1. INTRODUCTION

1.1 a) This document describes the specific requirements to be complied with by clinical microbiology sections before they can be accredited.

b) This document shall be studied in conjunction with ISO 15189 Medical laboratories – Particular requirements for quality and competence, other MEDICAL Series Technical Notes published by PNAC and Guidance Notes such as “ISO15190 Medical Laboratories – Requirements for Safety”.

2. MICROBIOLOGY LABORATORY

2.1 Microbiology includes bacteriology, mycobacteriology, mycology, parasitology, virology and serology. The tests may be performed in subspecialty laboratories, in general microbiology laboratories, or as part of a general or core laboratory. Laboratories shall be accredited according to their scope of tests.

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

3.1 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

4. SAFETY

4.1 Sealed buckets should be used in centrifuges when infectious organisms are present or are likely to be present. Where infection may be acquired by aerosolization, the bucket should be unloaded in a biological safety cabinet.

4.2 Autoclave: All persons using the autoclave should have been trained in its operation. Face shields and protective aprons should be used for unloading liquids. Heat-proof gloves should be available for unloading the autoclave.

4.3 There should be documented policies for handling spills of contaminated materials.

5. SPECIMENS

5.1 There should be suitable facilities for storage of specimens that may need re-testing. There should be a policy on the duration of storage, to allow retrieval of specimens or significant isolates for retesting or further testing.

6. REAGENTS/STAINS/MEDIA/KITS/ANTIMICROBIALS

6.1 Refer to Reagents in **General Technical Note: Medical - G-23/01** and the following additional requirements

6.2 All reagents, stains, media, kits and antimicrobials should be stored as recommended by the manufacturers and used within their indicated expiry dates.

6.3 They should be labeled, as applicable and appropriate, with the content and quantity, concentration or titre date received or prepared, date placed in service, storage requirements and expiry date. If there are multiple components of a reagent kit, the laboratory must use components of reagent kits only with other kits that are of the same lot number unless otherwise specified by the manufacturer.

6.4 The use of commercial reagents and controls must comply with manufacturer's instructions.

6.5 The laboratory must have documented records of quality control results of test procedures, reagents, stains, media, kits, antimicrobials, etc. These should be checked prior to being placed in service and subsequently be monitored at specified intervals for performance or limits of acceptability. Corrective actions should be documented when such results are unacceptable.

7. EQUIPMENT

7.1 The laboratory should have proper documentation and records of instruments

and equipment used for tests and/or calibrations, including schedules of preventive maintenance and function checks. All instruments and equipment used in the laboratory shall be calibrated by a PNAC accredited calibration laboratory, wherever possible.

7.2 Pipettes, microdiluters and automatic dispensers that are used for precise quantitative dispensing of material must be checked at specified intervals for accuracy and reproducibility and results recorded. The intervals are to be determined by the laboratory, unless otherwise specified by PNAC.

7.3 The temperature of temperature-dependent instruments/equipment must be checked and recorded on each day of use.

7.4 Corrective actions must be documented when an unacceptable tolerance limit or Instrument/equipment malfunction is detected.

7.5 Instructions on the use and maintenance of instruments/equipment should be readily available for use by appropriate and trained personnel.

8. QUALITY CONTROL AND PROFICIENCY TESTING

8.1 Refer to Quality control and proficiency testing in **General Technical Note: Medical - G-23/01**.

8.2 In addition to the above, the microbiology laboratory has to meet the following on quality control.

8.3 There should be records of at least weekly review of quality control results by the microbiology laboratory supervisor and monthly review by the microbiology laboratory head or designee.

8.4 Corrective actions taken when errors or unacceptable results/tolerance limits are detected should be documented.

8.5 For areas where external proficiency testing programme is not available, test performance must be checked at least semi-annually with appropriate procedures. The microbiology laboratory head or designee on receipt must review results of proficiency testing programme/s and prompt corrective actions taken in response to unacceptable results on the survey report form should be documented.

9 BACTERIOLOGY

9.1 Media

9.1.1 The laboratory must ensure that all media prepared in-house are sterile, able to support growth and are appropriately reactive biochemically. This will require that the laboratory maintains stock reference organisms and tests the media before or concurrent with use.

9.1.2 For purchased media, the manufacturer must document to the user that their quality control activities meet the criteria described as above, 9.1.1. However, the laboratory must continue to test the batches that were not previously quality controlled, as well as the media that are known to show significant variability in performance e.g. chocolate agar (for *H. influenzae*), campylobacter agar, Thayer-Martin agar

9.1.3 The user must visually examine each batch of media for breakage, contamination, appearance, or evidence of freezing or overheating.

9.1.4 It is necessary to document all the quality control procedures that were carried out in the laboratory.

9.1.5 A record should be kept of all lot numbers and expiration dates of the media received for the past two years.

9.2 Stains

9.2.1 Each new batch of stains (Gram stain, special stains, and fluorescent stains) should be checked at least weekly with known positive and negative control organisms for intended reactivity and results recorded.

9.3 Reagents / Kits / Antibiotic Discs

9.3.1 New reagent lots should be checked against old reagent lots or with suitable reference material before being placed in service.

9.3.2 The laboratory should perform and record results with positive and negative controls at specified periodic intervals.

9.3.3 Guidelines must be established for the number and type of antibiotics to be reported for organisms isolated from different sites of infection.

9.3.4 Only single isolates or pure cultures should be used for the final performance of antibiotic susceptibility testing. Each new lot of antibiotic discs should be checked for activity before being placed in service and at least weekly thereafter with reference cultures.

9.3.5 Inoculum's size should be controlled using a turbidity standard or other

acceptable method and tolerance limits for potency of antimicrobials (criteria for out of control) should be established.

9.3.6 Written criteria should be available for interpretation of the end point or zone size.

9.4 Equipment

9.4.1 Anaerobic jars should be checked with methylene blue strips, fastidious anaerobes or other appropriate procedure. The function of autoclaves, incubators and refrigerators should be monitored. Evidence of corrective actions taken when results are out of control should be documented.

9.5 Procedures and Tests

9.5.1 Positive and Negative control specimens should be tested in the same manner as patient samples and control results must be verified for acceptability before reporting patient results.

9.5.2 Respiratory cultures: All sputa should be assessed for adequacy of specimen (i.e. whether good quality sputum was obtained). Laboratories handling throat swabs should have facilities to identify *C. diphtheriae* and *N. gonorrhoeae* in-house or via a referral laboratory, when that is requested by the physician.

9.5.3 Urine cultures: The laboratory should perform and report quantitative cultures and use media and procedures that permit isolation of both Gram positive and negative bacteria. Specimens should be processed in a timely manner so that bacterial overgrowth does not occur in the urine. Where delay in plating is expected, provision should be made to prevent overgrowth: viz. storing at 4 ◦C, adding preservative to the container, and using dip slide cultures.

9.5.4 Urethral and Cervical cultures: Transport and culture conditions should be satisfactory for the isolation of *N.gonorrhoeae*.

9.5.5 Stool cultures: The procedure should permit isolation and identification of enteric pathogens in patients with diarrhoea (selective media and enrichment media).

9.5.6 Cerebrospinal fluid: Specimens should be processed and cultured immediately on receipt. A Gram stain should be performed routinely on sediments and results reported directly to the physician. Media and incubation conditions must permit recovery of fastidious bacteria (e.g.

Neisseria meningitidis, *H. influenzae*) and cultures should be performed on both smear positive and negative CSF specimens.

9.5.7 Blood cultures: Sterile technique for drawing and handling blood cultures should be defined. The blood culture system should be designed to recover both aerobic and anaerobic organisms. All negative blood cultures should be sub-cultured before discard. Sub-cultures and/or stains need not be done on blood cultures performed by automated methods if the bottles are monitored for five days. Information on positive cultures should be conveyed to the physician as soon as it is available.

9.5.8 Wound cultures: When indicated, Gram stain of direct smears should be examined and reported. Both aerobic and anaerobic cultures should be performed on specimens from appropriate sites.

9.5.9 Anaerobic cultures: Specimens and cultures should be placed in an anaerobic atmosphere as soon as practicable.

9.6.1 Reports should be available in a timely manner, and preliminary reports issued if specimens take a longer time to work up.

9.6.2 Reports with abnormal results should be reviewed by senior personnel for consistency and clinical relevance.

10. MYCOBACTERIOLOGY

10.1 The laboratory shall be assessed according to the level of mycobacteriological service offered.

10.1.1 Level I: Collect and/or transport specimens to a higher-level laboratory for isolation and identification; perform microscopic examination.

10.1.2 Level II: Perform all procedures of Level I; isolate organisms in culture; identify Mycobacterium tuberculosis; perform susceptibility tests; refer "other mycobacterial isolates" to Level III laboratory for identification.

10.1.3 Level III: Perform all procedures of Level II; identify all mycobacterium; perform susceptibility tests;may perform molecular diagnosis of *M. tuberculosis* complex.

10.2 Procedures and Tests

10.2.1 Rapid and reliable methods should be used for microscopic examination, isolation, identification and antimycobacterial susceptibility testing of

Mycobacterium tuberculosis complex.

10.2.2 Certain specimens (e.g. sputum) should be concentrated before AFB smear examination and culture. Control specimens should be tested in the same manner as test specimens.

10.3 Stains

10.3.1 Stains for acid-fast bacilli should be checked with known positive and negative control organisms and the results recorded for each new batch and thereafter at least weekly or on each day of use(whichever is less frequent)

10.4 Media

10.4.1 New lots of prepared media should be checked for sterility, ability to support growth (when applicable) and/or biochemical reactivity by means of reference cultures or parallel testing with previous batches.

10.4.2 For purchased, prepared media, a document should be obtained from the manufacturer to certify that quality control performance was acceptable.

10.5 Identification

10.5.1 A known strain of *M. tuberculosis* should be run whenever identification of M. tuberculosis complex is performed.

10.5.2 Biochemical tests used for identification should be checked each day of use with appropriate positive controls.

10.5.3 Nucleic acid probes or nucleic acid amplification technique for mycobacterial identification should be accompanied by appropriate positive and negative controls on each day of use.

10.5.4 Temperature growth requirements and photo-reactivity studies must be done when appropriate if complete identification of mycobacterial organisms cultured is performed.

10.6 Susceptibility Testing

10.6.1 Control strain of *M. tuberculosis*, which is sensitive to all antimycobacterial agents, should be included with each run.

10.7 Reporting of Results

10.7.1 Results of quality controls should be documented and reviewed for

acceptability before reporting patient results. Corrective action taken for unacceptable control results should be documented.

10.8 Laboratory Safety

10.8.1 Refer to Laboratory safety in **General Technical Note: Medical - G-23/01**. In addition to this the microbiology laboratory should comply with the following safety requirements.

10.8.2 Safety procedures should be designed to protect all personnel from biological hazards.

10.8.3 Specimens for microscopy/culture for mycobacteria must be collected and/or received in properly tightened screw capped, leak - proof containers.

10.8.4 Centrifugation should be carried out in aerosol proof safety carriers that are opened within the biosafety cabinet.

10.8.5 A biological safety cabinet must be provided for handling specimens and must meet minimum requirements for mycobacteriological work.

10.8.6 Biosafety cabinets must be certified at least annually to ensure that filters are functioning properly and that airflow rates meet specifications.

11. MYCOLOGY

11.1 Quality Control

11.1.1 In-house prepared media must have quality control checks for sterility, ability to support growth and appropriate biochemical reactivity.

11.1.2 Commercially prepared media must have documentation that quality control checks have been performed, and results are satisfactory.

11.1.3 Reference cultures and sera should be maintained for the proper control of stains, media, reagents, antimicrobial susceptibility tests and serological tests.

11.1.4 All stains must be checked with appropriate positive and negative controls for each new batch of preparation and at least daily. (For stains like Gomori's methenamine silver, the slide itself serves as the negative control).

11.1.5 Serological (antibody and antigen) and nucleic acid tests should be run with known positive and negative control organisms or sera with each new batch

of preparation and when appropriate.

11.1.6 Quality control tests with control specimens must be tested in the same manner as patient specimens. All results of quality control checks must be documented.

11.1.7 Results of control tests must be reviewed before reporting patient results.

11.1.8 Tests are not to be released if controls are unacceptable. Unacceptable control tests must be reported to the supervisor and corrective action documented.

11.2 Procedures and Tests

11.2.1 Technical procedures for isolation and identification of fungi directly from specimens and cultures should be available.

11.2.3 These include the use of microscopy, stains, selective media, biochemical tests, serological tests and nucleic acid tests.

11.3 Laboratory Safety

11.3.1 Refer to Laboratory safety in **General Technical Note: Medical - G-23/01**. Additional safety precautions are needed to protect personnel from biological hazards.

11.3.2 A biological safety cabinet must be provided for handling cultures.

11.3.3 The biological safety cabinet must meet minimum requirements for microbiological work. It must be certified annually to assure that the filters are functioning properly and the airflow rates meet specifications.

11.3.4 Handling of cultures with myecelial growth during laboratory investigations should be done in a biological safety cabinet.

12. PARASITOLOGY

12.1 Quality Control

12.1.1 Quality control checks of the specific gravity of concentrating solution (e.g. Zinc sulphate) should be done periodically.

12.1.2 All permanent stains must be checked, together with controls, for intended staining results at least monthly, or with each test if test is performed less

frequently. Special stains used to detect specific organisms (e.g. acid-fast, fluorescent stains) must be checked with appropriate control organisms each time the stain is used.

12.1.3 All results of quality control checks must be documented.

12.1.4 Results of control tests must be reviewed before reporting patient results. Tests are not to be released if controls are unacceptable. Unacceptable control results must be reported to the supervisor and corrective action documented.

12.2 Equipment

12.2.1 An ocular micrometer is required for the measurement of eggs, larva etc. An accredited calibration laboratory for the microscope in which it is used must calibrate it.

12.3 Procedures and Tests

12.3.1 A concentration method and a permanent mount should be used to examine stools for optimal detection of parasites. The method should fit the purpose according to clinical indication. If a wet mount is performed, the limitation should be communicated in the report.

12.3.2 Both thick and thin blood films should be used in the examination for malarial parasites in suspected cases of malaria.

12.3.3 Stained films should be washed with a buffer of known pH (6.8 - 7.2). Thick film examination should include at least 100 oil immersion fields (approximately 10-15 mins).

12.3.4 The physician must be informed immediately of a blood film positive for malaria.

13. VIROLOGY

13.1 This section applies to any laboratory providing facilities for the diagnosis of viral infections, which include cell culture, antigen detection, serology or molecular diagnosis. . (Refer to “MOLECULARPATHOLOGY” section).

13.2 Sterility of all culture media must be ensured following the addition of ingredients post sterilization.

13.3 All cell cultures must be tested for mycoplasma contamination immediately upon receipt, after recovery from the deep freezer and at regular intervals as the cultures are maintained in the laboratory.

13.4 Animal sera for use in culture media must be tested to exclude toxicity to cells.

13.4 Appropriate cell lines should be available to support the services offered by the laboratory.

13.5 Tube monolayer cultures should be incubated for a sufficient time to recover the relevant viruses.Tube cultures should be checked for cytopathic effect every other day for the first two weeks,unless other additional diagnostic methods are used (e.g. shell vials), in which case the observation schedule may be modified as appropriate.

13.6 Media and diluents must be checked for sterility and pH.

13.7 There must be documentation of cell types, source, passages and media used in their propagation.

13.8 Worksheets or records must indicate titres, when known, of reagents and control sera.

13.9 There must be known positive and negative controls with each run of test specimens for all direct antigen tests on patient specimens.

14 SEROLOGY FOR INFECTIOUS AGENTS

14.1 In addition to the general requirements described in the “IMMUNOLOGY” section, the following points apply to infectious disease serology.

14.2 The method used should be appropriate to the clinical indication. Relevant interpretive comments should be inserted, and request for a follow up specimen made where necessary.

14.3 Serum should be stored for an appropriate length of time when paired titres are expected to be performed.

14.4 Qualified personnel should be available to provide consultation regarding the interpretation of results.