1. INTRODUCTION

1.1 a) This document describes the specific requirements for Molecular Pathology diagnostic testing services.

b) This document shall be studied in conjunction with ISO15189 Medical laboratories – Particular requirements for quality and competence, other MEDICAL Series Technical Notes published by PNAC and Guidance Notes such as "ISO 15190 Medical Laboratories – Requirements for Safety".

2. GENERAL TECHNICAL NOTE : MEDICAL G-23/01

2.1 Please refer to **General Technical Note: Medical - G-23/01** for the following:

- PERSONNEL
- COLLECTION AND HANDLING OF SPECIMENS
- PHYSICAL FACILITIES
- REAGENTS
- REFERENCE MATERIALS
- REQUISITIONS TEST METHODS AND METHOD VALIDATION
- MAINTENANCE OF EQUIPMENT
- CALIBRATION OF EQUIPMENT
- QUALITY CONTROL AND PROFICIENCY TESTING
- LABORATORY SAFETY
- RETAINED SAMPLES
- WASTE DISPOSAL
- REPORTING OF RESULTS

3. SCOPE OF TESTING

3.1 In clinical molecular diagnostic laboratories, molecular biology methods are used to test nucleic acids (DNA or RNA) from patient samples. Molecular diagnostic tests are used to diagnose disease or disease risk, direct choice of therapy, detect residual disease after therapy, provide prognostic information, and distinguish one person from another. Over approximately 15 years since the advent of the polymerase chain reaction (PCR) technology in the late 1980s, the practice of diagnostic molecular pathology has developed to include clinical testing for genetic (inherited) diseases, infectious diseases, solid tumours, leukaemias and lymphomas, and human identification.

3.2 Infectious diseases

3.2.1 Molecular diagnostic methods are used to detect the presence of infectious organisms, quantify pathogens in blood or other specimens, study the relationship among organisms from an infectious outbreak, and identify clinically relevant characteristics of pathogens. Molecular methods for infectious diseases include the detection of nucleic acid through hybridization or amplification of nucleic acid or the use of probes. The degree of stringency in the design and facilities of a molecular laboratory varies according to the methodology used in each laboratory.

3.3 Genetic diseases

3.3.1 In the post-genomic era, molecular diagnostic tests will be used not only for classic single-gene genetic diseases, but also for complex disease traits such as asthma, diabetes, atherosclerotic heart disease, and most other medical conditions predicted by family history. Once therapies are based on genetically variant targets, the need to use the right drug in the right patient will link molecular diagnostic testing with drug selection.

3.4 Cancers and oncological risk assessment

3.4.1 Understanding the underlying genomic changes that cause cancer has led to the development and use of molecular diagnostic tests for the diagnosis, prognosis and monitoring following treatment of cancers. Molecular diagnostic tests are used for leukaemias, lymphomas, sarcomas and carcinomas.

3.5 Identity testing

3.5.1 Molecular methods are used to distinguish one person from another. Molecular identity testing methods were first developed for forensic investigations but are now widely used for medical testing related to bone marrow transplantation, genetic assessment of hydropic and molar pregnancies, and maternal contamination contamination analysis for prenatal genetic testing, as well as laboratory uses for assessment of specimen identification and of paternity.

4. PROFICIENCY TESTING AND QUALITY ASSURANCE

4.1 The laboratory shall participate in proficiency testing for the tests offered. Where proficiency testing for an analyte is not available, performance assessment must be conducted at suitable intervals (e.g., six-monthly) by

appropriate procedures. These procedures include: split sample analysis with other laboratories, split samples with an established in-house method, assayed and reference materials, clinical validation by chart review, or other suitable and documented means.

4.2 The sample turnaround times should be appropriate for the intended purpose of the test.

4.3 There should be active review of records of controls, instrument maintenance and function on all shifts. There should be active review by the supervisor (monthly) and the laboratory head or designee (at regular intervals) of the records. A technologist, supervisor or pathologist should check all results before reporting.

4.4 Discrepancies between the molecular pathology laboratory's results, other laboratory findings, and the clinical presentation should be investigated and documented, along with any corrective action.

5. QUALITY CONTROL

5.1 Refer to Quality control and proficiency testing in **General Technical Note: Medical G-23/01**. In addition to that the laboratory should comply with the following:-

5.2 The laboratory must have a written programme defining the general quality control policies and procedures in use.

5.3 The programme should be under surveillance by the section laboratory supervisor and reviewed at least monthly by the laboratory head or designee.

5.4 Appropriate tolerance and acceptability limits must be defined for all control procedures, control materials and standards. Only when the controls are acceptable can results be reported. There should be documentation of corrective action taken when controls or measurements of instruments are out of range.

5.5 For electrophoretic separations, known molecular weight markers should be used for each run and they should span the range of the expected bands.

5.6 Positive (including low positive) and negative controls should be run for each assay, when available, appropriate and practical. For panels with multiple targets, systematic rotation of controls may be acceptable.

5.7 False negatives should be excluded. This may be done by one or more of the following:

. using an inhibitor control with each run
. establishing the false-negative rate during the analytical validation phase, and during
continual monitoring of test result trends
. establishing the suitability of specimen type for the particular test in prior studies
. using reagents tested for inhibitory properties for the specimen type
. referring to published literature using the same reaction conditions

5.8 The use of an internal inhibitor control in each run should be determined on a case-by-case basis, depending on the following factors:
. likelihood of encountering inhibitors e.g. crude or contaminated clinical specimens
like sputum or stool
. performance (sensitivity, specificity, precision) related to conventional methods
. clinical implications of a false-negative result
. the degree to which the clinical diagnosis rests on the result of the laboratory procedure

5.9 For commercial kits approved by an acknowledged regulatory body, it is sufficient to follow the manufacturer's instructions and use the manufacturer's controls.

6. VALIDATION OF TEST PERFORMANCE

6.1 Test validations should be performed before new tests are introduced for clinical use. The procedure for such validation should be documented. New tests may be validated by comparison with gold standard methodologies (eg, culture or western blot assays). If this is not possible, nucleic acid testing (NAT) results may be confirmed by hybridization, sequencing of amplified products, or by using alternative NATs. Participation in external proficiency testing or split samples for inter-laboratory testing may also be used. In situations where all these are not possible, using specimens from patients with clinically documented infections may perform validation of NAT results.

6.2 Commercial kits should perform according to specifications. Modifications to manufacturer's instructions should be validated and documented.

6.3 There should be a documented process for validating in-house methods.

6.4 In-house methods should be validated in ways commensurate with the intended clinical purpose of the test. Validation parameters may include:

. comparison with other molecular and conventional laboratory tests
. use of control or reference material
. test for inhibitors and contamination
. limit of detection
. sensitivity (with reference to a second method or clinical studies)
. specificity
. precision and reproducibility
. correlation with clinical diagnosis

6.5 The validation of in-house nucleic acid tests for infectious diseases is used as an illustration of basic principles.

6.5.1 A written procedure giving adequate and precise details on the reagents (source, preparation, storage, and stability) and methods used must be produced for each test.

6.5.2 In molecular diagnostic methods for pathogen detection, the chosen target sequence should be checked against a databank for homology to the desired pathogen and to ensure lack of cross reactivity to other closely related organisms, prior to adoption of the method.

6.5.3 Limit of detection: A cut-off limit that separates a positive from a negative result should be determined to minimize false positives and false negatives.

6.5.4 Assay specificity should be evaluated (using preferably 100 samples) against a reference method and/or using known negative samples.

6.5.5 The assay should be tested for cross-reactivity against at least 10 samples containing commensal or potentially cross-reacting organisms.

6.5.6 If the assay specificity is sub-optimal, a confirmatory test targeting a different region of the pathogen genome should be used.

6.5.7 Assay sensitivity should be tested (using preferably 100 samples) against a reference method, known positive specimens, or spiked specimens. For disease of low occurrence rates, at least 20 samples should be tested.

6.5.8 Dynamic range of quantitative assays: The quantitative limit (limit of detection with reportable titres), where applicable, should be determined using known standards.

6.5.9 Linearity range of quantitative assays: The assay linearity should be established using at least 4 replicates for each dilution.

6.5.10 Assay precision and reproducibility: Between-batch (day) variation should be evaluated using at least two dilutions near the cut-off limit and tested in 20 individual runs.

6.5.11 For assays relying on presumptive identification based on a single band on a gel, or on melting point for real-time PCR, the identity of the product should be checked during validation by sequencing, probe recognition or other methods.

6.6 Post-validation monitoring.

6.6.1 This should be done for both in-house and commercial methods, and includes review of clinical cases and further published reports in the scientific literature. A logbook may be kept for unusual or instructive cases.

6.6.2 An annual review should be done to investigate possible cross-reactivity of amplified target sequence to newly published sequences deposited in gene databanks.

6.6.3 Changes in procedure or reagents should be re-validated in an appropriate manner and Documented.

7. SPECIMEN PROCESSING

7.1 Refer to Collection and Handling of Specimens in **General Technical Note: Medical – G-23/01**.

7.2 Amplification procedures should be designed to minimize carryover using appropriate physical containment and procedural controls. Reagents must be prepared and aliquotted in an area separate from the specimen preparation and post-amplification areas. There should be a unidirectional workflow from "clean" to "dirty" areas, with change of laboratory coats and gloves where appropriate.

7.3 Sample identification should be traceable through all phases of analysis, such as specimen receipt, nucleic acid extraction, endonuclease digestion, amplification, electrophoresis, photography and storage.

8. STORAGE OF NUCLEIC ACIDS

8.1 Nucleic acids should be processed promptly and stored adequately to minimize degradation. Isolated DNA and RNA should be stored in tightly capped containers. Long-term storage should be carried out at –20_ or –70_ C to prevent degradation. Stability of the sample can be maintained for several months by storing at 4_ C. Integrity of samples should be re-evaluated before use if stored for prolonged periods of time at any temperature.

9. REPORTING OF RESULTS

9.1 Refer to Reporting of Results in **General Technical Note: Medical - 001**.

9.2 The final report should include sufficient information about the method used, and for certain tests, subjective or interpretative comments, including references where relevant. For in-house tests the method used must be traceable. The report should be formatted in such a way as to allow easy and correct clinical interpretation.

10. REAGENTS

10.1 Refer to Reagents in **General Technical Note: Medical - G-23/01**.