Tissue:
  - Cultured primary fibroblasts with low passage number, saliva, or buccal swab.

- Caution: analysis of transformed cell lines (i.e. EBV transformed lymphoblasts) should be avoided for clinical studies since there is an increased risk of detecting genomic imbalances acquired during transformation/culturing.

6. Platform Requirements - Minimum

- The manufacturer must provide details regarding the distribution and sequence of all probes, as well as quality control measures performed prior to shipment of new lots of arrays.
- Constitutional Postnatal:
  - Genome-wide backbone coverage at a minimum effective resolution of 400 Kb.

- o Oligonucleotide-based array platforms with non-polymorphic probes or platforms which combine SNP and non-polymorphic probes are recommended.

7. Reference DNA

- The laboratory must establish a reliable source of male and female reference DNA – either commercially available mixtures or internal review board (IRB) approved volunteer source are acceptable.
- Longitudinal consistency of reference DNA is recommended.
- For constitutional microarray studies, sex - matched comparisons with patients' DNA are recommended.

8. Procedure

- The laboratory must have written procedures and a quality management program for all aspects of microarray testing.
- The laboratory must document all analytic parameters in the patient record.

9. Monitoring of Analytic Standards

- Pre-analytic:
  - o Evaluate the quality of DNA (e.g. concentration/quality by fluorometer/spectrophotometer and by agarose gel electrophoresis).
  - o Document equipment monitoring and maintenance.
  - o Validate new lots of arrays by repeating hybridization of an abnormal sample analyzed by the previous lot of arrays. Data quality measures should be used to inspect the quality of new lots of arrays.
  - o Compare each new lot of reference DNA to a previous lot by testing a positive case.
  - o Assess quality parameters for each lot of control DNA in the same manner as the patient DNA (e.g. concentration/quality by fluorometer/spectrophotometer and by agarose gel electrophoresis).
  - o Analytic:
    - Assess fragmentation of DNA by sonication/enzyme digestion if applicable (e.g. by agarose gel electrophoresis)
    - Assess labeling efficiency of DNA samples (e.g. spectrophotometer).
  - o Post-analytic:
    - Perform visual inspection of array image if possible, to check for hybridization of probe mixture across the entire array – check for uneven hybridization due to stationary bubbles or leaks.
    - Ensure that there is no significant wave artifact in the microarray log2 ratio plot, which may potentially result in missed abnormality calls.
    - Evaluate QC data calculated from analysis software and establish minimum requirements to proceed with data interpretation.
    - Monitor ongoing FISH/QPCR/MLPA validation of genomic gains/losses called by the microarray software algorithm.

10. Analysis of Microarray Data

- The laboratory geneticist should be familiar with the principles of the algorithm/software processing the data.
- Establish the appropriate software algorithm and parameters for the diagnostic

assay during the initial validation of the microarray platform.
- Re-analyze all data used for initial validation if switching to an alternate analysis software/algorithm.
- Determine the sensitivity of the assay to detect mosaicism OR indicate in the report the limitations of the assay to detect mosaicism.

11. Interpretation of Microarray Data

- The laboratory geneticist should be familiar with current literature and databases available for interpretation of CNV data, and must interpret patient results using tools such as PUBMED, UCSC Genome Browser (http://genome.ucsc.edu/), Online Mendelian Inheritance in Man (OMIM) (www.omim.org) Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home), DECIPHER (https://decipher.sanger.ac.uk/browser), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), ClinGen (http://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/ and ECARUCA (www.ecaruca.net).
- The laboratory should establish an internal database to identify common CNVs specific to their patient population and/or recurrent false-positive calls associated with the particular microarray platform (Qiao et al. 2008; Friedman et al. 2009).
- The laboratory geneticist must ensure the databases used for interpreting CNVs are based on the same reference human genome build (e.g. NCBI 36, March 2006 versus NCBI 37 February 2009), as the data generated from the array platform.
- Interpretation of a CNV should consider the gene content, size of the imbalance, whether it is inherited or *de novo*, complete or partial overlap with a known clinically relevant region, or whether it has been reported in databases of healthy control populations (Rodriguez-Revenga et al. 2007; Friedman et al. 2009; Koolen et al. 2009). As the data generated from healthy control populations is often not validated, a specific CNV region should appear in at least two independent studies to be considered a common benign variant.
- The laboratory geneticist should be aware of the current information regarding novel recurrent CNVs that may be associated with susceptibility to developmental disorders such as 16p11.2 (Shinawi et al. 2010)), but with limited information regarding penetrance, expressivity and recurrence risk. These associations must be interpreted appropriately; and family studies to determine the segregation of the CNV with disease state are recommended.
- When possible, FISH and/or G-band analysis is recommended to provide structural information of clinically significant CNVs (e.g. insertion, tandem duplication, marker).
- Microarray, QPCR or MLPA analysis can be used to perform parental follow-up studies to determine the inheritance of CNVs. FISH studies of parental samples should be performed when possible, for suspected *de novo* CNVs to investigate the possibility of a parental balanced rearrangement.
- For parental follow-up studies, FISH or targeted molecular techniques should be preferred over whole genome array testing.

12. Reporting Results

- The report should be written with the assumption that it will be read by Geneticists and non-Geneticists. The report must include the genomic coordinates and size of CNVs with information regarding the gene content of the affected genomic region. The number of known genes, as well as identification of clinically significant genes (e.g. OMIM Morbid Map Genes;

Amberger et al. 2015) should be included in the report. Copy number variants that are likely benign, do not need to be included in the final report. A brief description of the laboratory reporting standards for this type of CNVs should be stated in the final report.

- The deletion or disruption of genes associated with a autosomal recessive disorders that are consistent with the patient's phenotype should be reported with recommendations for analysis of the unaffected allele. Although rare, microarray analysis has led to diagnosis of a recessive disorder by unmasking of a recessive mutation (Ghai et al. 2011).
- The laboratory should have a policy for reporting carrier status of a gene associated with an autosomal recessive disorder. Although reporting carrier status is not generally recommended, it may be reported for disorders with a high carrier frequency in the population being tested.
- Laboratories using a microarray platform that includes SNPs should have a policy in place for reporting regions of AOH.
  - Multiple genomic regions of AOH due to consanguinity:
    - The laboratory should establish criteria for including regions of AOH in the report (e.g. proportion of autosomal genome that is homozygous is $\geq$ 3%), as well as a minimum size cut-off of AOH regions to be reported (e.g. $\geq$ 5 Mb).
    - It is recommended that the following information be provided in the report: *Multiple chromosomes with regions of AOH are often seen in normal individuals and are typically associated with parental consanguinity or ancestry from an isolated population. This result is not diagnostic but raises the possibility of a recessive disorder due to a homozygous mutation within a region of AOH.*
  - AOH affecting a single chromosome in the absence of consanguinity:
    - The laboratory should establish a minimum size threshold for reporting AOH affecting a chromosome known to be associated with an imprinting disorder (chromosomes 6, 7, 11, 14, and 15).
    - There is not sufficient evidence to determine the minimum size of AOH that is most predictive of UPD, however current practices commonly use greater than 8 to 10 Mb for reporting purposes.
    - Since AOH affecting one chromosome does not necessarily correlate with actual UPD, standard molecular testing for UPD (e.g. analysis of polymorphic markers in proband and parents/methylation studies) should be pursued if clinical features are consistent with an imprinting disorder.

- Elements of the report should include:
  - Patient demographics as in other cytogenetic reports.
  - ISCN nomenclature describing the result of the analysis.
  - A written description of the results indicating clinical significance, gene content (e.g. number of known genes, list of OMIM Morbid Map genes), size and location of imbalance, and follow-up recommendations.
  - A description of the array platform with information regarding probe coverage and the effective resolution of analysis across the genome. If the effective resolution in regions known to be clinically significant differs from the remainder of the genome, this information should be provided.
  - The genome build used as the reference (e.g. GRCh37).
  - The software program used for analysis of the microarray data.

  - Information regarding control DNA or *in silico* control reference data set used in the microarray analysis.
  - Information regarding limitations of microarray testing e.g. mosaicism, balanced rearrangements, etc.
- Qualifications for reporting
  - The laboratory geneticist should be CCMG/ABMG certified in clinical cytogenetics and/or molecular genetics
  - The laboratory geneticist should be familiar with the principles of chromosome structure, heteromorphisms, chromosomal imbalance and cytogenetic nomenclature.
  - If microarray technology is used in the analysis of malignancies, the laboratory geneticist should be CCMG/ABMG certified minimally in clinical cytogenetics, or have extensive training in molecular pathology.

13. Turnaround Time

- Routine Constitutional – 90% of samples should be reported within 4 weeks of specimen collection.

Expedite (Newborn) - 90% of samples should have a preliminary report with non-validated microarray results within 2 weeks.

14. **Documentation to be Maintained in the Laboratory Records**

- Quality indicators i.e. DNA sample quality, labeling efficiency, microarray QC measures
- Lot numbers for all reagents
- Equipment maintenance records
- All validated and non-validated abnormalities called by microarray software
- Failed experiments and repeats
- Maintained for 20 years

15. Proficiency Testing

- Required – minimum 4 samples per year
- CAP/QMPLS suggested as PT providers

16. Suboptimal specimens

- When possible, a repeat specimen should be obtained.
- If a repeat sample is not available (e.g. post-mortem sample) microarray analysis may be performed with the limitations of the results indicated in the body of the report.

17. Validation of platform

- Validation is required when the laboratory is introducing the technique as a diagnostic test or when changing platforms:
  - Correct identification of thirty known abnormal specimens is suggested as a minimum requirement.

- For validation of an enhanced (updated) version of a microarray platform
  - Correct identification of 5 known abnormal samples is suggested.

18. Other Guidelines

- American (Shaffer et al. 2007) and European (Vermeesch et al. 2007) guidelines

- Clinical Laboratory Standards Institute (CLSI) document MM12-A (2006)
- International Standard for Cytogenomic Arrays (ISCA) consensus statement (Miller et al. 2010)
- ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013 (South et al. 2013)

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