HOKLAS Supplementary Criteria No. 30

'Medical Testing' Test Category – Molecular Genetics

1. Introduction

1.1 This document is an application document for the requirements of HKAS 002 and HOKLAS 015 accrediting molecular examinations within the test category of Medical Testing. It sets out the general requirements applicable for the performance of molecular diagnostics in all test areas of Medical Testing. This document only details those requirements that require further elaboration but does not include all the accreditation requirements. Therefore, it has to be read in conjunction with HKAS 002, HOKLAS 015, HOKLAS SC-33 as well as respective supplementary criteria of the relevant pathology discipline.

1.2 The checklist given in the Annex serves as guidance for laboratories to self-assess their management system and operation procedures against the requirements given in HOKLAS 015 and this document.

2. Scope of accreditation

2.1 HKAS provides accreditation under HOKLAS for the following areas:

2.1.1 Nucleic acid amplification testing (NAT)

2.1.2 Hybridisation (including chromogenic and fluorescence in situ hybridisation in Anatomical Pathology)

2.1.3 Electrophoresis for nucleic acid analysis

2.1.4 DNA sequencing

Note: For chromosomal microarray analysis (CMA), please refer to Supplementary Criteria No. 35 for Cytogenomics.

3. Personnel

3.1 A qualified pathologist providing consultation and clinical interpretation for test results in molecular genetics shall have obtained the Fellowship of the Hong Kong College of Pathologists or equivalent as advised by the College in an appropriate specialty plus structured training in molecular genetics from a university and/or professional body and 2-years full-time equivalent laboratory experience in a laboratory providing molecular genetic service. The relevant test scope in molecular genetics where consultation and clinical interpretation are provided shall be covered in the scope of service of the laboratory training centre(s) and there shall be objective evidence that the training has taken place in an appropriate centre. A grandfathering provision for exemption from the above criteria applies to Fellows of the Hong Kong College of Pathologists with two years of training and experience in the relevant test area who meet the exemption requirements at the time of implementation of the new requirement on 23 March 2015. Furthermore, Fellows of the Hong Kong College of Pathologists conferred before 23 March 2015 and trainee pathologists registered with the Hong Kong College of Pathologists before 18 October 2012 shall also be exempted if the grandfather exemption is satisfied.

3.2 A pathologist nominated by a laboratory to be its signatory for molecular tests shall seek endorsement from the Hong Kong College of Pathologists that his/her training and experience meet the requirements of a signatory for such molecular 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 Other than being a specialist in Pathology, a medically qualified person nominated by a laboratory to be its signatory to provide consultation and clinical interpretation for test results in molecular genetics (for prenatal and postnatal diagnosis of constitutional disorders) shall have obtained Fellowship in an appropriate specialty (e.g. Obstetrics and Gynaecology, Paediatrics) plus qualification in molecular genetics from a university and/or professional body and 2-years post-fellowship full-time equivalent laboratory experience in a laboratory providing molecular genetic service. The relevant test scope in molecular genetics where consultation and clinical interpretation are to be provided shall be covered in the scope of service of the laboratory training centre(s) and there shall be objective evidence that the training has taken place in an appropriate centre.

3.4 A technically qualified approved signatory for molecular genetics shall have qualification in molecular genetics from a university and/or professional body in addition to the basic requirements described in HOKLAS Policy on Personnel,

HOKLAS 015.

4. Accommodation and environmental conditions

4.1 For NAT, there shall be compartmentalisation and appropriate procedures and controls to prevent cross-contamination. Requirements to use separate rooms do not apply if reagent preparation, sample preparation, amplification, and detection are performed on the same instrument system. Separate rooms or clearly designated areas should be provided for the following processes:

- preparation of reagents and dispensing of master mix,
- sample preparation and extraction,
- amplification, and
- manipulation of amplified product e.g. gel electrophoresis or sequencing

The movement of nucleic acid samples should as far as possible be unidirectional i.e. from pre-amplification to post-amplification areas. Arrangements shall be made to ensure that printouts from post-amplification areas are protected from cross contamination.

4.2 The primary specimens, nucleic acid extracts and post-amplification products shall be stored in separate compartments or refrigerators. Nucleic acid samples shall be kept in designated refrigerated compartments after sample preparation. They shall not be kept in areas where activity such as gel electrophoresis or PCR work is conducted.

5. Laboratory equipment, reagents and consumables

5.1 There shall be no sharing of general instruments (e.g. micropipettes, vortex mixers, heating block and micro-centrifuges) among designated areas listed in 4.1 unless reagent preparation, sample preparation, amplification, and detection are performed on the same instrument system. Aerosol resistant pipette tips are mandatory and positive displacement pipettes are strongly recommended.

6. Examination processes

6.1 The laboratory shall establish the type of specimen acceptable for molecular testing such as the minimum amount of different tissue types. The method validation study shall include each type of specimens for the test and the amount

of material sufficient for testing shall be validated.

6.2 Nucleic acid shall be processed promptly and stored appropriately to minimise degradation. The specimen integrity should be re-evaluated before use after prolonged storage.

6.3 Methods for nucleic acid extraction shall ensure minimal number of tube-to-tube transfers. Acceptance criteria for the quality and quantity of nucleic acids suitable for subsequent analysis shall be defined and validated.

6.4 For prenatal testing based on foetal cell cultures, it is important to maintain backup cultures until the molecular diagnostics is completed. There shall also be postnatal follow-up measure(s) to monitor the accuracy of the test.

6.5 For prenatal testing based on genetic materials from foetus, the laboratory shall document how its testing methods are affected by the presence of maternal cell contamination and shall have procedures to assess and minimise it.

6.6 For prenatal testing, it is recommended that the mutation status of one or both parents, as appropriate, be tested prior to testing of foetal specimens, preferably by the same laboratory. Duplicate tests using DNA extracted from two separate specimens shall be performed when there is adequate material.

6.7 Discrepancies between the molecular diagnostic results, clinical and other laboratory findings shall be recorded and investigated, together with any corrective actions taken.

6.8 There shall be a clear and consistent definition of in situ hybridisation (ISH) signals and also defined criteria for false positive and false negative signals.

6.9 For ISH in Anatomical Pathology, it is important to correlate the result with the histology of the tissue concerned and to distinguish between the target or malignant cells from carcinoma-in-situ or other bystander cells. For enumeration of hybridisation signals, an internationally accepted scoring scheme shall be used if available.

6.10 For in-house developed nucleic acid amplification assays, the development and validation of the assay shall be well documented to include:

1. Primer and probe design (re-evaluated at least once a year)
2. Target gene
3. Analytical sensitivity (Limit of Detection – LOD)
4. Analytical specificity

5. Precision
6. Linear range (applicable only for quantitative assays such as viral load monitoring for HBV, HCV, HIV and CMV)
7. Accuracy
8. Cutoff values
9. Diagnostic sensitivity
10. Diagnostic specificity
11. Pre-nucleic acid amplification decontamination procedures
12. Detection of inhibitors and interfering substances (optional)

6.11 For commercial diagnostic assays approved by international or national regulatory bodies (e.g. FDA or CE-IVD), verification of assay performance shall, at least, cover items (3) to (8) under 6.10 if information on assay performance is provided by the manufacturers. Clinical samples shall be included where applicable.

6.12 For modified protocols of FDA and CE-IVD approved kits, research use only (RUO) or investigation use only (IUO) diagnostic kits, validation shall be conducted as extensive as for in-house developed assays (see 6.10). Similarly, modification of any of the reagents used or platform used by the laboratory for any procedural steps from the adopted method or from those recommended by the in vitro diagnostic manufacturer shall be re-validated to ensure that the modification is fit for the intended use.

6.13 For quantitative tests, the laboratory shall determine the uncertainty of measurement and document the uncertainty components. Examples of such tests include quantitative nucleic acid amplification assay such as viral load monitoring, and FISH study with quantitative results. The MU should also be estimated around the cut off of an assay for reporting qualitative results.

7. Ensuring quality of examination results

7.1 There shall be a set of defined criteria for internal quality control to determine the quality and acceptance of each run.

7.2 Laboratories offering molecular diagnostic testing shall participate in appropriate external quality assurance programme(s) for each specific molecular test for each target gene/microorganism or interlaboratory comparison if this is not available.

7.3 If the molecular diagnosis is not based on definitive method or nucleotide sequencing, positive and negative controls of the interrogated nucleotide or nucleotides shall be included in the same run of the assay.

8. Post-examination processes

8.1 The primary sample, DNA and RNA extract, and amplified DNA products shall be retained for a minimum of one month after reporting.

8.2 Technical records to be retained for establishing traceability shall also include the gel images and hybridisation blots.

9. Reporting of results

9.1 For all molecular tests related to human genome carried out in laboratories of Anatomical Pathology, Chemical Pathology, Haematology or Immunology, they shall have direct input from and be reported by a qualified pathologist with appropriate training and laboratory experience in that discipline.

9.2 For Clinical Microbiology and Infection, positive results for certain molecular tests shall have direct input from a qualified clinical microbiologist (or qualified pathologist as advised by the HKCPath) before reporting. The laboratory shall ensure that the relevant requirements in HOKLAS SC-27 are met when reporting and releasing results of such molecular tests.

9.3 It should be noted that, for laboratory investigation of HIV, HBV and HCV infections, qualitative molecular test results alone are inadequate for clinical management. The laboratory shall remind the physicians ordering the tests to take into account of other parameters such as serology findings and clinical features before initiating any definitive treatment.

9.4 A laboratory unable to provide direct input from a qualified pathologist in the report could also be accredited for the molecular tests listed above in clauses 9.1 and 9.2 if the samples are referred from an accredited laboratory with a qualified pathologist of the relevant discipline. However, the laboratory shall provide the test reports only to the pathologist of the laboratory that initiated the referral. To ensure that the test results on such reports would not be misinterpreted, the following mandatory remark shall also be shown on each of these reports: "Test results shown on this report require clinical interpretation and comments by a qualified pathologist of respective discipline".

9.5 For prenatal and postnatal diagnosis of constitutional disorders, the test results shall be reported by a qualified pathologist or medically qualified person with appropriate training and laboratory experience.

10. Release of results

10.1 Where appropriate, the test report shall include a recommendation that the patient should obtain genetic counselling from a qualified healthcare professional regarding the benefits, limitation, and result of the genetic tests.

10.2 Access to the test report shall be restricted to the clinician who has the responsibility to provide comprehensive pre- and post-test counselling to the patient or to refer the patient to appropriate clinical service.

Table 1 Retention of Laboratory Records and Materials

	Record/material	Requirement
General	Referring doctor's request	3 years after dispatch of final report. Indefinite for request forms that contain 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.
Molecular genetics	Copies of reports	Indefinite
	Patient information	Indefinite
	Original specimen and container/wet specimen/tissue	1 month after reporting
	DNA and RNA extract, and amplified DNA product	1 month after reporting, and indefinite where appropriate
	Gel images and hybridisation blots.	Indefinite

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

HOKLAS Requirement	Clause (HOKLAS 015, 5th edition and relevant SC)	*[1]	Y	N	NA	Lab's Document Reference or Remarks[2]	Assessment Team's remarks / questions to be asked at the laboratory
Discipline Specific Technical Requirements							
Accommodation and environmental conditions							
For Nucleic Acid Amplification Testing (NAT)							
Are there separate rooms or clearly designated areas provided for the following processes: A. preparation of reagents and dispensing of master mix; B. sample preparation and extraction; C. amplification; and D. manipulation of amplified products?	5.2.6	•	☐	☐	☐		
Is there a documented policy describing the movement of nucleic acid samples or specimens should as far as possible be unidirectional i.e. from pre-amplification to post-amplification areas?	5.2.6, 5.5.3	•	☐	☐	☐		
Are nucleic acid samples kept in designated refrigerated compartments after sample preparation away from areas where activity such as gel electrophoresis or PCR work is conducted?	5.2.6	•	☐	☐	☐		

Note: 1. The assessor should concentrate on items marked with a ●; other items will be checked by the team leader.
2. Please put down the laboratory's document reference(s) where there are descriptions or procedures related to the requirement.

HOKLAS Requirement	Clause (HOKLAS 015, 5th edition and relevant SC)	*[1]	Y	N	NA	Lab's Document Reference or Remarks[2]	Assessment Team's remarks / questions to be asked at the laboratory
Laboratory equipment, reagents and consumables							
For Nucleic Acid Amplification Testing (NAT)							
Are aerosol resistant pipette tips or positive displacement pipettes used in the whole process?	5.3.1.1	•	☐	☐	☐		
Is there a documented policy preventing the sharing of general instruments (e.g. micropipettes, vortex mixers, heating block and micro-centrifuges) among designated areas? A. preparation of reagents and dispensing of master mix; B. sample preparation and extraction; C. amplification; and D. manipulation of amplified products.	5.3.1.3	•	☐	☐	☐		
For Signal Detection Instruments (e.g. chemiluminescence / fluorescence detector)							
Is stray light checked periodically with extinction filters or appropriate solutions as required by the manufacturer?	5.3.1.5	•	☐	☐	☐		
Are background levels taken and recorded daily or with each use of the instrument?	5.3.1.7 (j)	•	☐	☐	☐		
Are there written criteria for the acceptable background levels?	5.3.1.3	•	☐	☐	☐		
Are all curves rerun regularly and/or verified after servicing or recalibration of the instrument for procedures using calibration curves?	5.3.1.5	•	☐	☐	☐		

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HOKLAS Requirement	Clause (HOKLAS 015, 5th edition and relevant SC)	*[1]	Y	N	NA	Lab's Document Reference or Remarks[2]	Assessment Team's remarks / questions to be asked at the laboratory
Pre-examination processes							
Is there an aliquoting protocol that prevents cross-contamination of different specimens and contamination of original specimen?	5.4.7	•	☐	☐	☐		
Examination processes							
Does the laboratory verify the performance of commercial diagnostic assays (e.g. analytical sensitivity, analytical specificity, precision, linear range, accuracy and cutoff values)?	5.5.1.2	•	☐	☐	☐		
Does the laboratory perform validation studies to establish performance characteristics of laboratory-developed assays?	5.5.1.3	•	☐	☐	☐		
Does the laboratory include all specimen or tissue types it normally encounters in the validation / verification study? Is the amount of material sufficient for testing determined?	5.5.1.2 5.5.1.3	•	☐	☐	☐		
Are published protocols for nucleic acid extraction and purification used, and are in-house procedures validated and documented if they are used?	5.5.1.1 5.5.1.3	•	☐	☐	☐		
Is there a written policy for the short and long term storage of DNA and RNA extracts to prevent degradation?	5.5.1.1, 5.5.3	•	☐	☐	☐		
Is specimen integrity re-evaluated after prolonged storage?	5.5.1.3	•	☐	☐	☐		

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2. Please put down the laboratory's document reference(s) where there are descriptions or procedures related to the requirement.

HOKLAS Requirement	Clause (HOKLAS 015, 5th edition and relevant SC)	*[1]	Y	N	NA	Lab's Document Reference or Remarks[2]	Assessment Team's remarks / questions to be asked at the laboratory
Are the acceptance criteria for the quality and quantity of nucleic acids suitable for analysis defined and have they been validated?	SC-30 6.3	•	☐	☐	☐		
For prenatal testing based on foetal cell cultures, are backup cultures maintained until the molecular testing is completed?	SC-30 6.4	•	☐	☐	☐		
For prenatal testing based on foetal genetic materials, are there procedures to assess the presence of maternal cell contamination?	SC-30 6.5	•	☐	☐	☐		
Are records kept for discrepancies between molecular diagnostic results, clinical and other laboratory findings?	SC-30 6.7	•	☐	☐	☐		
Has the laboratory established a clear and consistent definition of in situ hybridisation (ISH) signals and also criteria for false positive and false negative signals?	SC-30 6.8	•	☐	☐	☐		
For enumeration of hybridisation signals, is the scoring scheme used internationally acceptable?	SC-30 6.9	•	☐	☐	☐		
Ensuring quality of examination results							
Does the laboratory have a quality control program covering the examination process?	5.6.2.1	•	☐	☐	☐		
Has the laboratory determined the Uncertainty of Measurement for quantitative molecular tests such as viral load monitoring, and FISH study with quantitative results?	5.5.1.4	•	☐	☐	☐		

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HOKLAS Requirement	Clause (HOKLAS 015, 5th edition and relevant SC)	*[1]	Y	N	NA	Lab's Document Reference or Remarks[2]	Assessment Team's remarks / questions to be asked at the laboratory
Does the laboratory participate in external quality assurance programme(s) covering each of the molecular tests for each target gene/microorganism?	5.6.3.1	•	☐	☐	☐		
Whenever an interlaboratory comparison is not available, does the laboratory develop other approaches and provide objective evidence for determining the acceptability of examination results?	5.6.3.2	•	☐	☐	☐		
Are proficiency testing samples processed and analysed by personnel who routinely test patient samples, using the same primary methods as for patient samples?	5.6.3.3	•	☐	☐	☐		
Are there ongoing review and evaluation of external quality assurance results, with prompt corrective action taken for unacceptable results?	5.6.3.4	•	☐	☐	☐		
Post-examination processes							
Are the primary samples, DNA extracts, RNA extracts and amplified DNA products retained for a minimum of 1 month after reporting?	SC-30 8.1	•	☐	☐	☐		
Are gel images and hybridisation blots kept as part of the technical records retained?	SC-30 8.2	•	☐	☐	☐		
Reporting of results							
Does the test report provide comments on the limitation of the test results when the presence of inhibitors is detected in Nucleic Acid Amplification testing?	5.8.3 (l), 5.9.1 (a)	•	☐	☐	☐		

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HOKLAS Requirement	Clause (HOKLAS 015, 5th edition and relevant SC)	*[1]	Y	N	NA	Lab's Document Reference or Remarks[2]	Assessment Team's remarks / questions to be asked at the laboratory
Are all reports, including those requiring clinical interpretation, reviewed and signed by appropriate personnel with appropriate qualification, training and experience in molecular testing?	SC-30 9.1, 9.2, 9.5	•	☐	☐	☐		
If the laboratory is performing a referred test from a pathologist and that the test requires clinical input from a qualified pathologist, does the report contain the mandatory remark: "Test results shown on this report requires clinical interpretation and comments by a qualified pathologist of respective discipline"?	SC-30 9.4	•	☐	☐	☐		
Release of results							
Is there a remark on the test report recommending the patient to obtain genetic counselling from a qualified healthcare professional?	SC-30 10.1	•	☐	☐	☐		
Is the access of the test report restricted to the requesting clinician?	SC-30 10.2	•	☐	☐	☐		

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2. Please put down the laboratory's document reference(s) where there are descriptions or procedures related to the requirement.