HOKLAS Supplementary Criteria No. 35

'Medical Testing' Test Category – Cytogenomics

1 Introduction

1.1 This document is an application document for the requirements of HKAS 002 and HOKLAS 015 accrediting cytogenomic examinations within the test category of Medical Testing. This document sets out only those specific requirements which require further elaboration but does not include all the accreditation requirements. Therefore, it has to be read in conjunction with HKAS 002, HOKLAS 015 and HOKLAS SC-33.

2 Scope of accreditation

2.1 HKAS provides accreditation under HOKLAS for cytogenomics in the following areas:

2.1.1 Constitutional Cytogenetics (prenatal and postnatal)

2.1.2 Cancer Cytogenetics (excluding solid tumours)

2.1.3 Fluorescence in situ hybridisation (FISH)

2.1.4 Chromosomal Microarray Analysis (CMA)

2.1.4.1 array comparative genomic hybridisation (array CGH)
2.1.4.2 single nucleotide polymorphism array (SNP array)

3 Personnel

3.1 A qualified pathologist providing consultation and clinical interpretation for test results in any test areas of cytogenomics shall have obtained the Fellowship of the Hong Kong College of Pathologists in an appropriate specialty plus qualification in relevant subjects in cytogenomics from a university and/or professional body and two years' post fellowship full-time equivalent laboratory training in an ISO 15189-accredited cytogenomic laboratory, or at least 5 years of laboratory experience in a laboratory providing the cytogenomic test services before 1 September 2008 (the

launching date of HOKLAS for the accreditation of tests and examinations in Medical Genetics).

3.2 A pathologist nominated by a laboratory to be its signatory for any test areas in cytogenomics shall seek endorsement from the Hong Kong College of Pathologists that his/her training and experience meet the requirements of a signatory for such tests. It will be the responsibility of the pathologist to seek endorsement from the Hong Kong College of Pathologists and provide HKAS Executive with the written confirmation from the Hong Kong College of Pathologists.

3.3 A medically qualified person nominated by a laboratory to be its signatory to provide consultation and clinical interpretation for test results in constitutional cytogenetics and cytogenomics shall have obtained Fellowship in an appropriate specialty (e.g. Obstetrics & Gynaecology, Paediatrics) plus qualification in the relevant subjects in cytogenomics from a university and/or professional body and two years' post fellowship full-time equivalent laboratory training in an ISO 15189-accredited cytogenomic laboratory, or at least 5 years of laboratory experience in a laboratory providing the cytogenomic test services before 1 September 2008.

3.4 A biomedical scientist reporting test results in constitutional cytogenetics without clinical interpretation shall be MLT Board Part I registered (or exempted from such registration) and have obtained a BSc degree or above in a relevant subject plus 5 years of post-Part I registration supervisory experience in constitutional cytogenetics, or have obtained a recognised overseas qualification in cytogenetics from a university and/or professional body.

3.5 Both the medical personnel who provides clinical interpretation and the technical personnel involved in producing the test results for chromosomal microarray analysis (CMA) shall be familiar with the principles of the software programme being applied, and up-to-date information on the annotation and clinical significance of copy number variation (CNV).

4 Laboratory equipment, reagents, and consumables

4.1 Procedures to assure and verify the proper functioning of equipment shall meet acceptable standards, e.g. HOKLAS Supplementary Criteria No. 38 "'Medical Testing' Test Category - Performance Verification of Automated Analysers".

4.1.1 Tissue culture incubators shall have a system to monitor the temperature continuously, and an alarm system to alert laboratory staff of abnormal culture conditions.

5 Pre-examination processes

5.1 Both the request form and specimen submitted for cytogenomic studies shall each contain at least two independent identifiers for unique identification of the patient. The identifying information on the request form shall be identical to that on the specimen tube label. There shall be a system to identify the person collecting the specimen for the test.

5.2 The laboratory shall only accept test requests for CMA, where relevant, with appropriate genetic counselling and consent documented.

6 Examination processes

6.1 Each prenatal specimen for cytogenetic studies shall be divided, cultured in two separate incubators and maintained with independent cell cultures, media and reagents. Duplicate or independently established cultures should be included if adequate specimen is available.

6.2 For cytogenetic studies, adequate number of banded metaphases should be examined. In general, a minimum of 5 cells (10 for cancer cytogenetics) should be analysed, and at least two cells shall be checked by a qualified personnel as defined in clauses 3.1 to 3.4. When there is evidence of mosaicism or clonal evolution, further cells should be examined.

6.3 For FISH studies, an effort should be made to examine a few metaphases with reverse DAPI chromosome staining to confirm that the correct probes have been used and to identify any unusual signal pattern. If metaphase is absent or inherent control signal is not available, the test should be repeated in parallel with another sample known to have the target of the probes.

6.3.1 There shall be a clear and consistent definition of fluorescence signals and also criteria for false positive and false negative signals.

6.3.2 Adequate number of interphase nuclei or metaphases should be examined. In general, a minimum of 5 metaphases and/or 100 interphase nuclei should be studied and scored. FISH signals shall be scored independently by 2 people.

6.4 For CMA, the laboratory shall have written procedures for DNA extraction, quality, yield and quantitation, proper fragmentation and fluorescent labelling.

6.5 CMA of cancers often requires the testing of small genomic aberrations in a mixed cell population. The laboratory shall understand the limitations of the test and in particular, the type of specimens (such as blood, fresh and formalin-fixed paraffin embedded tissues), tumour heterogeneity, contamination by normal DNA, selection of CMA platforms, coverage of the region of interest and the need for reference DNA (matched or pooled).

6.6 For CMA, the following experiment design and validation shall be considered:

6.6.1 All processes and parameters of CMA, including new array designs, shall be validated using DNA from a range of known abnormal samples, including numerical and structural variation, microdeletion and microduplication. Correct identification of a defined and reasonable number of abnormal specimens is suggested. Where a limited number is used for validation, there shall be documentation of justification and on-going data accumulation to complete the validation process. For validation of an updated version of a microarray platform, correct identification of at least five known abnormal samples is suggested.

6.6.2 If an enhanced (or a new) version of platform is used, it shall be validated with abnormal samples that reflect the additional capacity of the platform. A single algorithm or software programme shall be used throughout.

6.6.3 If an in-house assay is developed, the laboratory shall validate each clone or region using alternative method and reference DNA.

6.6.4 For an individual application, a single algorithm and set of parameters shall be used for all data analysis. The laboratory shall re-analyse all data used for initial validation if an alternative algorithm is used.

6.6.5 Software packages should produce diagrammatic and numerical output for analysis. Software parameters shall be defined and documented to ensure detection of imbalance at, or greater than, the level specified by the laboratory. A minimum genome-wide resolution at 400 kb is recommended.

7 Ensuring quality of examination results

7.1 For tests that give quantitative results, the laboratory shall determine the uncertainty of measurement and document the uncertainty components. An example is FISH study with quantitative results.

7.2 All staff taking part in the testing activities shall participate in appropriate external quality assessment or interlaboratory comparison programme.

7.3 In the absence of EQAP, both normal and abnormal samples shall be included in the interlaboratory comparison programme.

7.4 The laboratory shall have a standard protocol for the confirmation of abnormal or ambiguous results for CMA by alternative techniques, such as FISH, PCR, as far as is reasonably practicable.

8 Post-examination processes

8.1 A minimum of two karyotypes/images shall be prepared and archived.

8.2 Storage of the primary specimens and other laboratory samples shall be in accordance with the requirements given in Table 1. Laboratories should retain records and/or materials for a longer period of time than specified when such is appropriate for patient care, education, quality improvement needs or legal requirements, etc.

9 Reporting of results

9.1 A cytogenetics report shall contain:

a. biological sex of the patient;
b. description of specimen / tissue studied;
c. number of cells (metaphases) analysed, counted and karyotyped;
d. banding method(s) used; and
e. diagnosis and classification according to the WHO classification (for haematology malignancies)

9.2 For FISH studies, information on the limitations of the test applied, on the source of the probe and on the implications of the result shall be included in the report.

9.3 The description of test results shall follow the latest version of the International System for Human Cytogenomic Nomenclature (ISCN). For

cancer cytogenetics and FISH studies in haematology, the tests shall be reported by a qualified haematologist with appropriate training and laboratory experience.

9.4 For FISH studies in Anatomical Pathology, the tests shall be reported by a qualified anatomical pathologist with appropriate training and laboratory experience.

9.5 For constitutional cytogenetics, the tests shall be reported by a medically qualified person with appropriate training and laboratory experience as required in sections 3.1 to 3.3. For those tests with normal results, numerical autosomal or sex chromosome abnormalities of well-recognised syndromes and common chromosomal polymorphism (an indicative list is given in Appendix 1), they may be reported by a biomedical scientist. Clinical interpretation shall not be provided in such reports. Communication of the significance of the finding to referring doctor is advised.

9.6 For CMA, the following information shall be provided in the report for the diagnosis of constitutional disorders in addition to the information described in clause 5.8.3 of HOKLAS 015 (5th edition):

a. Date of specimen reception at the laboratory;
b. Pedigree information (optional);
c. Clinical disorder or indications for testing;
d. Gene(s) and/or specific region or mutation(s) of interest analysed, if applicable (standard nomenclature of Human Genome Organisation Gene Nomenclature Committee (HGNC) shall be used);
e. Brief description of the technical information, such as commercial source, the number and type of clones and loci tested, the version number of array chip and software programme used for the analysis;
f. Database accession number and version number for the reference sequence or genome, if applicable;
g. Test performance specifications and limitations (e.g. mosaicism, balanced rearrangements, etc);
h. Test sensitivity with quoted source reference, if available (e.g. the proportion of affected individuals likely to be detected);
i. A remark to notify the requesting clinician that post-test genetic counselling service has to be provided to the patient if necessary;
j. Comments or recommendations on family study;
k. A summary statement, where applicable (e.g. current ISCN nomenclature for copy number variant identified).

9.7 The laboratory shall have established criteria to identify copy number variation (CNV) and the corresponding reportable range in CMA with

reference to international guidelines such as the American College of Medical Genetics and Genomics (ACMG).

a. Standard abnormality and mutation nomenclature (e.g. The Human Genome Variation Society (HGVS) nomenclature and An International System for Human Cytogenomic Nomenclature) shall be used for result reporting. Commonly used name for the abnormality and mutation may also be included in the report for further clarification where appropriate.
b. When reporting negative test results, “no abnormality has been detected” shall be used instead of “normal”.
c. Disclaimers such as the possibility of errors due to factors beyond the control of the laboratory (e.g. the risk of mistaken paternity and the knowledge of family relationship as stated), where appropriate, should be mentioned in the report.
d. Interpretation of the residual risk remaining after a negative CMA result, when applicable, should be included in the report. This risk assessment is a quantitative statement of the predictive value of the result. It is usually based on the CNV frequency and test sensitivity. Bayesian calculation can be used to estimate the residual risk.
e. References should be given when published data have a bearing on the interpretation or risk calculation. In general, inclusion of references is only necessary when the data are not widely known or accepted. When different publications have shown conflicting results, it is important to specify which has been used as the basis for the interpretation.
f. The laboratory should have a written policy on the reporting of absence of heterozygosity (AOH) for SNP array.

9.8 The laboratory shall keep information of available genetic counselling service.

9.9 Where appropriate, the test report shall include a recommendation that the patient should obtain genetic counselling from a qualified healthcare professional.

- End -

Table 1 Retention of Laboratory Records and Materials

	Record/material	Requirement
General	Records of employee signatures, initials, and identification codes	10 years
	Referring doctor's request	3 years after dispatch of final report. Indefinite for request form which contains clinical information not readily accessible in the patient's notes but used in the interpretation of test result. Where the request form is used to record working notes or as a worksheet, it should be retained as part of the laboratory record.
Cytogenetics/Fluorescence in situ hybridisation/ Chromosomal microarray analysis	Copies of reports	Indefinite
	Patient information/karyotypes	Indefinite
	Slides	3 years after final report if photographic record kept; 5 years otherwise, unless degeneration evident
	Original specimen and container/wet specimen/tissue	1 month after reporting
	Fixed chromosome preparation (blood, bone marrow)	Abnormal: indefinite Normal: 6 months
	Tissue culture/cell culture	Cryopreserved, and indefinite when appropriate
	Diagnostic images (digitised or negatives)	Indefinite
	CMA data	Indefinite

N.B.: Indefinite means without limit of time, but not less than 30 years.

Appendix 1

An indicative list of the numerical autosomal or sex chromosome abnormalities of well-recognised syndromes and common chromosomal polymorphism.

A. Non-mosaic numerical abnormalities:

1. 47,XX,+21 or 47,XY,+21
2. 45,X
3. 47,XX,+13 or 47,XY,+13
4. 47,XX,+18 or 47,XY,+18
5. 47,XXY
6. 47,XXX
7. 47,XYY
8. 69,XXX or 69,XXY or 69,XY Y

B. Common chromosomal polymorphism:

1. 46,XX,inv(9)(p11q13) or 46,XY,inv(9)(p11q13)
2. 46,XX,inv(9)(p11q12) or 46,XY,inv(9)(p11q12)