



Association for
Clinical Cytogenetics

PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS

CONSTITUTIONAL POSTNATAL CHROMOSOMAL MICROARRAY BEST PRACTICE GUIDELINES (2011) v2.00

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1 INTRODUCTION

These guidelines should be used in conjunction with all other Association for Clinical Cytogenetics Best Practice Guidelines.

Professional guidelines for Genetics laboratories incorporate the standards imposed by regulatory bodies (Clinical Pathology Accreditation (CPA) [1] and by statute (Clinical Governance) while taking into account current practice in the U.K.

Elements of the service not subject to statute may be varied in order to comply with local constraints and agreements. It must be noted that these guidelines are minimum requirements and that professional judgement is of paramount importance for many circumstances.

The use of must in this document indicates a requirement and the use of 'should' or 'may' indicates a recommendation.

Where there appears to be contradiction between available active ACC guidelines, the most recently published should be taken to apply to all.

All diagnostic Genetics laboratories must be accredited to nationally or internationally accepted standards. Laboratories must participate in an External Quality Assessment Scheme for all aspects of their service for which a scheme is available [2].

2 SERVICE OVERVIEW

Array identifies genomic imbalance at a level of resolution significantly higher than that achievable by G-band karyotyping and is currently employed to investigate selected patients with learning disabilities and dysmorphism/congenital abnormalities.

The higher resolution of genomic screening achievable by arrays has allowed more detailed evaluation of breakpoint locations and gene content.

Microarray testing may be used as a 'first line test' replacing conventional karyotyping for postnatal referrals with learning difficulties, developmental delay, dysmorphic features and/or congenital abnormalities [3, 4].

The screening resolution is determined by the array platform of choice and the analysis criteria used by the laboratory. This screening resolution should be identified to clinical users in the report.

Laboratories may wish to consider adopting array designs that have been specifically developed for clinical situations (eg by the International Standards for Cytogenomic Arrays Consortium (ISCA) [5]).

3 SAMPLE PROCESSING

Laboratories must have Standard Operating Procedures (SOPs) for all relevant techniques. SOPs must minimise the risk of sample mix-up. All manual sample transfers or loading of robotic systems must be checked by a second individual or validated by another method and suitably documented.

All processes and parameters should be validated using DNA from a range of known abnormal cases before initiating an array service.

New array designs or commercial kits must be validated as suitable for diagnostic use, using DNA from a range of known abnormal cases, prior to introduction into routine service.

3.1 DNA EXTRACTION

All methods must ensure a minimal number of tube-tube transfers and produce a standard quality of DNA that is reliable for use in array assays.

There must be internal criteria for deeming the DNA quantity or quality as unsuitable.

3.2 LABELLING AND HYBRIDISATION

All techniques employed should be subject to internal quality control.

3.3 SCANNING AND DATA EXTRACTION

Hardware and software must be suitable for the array platform and operate with appropriate levels of sensitivity and specificity to detect imbalances at or above the size cited by the diagnostic service.

There must be internal criteria for deeming the data produced as suitable for analysis.

4 ANALYSIS

Either the analyst, or the independent checker of analysis, must be a state registered Clinical Scientist. Analytical procedures and the checking systems used must be documented and specify the minimum level and experience of the staff involved, with reference to relevant scopes of practice for Clinical Scientists and Genetic Technologists.

Software packages should produce diagrammatic and numerical outputs for analysis. Software parameters must be set to ensure detection of imbalance at, or greater than, the level specified by the laboratory.

Analytical parameters should be set to ensure unequivocal identification of copy number changes at the resolution cited in the patient report.

5 INTERPRETATION AND REPORTING

It is the responsibility of the Clinical Scientist to provide a clear and unambiguous description of the array imbalance(s), and whenever possible, an explanation of the clinical implications of the results. The report will be inserted into the patient's notes and may be seen, not only by the referring clinician, but also by other healthcare workers, some of whom may not have a clear understanding of array investigations. When writing a report it is important to remember that it may also be made available to the patient. It is acknowledged that many reports will be complex and may only be fully understandable to referring Clinical Geneticists. Complex reports to other referring clinicians should include a recommendation for referral for genetic counselling.

Handwritten alterations must never be made to the report; accreditation standards insist that validation procedures are in place to ensure no alteration of reports can be made after issue.

It is not necessary to include details of practical processing, unless relevant.

Authorisation of reports must be carried out as recommended in the current version of ACC General Best Practice Guidelines [6]

Interpretation of the clinical significance of the detected genomic imbalance is influenced by the size, genetic content, position, whether de novo or inherited and the carrier parent's phenotype.

The published literature and public databases such as ENSEMBL, Database for Genetic Variants, DECIPHER, UCSC, OMIM etc should be used when evaluating the clinical significance of a detected imbalance. By its very nature, this evolving technology will produce significant numbers of results where interpretation remains difficult e.g. families with rare CNVs whose significance may be uncertain until further examples are reported. Proband samples may be referred singly or with parental blood samples, depending on local referral policy.

Records of apparently benign copy number variants detected should be retained for future review. These may be listed in the final report as required by local policy.

A preliminary report may be issued, identifying detection of an imbalance in the proband and requesting parental bloods for follow-up investigations. Comment on the clinical significance may be made in this preliminary report if a phenotypic association is supported in the published literature, otherwise it is appropriate to report the imbalance as having unknown significance in the absence of further information from parental investigations.

Follow-up studies should be performed by a suitable technique and may include karyotyping, FISH, MLPA, q-PCR or further array studies. The accuracy of the follow up technique to detect the imbalance must be confirmed on the proband prior to reporting parental samples as normal and hence reporting the change as de novo.

Follow up studies will confirm the imbalance in the proband and parental studies will establish if the imbalance is de novo or inherited. Quantitative follow-up studies do not differentiate between normal or balanced carrier status in the parents. FISH studies should be carried out, whenever possible, as these 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.

Report:

The report of a case with no significant imbalance (as defined by the laboratory screening criteria) must include the following:

- summary statement and/or karyotype designation using current ISCN nomenclature [7]
- description of array (manufacturer, array version, software used for analysis and minimum size of imbalance routinely reported)
- limitations of the test

The report of an abnormal case, issued after completion of follow-up studies, must include the following:

- summary statement and/or karyotype designation using current ISCN nomenclature [7]
- description of array (manufacturer, array version, software used for analysis and minimum size of imbalance routinely reported)
- a clear written description of the genomic imbalance
- the location of the genomic imbalance in base pairs indicated by the start and end positions of the informative probes
- the size of the genomic imbalance
- identification of genome build used
- reference to other investigations to clarify significance
- identification of methods used in follow-up studies
- clinical interpretation to include (as appropriate):
 - a) the gene content * of the genomic imbalance including the name of any known syndrome(s) in the region
 - b) if possible to determine, whether the result is consistent with the clinical findings and/or an indication of the expected consequences of the abnormality.
 - c) request for follow up of family members at risk of the abnormality, starting with closest available relatives
 - d) if possible to determine, an assessment of risk/recurrence.
 - e) recommendation for consideration of prenatal diagnosis in future pregnancies, where appropriate.
 - f) onward referral for genetic counselling, if the referral has not been initiated by Clinical Genetics.
- identification of published reference information/databases used
- limitations of the tests used

* 'gene content' may refer to specific genes in the case of obvious clinical relevancy or where there are few genes involved and listing each is not prohibitive, or a quantitative statement (there are many genes in this region; there are no genes in this region).

5.1 REPORTING TIMES

All laboratories should endeavour to maintain adequate reporting times. It is recognised that local clinical need may influence the reporting times for non-urgent work.

It is recognised that it is not possible to evaluate the true clinical significance of an imbalance detected in the proband without parental studies and there is variation in the time taken to obtain parental blood samples.

Referral package	Reporting guideline
Proband (no follow-up studies required)	95 % within four weeks from receipt of a suitable DNA sample
Proband & Parental blood samples referred together (follow-up material held by laboratory)	95 % within eight weeks from receipt of suitable DNA samples
Parental blood samples requested after detection of imbalance in proband	95% within four weeks from receipt of suitable DNA from parental blood samples and follow-up test materials (eg FISH probes)

Submission of imbalance data, both de novo and inherited, to international databases is strongly recommended. The collected data provides updated reference for pathogenicity of human CNVs. Patient consent may be required.

6 ARCHIVING AND STORAGE

Guidelines published by the Royal College of Pathologists (2009) for "The retention and storage of pathological records and archives" should be followed [8], including those for the retention of request forms, daybooks, worksheets, correspondence and electronic data.

7 DEFINITION OF TERMS

Analysis/checking	to independently scrutinise the data output via an appropriate software package that provides an indication (visual and numerical) of the relative ratio of proband DNA to normal control DNA or a SNP reference set.
Validation	Final authorisation for a report to be sent out and protection of the computer record
CNV	copy number variant
MLPA	multiplex ligation dependent probe amplification
FISH	fluorescence in situ hybridisation
q-PCR	quantitative polymerase chain reaction

8 REFERENCES

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9 VERSION CONTROL

issue date	Current document	summary of changes	Version replaced
16/12/2011	Constitutional Postnatal Chromosomal Microarray Best Practice Guidelines 2011 v2.00	Update to reporting times and references	Constitutional Array Best Practice Guidelines 2009 v1.00
13/01/2009	Constitutional Array Best Practice Guidelines 2009 v1.00		No previous document