



Information for Medical Staff on Microarray Testing

Molecular Karyotyping, also called Microarray analysis, screens patient DNA samples for genomic imbalance (chromosomal losses/deletions and gains/duplications) across the entire human genome. Microarray increases the detection rate of pathogenic chromosomal imbalances to approximately 15% as compared to approximately 3% using standard cytogenetics. Microarray analysis should now be considered the first line diagnostic genetic test for individuals with developmental delay/intellectual disability, autism spectrum disorder and multiple congenital anomalies.

The microarray platform used by LabPlus at Auckland City Hospital is the Affymetrix CytoScan Whole Genome 750K Array with Affymetrix Chromosome Analysis Suite (ChAS). This array provides whole-genome coverage with a density of 750,000 markers. This type of array can detect aneuploidy, chromosome copy number variants (deletions and duplications) and some intragenic deletions/duplications depending on the probe coverage. The Affymetrix 750K array includes 200,500 single nucleotide polymorphisms (SNPs) so provides additional genotyping information and can detect long contiguous stretches of homozygosity (LCSH/LOH), which may indicate consanguinity or uniparental isodisomy.

A microarray analysis will not identify intragenic mutations such as nucleotide base pair changes or very small indels. Therefore if a single gene condition is suspected in a patient, gene sequencing or a targeted gene dosage array may be required. Triplet repeat disorders such as Fragile X will not be identified using a microarray and will need to be requested separately.

This technique cannot detect balanced rearrangements or low level mosaicism, therefore this test should not be used with a family history of a balanced chromosomal rearrangement, couples experiencing infertility/multiple spontaneous pregnancy losses, or suspected mosaicism. Also it cannot be done as a rapid test, therefore other methods may be more appropriate where the patient has a recognizable syndrome and requires a more rapid result. (e.g. FISH for 22q11.2 deletion in a child due for surgery).

For advice regarding the appropriate genetic test to order please contact either the clinical genetic service (Genetic Health Service NZ-Northern Hub) or the LabPlus cytogenetics department at Auckland City Hospital (see contact details at end of this leaflet). The following examples provide guidance when choosing the appropriate initial test in common clinical scenarios.

Examples:

- Child with developmental delay/ID/autism spectrum disorders – request microarray and a separate Fragile X assay.
- Infant suspected of Trisomy 21 with typical features – follow current practice e.g. request FISH for T21 if rapid turnaround time required and standard (G-banded) karyotype to determine whether T21 is standard or the product of a translocation.
- Infant/child with multiple congenital anomalies and dysmorphism – request microarray.
- Child with suspected microdeletion/duplication syndrome e.g. Williams syndrome, 22q11 deletion – request microarray rather than specific FISH probe unless result needed within short time frame and clinical features strongly suggestive of a particular condition.
- Infant/child with suspected Prader Willi syndrome (PWS) or Angelman syndrome (AS) – in clear-cut cases request methylation studies and if these are abnormal the laboratory will direct further testing. A SNP-microarray will pick up the majority of cases of PWS and AS (deletion, uniparental isodisomy) but the turnaround time is longer. If PWS or AS are only one of many differentials, requesting microarray is likely to be more cost-effective. Please note, not all cases of Angelman will have abnormal methylation studies (refer LabPlus Test guide: Angelman Syndrome, [UBE3A]).

Limitations and Implications

The main limitation of a microarray analysis is the detection of CNVs (copy number variants) for which there is still limited information available regarding clinical significance. A variant of uncertain significance (VOUS) may be detected in up to 15% of cases. A large number of CNVs have been identified that are associated with a highly variable phenotype making it difficult to predict the phenotypic consequence of these CNVs. The interpretation of a VOUS may be aided by the evaluation of parental samples to determine whether a copy number variant is de novo or inherited. In most cases, the need for parental samples will be determined by the clinical genetic service.

In rare cases an abnormality will be detected that has implications for a child's health that is not related to the clinical indication for the microarray. For example, deletions involving cancer predisposition genes such as BRCA1/2 (familial breast and ovarian cancer syndrome) or adult onset neurological conditions such as spinocerebellar ataxia. It is important that families understand the possibility of this type of result even though the likelihood of this occurring is low. It is advised that these types of unexpected results are discussed with the clinical genetic service prior to disclosure to the family.

LCSH/LOH

A SNP-microarray can detect long contiguous stretches of homozygosity (LCSH). These represent regions of the genome that are identical on both homologues of a chromosome pair. *The laboratory will only report areas of LCSH that are above a certain length (>20Mb).* The detection of low levels of LCSH is considered a type of normal genomic variation. Areas of LCSH may be clinically relevant if they represent uniparental isodisomy e.g. Prader Willi syndrome due to maternal uniparental isodisomy for 15q11-13 will be detected on a SNP microarray. SNP probes cannot detect all types of UPD. Therefore haplotype analysis is still recommended as the first-tier test for UPD and to confirm UPD detected by array.

Widespread regions of LCSH suggest the parents of the individual tested are biologically related e.g. children born to 1st cousin mating are expected to share 1/16 of their DNA therefore a finding of 6% LCSH on a SNP-microarray is an expected result rather than an abnormality. The risk of autosomal recessive diseases is higher in individuals born to consanguineous matches but the finding of LCSH is not in itself diagnostic of an underlying disorder and in most cases is clinically benign. In some cases the finding of LCSH may direct specific genetic testing in an individual with features of a specific recessive genetic condition therefore can be a helpful adjunct to the clinical geneticist when trying to make or confirm a diagnosis.

Sample Requirements*

Adult: 8ml peripheral blood collected in EDTA (purple top) vacutainer.

Paediatric: 4ml peripheral blood collected in EDTA (purple top) vacutainer.

*Important Note: Collection of an additional sample in Lithium Heparin (for Chromosome Karyotype- cell storage) could also be considered for any future follow-up studies. This would incur a separate process fee.

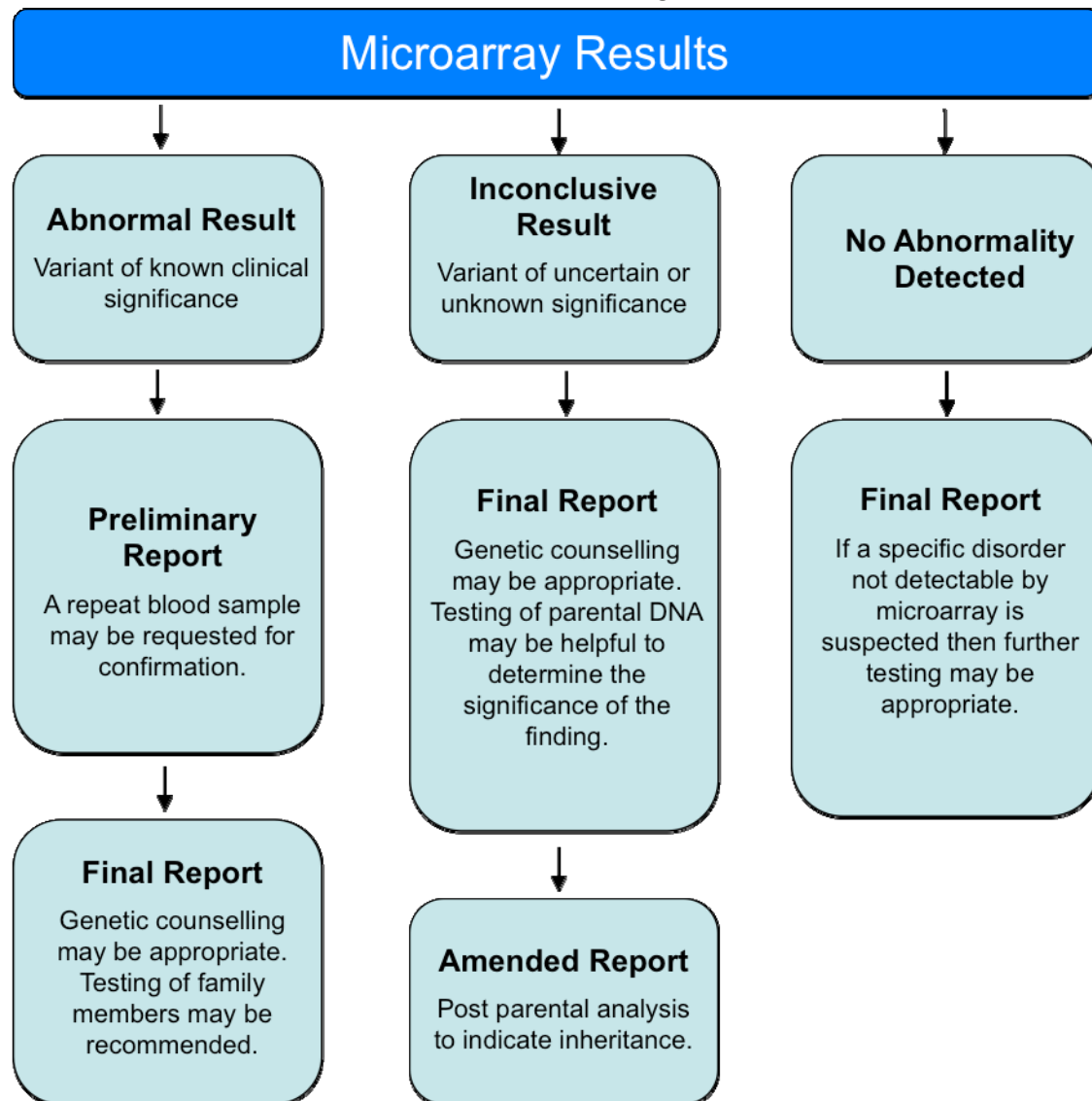
These requirements are subject to change, the most current and recommended source of collection requirements is the LabPlus Test Guide <http://testguide.adhb.govt.nz/EGuide> (test name Molecular Karyotype). Please also note the sample requirements for Molecular Karyotyping and Chromosome (G-band) Karyotyping are different and incompatible. Therefore particular care it is requested when indicating which of these test is requested on the laboratory referral form to avoid sampling errors.

Microarray Results

A microarray cannot be done as a rapid test. For current turnaround times please consult the online LabPlus test guide, but, depending on sample requirements, we aim to report samples in 4 weeks.

Microarray analysis shows a change or variation in the number of copies of genes in segments of DNA, i.e. are there more or less than the two expected copies of DNA. This is referred to as copy number variation (CNV).

The results will be classified into the three categories shown below:



When a copy number variant is detected, looking at the genes involved in the variant region assesses the significance of this variation. If a reported abnormality or significant genes are associated with the variant region the result is reported as a variant of known clinical significance. If no reported abnormality is associated with this region the result is reported as unclear. Testing of parental samples is often required to help clarify inconclusive results. Parental samples may also be requested in abnormal cases to confirm if the abnormality was inherited or de novo to assess heritability.

Microarray results are reported using ISCN nomenclature, as is chromosome karyotyping, but different nomenclature is used to explain the result. The molecular karyotype may look something like the example given below:

arr 3p26.3 (56,669-1,850,707)x1, 3q23q29 (141,829,104-199,355,203)x3

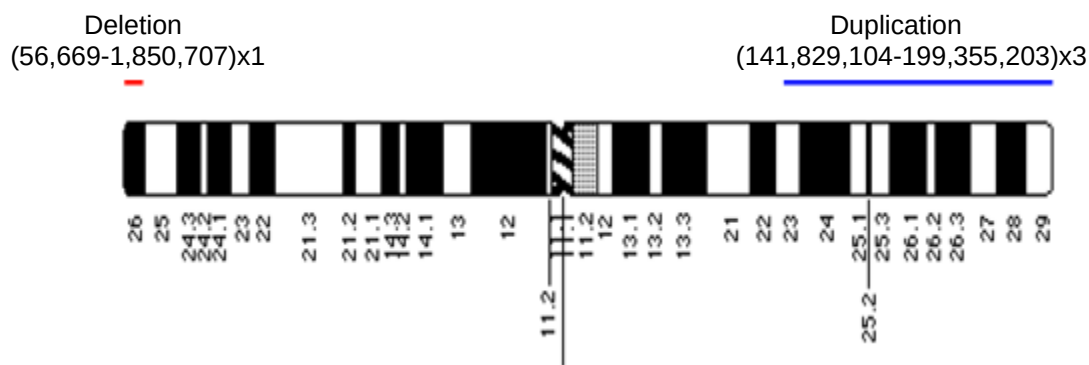
arr This is a molecular karyotype and the analysis was done by microarray

3p26.3 and 3q23q29 This is the chromosome location of the change

x1 The change was a deletion

x3 The change was a duplication

(56,669-1,850,707) The first number is the number of the first base pair shown to be missing, counting from the left of the chromosome. The second number is the last base pair shown to be missing.



Other terminology

hmz homozygosity

mat/pat denotes parental origin of the CNV

Follow-up studies from Molecular Karyotyping:

Please Note: Referral to Genetic Health Service NZ-Northern Hub for Genetic Counseling is recommended for most abnormal results and necessary before referral of samples for follow-up testing.

There are several indications for additional testing, these include:

- Parental/Family studies following detection of an imbalance in a child.
- Detection of LOH indicative of UPD.
- Equivocal results which required confirmation by another technique.

Most of these possibilities require sample recollection from the proband and/or from family members.

FISH compared to microarray for Parental Studies:

Parental studies will establish if the imbalance is de novo or inherited. Quantitative follow-up studies (such as microarray) do not differentiate between normal or balanced carrier statuses in the parents. In comparison, FISH studies will be able to identify chromosome location for deletions and larger duplications, and a balanced parental rearrangement and hence also provide assessment of recurrence risk. Therefore it is highly recommended FISH should be carried out, whenever possible. These studies will be arranged after consultation with genetic services.

We would encourage you to discuss the issues addressed above with the patient/parents prior to testing. There is also a LabPlus leaflet “Information for Patients on Microarray Testing”, available on the LabPlus Test Guide or from Genetic Health Service NZ-Northern Hub.

Contact and Information Details

<http://www.genetichealthservice.org.nz/> (Genetic Health Service NZ-Northern Hub)

Ph: (09) 307 4949 Ext. 25870

Toll Free: 0800 476 123

Email: GenSec@adhb.govt.nz

To contact the laboratory refer to the Cytogenetic contacts on the LabPlus Test Guide
<http://testguide.adhb.govt.nz/EGuide>

Further reading

- Palmer EE, Peters GB, and Mowat D. Chromosome Microarray in Australia: a guide for paediatricians. J Paediatr Child Health. 2012 48(2):E59-67.
- Hum Mutat. 2009 Mar;30(3):283-92. Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. Koolen DA et al. PMID:19085936
- Am J Hum Genet. 2010 May 14;86(5):749-64. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Miller DT *et al.* PMID:20466091
- Prenat Diagn. 2012 Apr;32(4):336-43. doi: 10.1002/pd.2933. Genomic microarrays: a technology overview. Brady PD, Vermeesch JR.