



ACCREDITATION SCHEME FOR LABORATORIES

Technical Notes MED 002

Specific Criteria for Microbiology Section

1. Introduction & Scope

- 1.1 a) This document describes the specific requirements for clinical microbiology laboratory to be accredited.
- b) The International Standard 'ISO 15189 Medical laboratories – Particular requirements for quality and competence', other MEDICAL Series Technical Notes published by SAC-SINGLAS shall be studied in conjunction with this document.
- 1.2 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.

2. General Technical Note : Medical 001

- 2.1 Please refer **General Technical Note: Medical - 001** for the following:
- Personnel
 - Accommodation & Environmental Conditions
 - Physical Facilities and Laboratory Safety
 - Laboratory Equipment – Calibration & Maintenance
 - Pre-examination Procedures
 - Requisitions, Collection and Handling of Specimens
 - Examination Procedures
 - Test Methods and Method Validation
 - Assuring Quality of Examination Procedures
 - Quality Control, Proficiency Testing, Reagents and Reference Materials
 - Post-examination Procedures
 - Retained Samples and Waste Disposal
 - Reporting of Results

3. Personnel

- 3.1 Refer to Personnel in **General Technical Note: Medical - 001**.

4. Accommodation and Environmental Conditions

- 4.1 Refer to Accommodation & Environmental Conditions - Physical Facilities and Laboratory Safety in **General Technical Note: Medical - 001**. In addition to that the following is applicable to microbiology laboratory.

4.2 Safety

4.2.1 Biological safety cabinet:

- a) A biological safety cabinet shall be provided for handling cultures.
- b) Handling of cultures with mycelia growth during laboratory investigations should be done in a biological safety cabinet.
- c) The biological safety cabinet shall meet minimum requirements for microbiological work. It shall be certified annually to assure that the filters are functioning properly and the airflow rates meet specifications.
- d) There shall be written procedures for staff handling microbiological cultures to ensure that they recognize potential risk groups 3 (RG3) or biothreat agents, and take further action to ensure safety of personnel and correct identification of the pathogen. There shall be procedures for handling suspected RG3 agent and other agents with special hazard of serious laboratory-acquired infection suspected e.g. *Burkholderia pseudomallei*, *Neisseria meningitidis*, *Brucella spp.*

4.2.2 Centrifuge: Sealed buckets shall be used in centrifuges when infectious organisms are present or are likely to be present. Where infection may be acquired by aerosolisation, the bucket shall be unloaded in a biological safety after waiting for a suitable time before opening the sealed buckets.

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

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

4.3 Mycobacteriology

4.3.1 The laboratory shall be assessed according to the types of tests offered: Acid-fast smear, Molecular diagnosis and Mycobacterial culture and identification. A risk assessment should be done and safe work practices, accommodation and environment provided to minimize risk of transmission to staff. The use of a BSL-3 or equivalent facility for TB culture work will need to be in compliance with requirements under the Biological Agents and Toxins Act.

5. **Equipment:**

5.1 Refer to Laboratory Equipment – Calibration & Maintenance in **General Technical Note: Medical - 001**. In addition to that the following is applicable to microbiology laboratory.

- 5.2 Anaerobic jars should be checked with methylene blue strips, fastidious anaerobes or other appropriate procedure.

5.3 Parasitology

- 5.3.1 A calibrated ocular micrometer is required for the measurement of eggs, cysts and larva.

6. Pre-examination Procedures

- 6.1 Refer to Pre-examination Procedures – Requisitions, Collection and Handling of Specimens in **General Technical Note: Medical - 001**.

- 6.2 There must be procedures/policies for appropriate collection, transport and rejection of samples including how to deal with samples submitted after office hours.

Clients should be instructed to send microbiological specimens in appropriate leak-proof containment. Specimens sent to a referral laboratory should also be packaged and labelled with proper containment.

7. Examination Procedures

7.1 Bacteriology

Suitable control strains, for identification and antibiotic susceptibility testing, should be traceable to type collections and the passage history documented. There should be procedures for maintenance of the control strains including when to change aliquots or re-characterize the controls.

- 7.1.1 Respiratory cultures: All sputa shall 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* in-house or via a referral laboratory, when that is requested by the physician.

- 7.1.2 Urine cultures: The laboratory should perform and report quantitative cultures and use media and procedures that permit isolation of clinically-relevant 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 deg C, or by adding preservative to the container, and or using dip slide cultures. For dip slide cultures, there must be evidence that the estimated counts correlate with actual colony counts.

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

- 7.1.4 Stool cultures: The procedure should permit isolation and identification of enteric pathogens in patients with diarrhoea (using appropriate selective media and enrichment media). The range of pathogens detected by the culture should be indicated to lab users, and procedures in place to cater to requests for gastroenteritis agents not routinely detected. The policy for reporting susceptibility test results should not lead to inappropriate antibiotic use.
- 7.1.5 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. There should be procedures to check and resolve discrepancies between Gram stain and culture. 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.
- 7.1.6 Blood cultures: Sterile technique for drawing and handling blood cultures should be defined. The blood culture system shall be designed to recover both aerobic and anaerobic organisms. Sub-cultures and/or stains need not be done on blood cultures performed by automated methods if the bottles are monitored as recommended by the vendors. There shall be a policy for immediate notification of positive blood results
- 7.1.7 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.
- 7.1.8 Anaerobic cultures: Specimens and cultures should be placed in an anaerobic atmosphere as soon as practicable.
- 7.2 Mycobacteriology
- 7.2.1 Rapid and reliable methods shall be used for microscopic examination, isolation, identification and antimycobacterial susceptibility testing of *Mycobacterium tuberculosis* complex.
- 7.2.2 Certain specimens (e.g. sputum) should be concentrated before AFB smear examination and culture.
- 7.3 Mycology
- 7.3.1 Technical procedures for isolation and identification of fungi directly from specimens and cultures shall be available.
- 7.3.2 These include the use of microscopy, stains, selective media, biochemical tests, serological tests and nucleic acid tests.

7.4 Parasitology

- 7.4.1 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.
- 7.4.2 Both thick and thin blood films should be used in the examination for malarial parasites in suspected cases of malaria.
- 7.4.3 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).
- 7.4.4 The physician shall be informed immediately of a blood film positive for malaria. Where infection by *P. knowlesi* is suspected, there should be a process for identification.

7.5 Serology for Infectious Agents

- 7.5.1 In addition to the general requirements described in the "IMMUNOLOGY" section, the following points apply to infectious disease serology.
- 7.5.2 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.
- 7.5.3 Qualified personnel shall be available to provide consultation regarding the interpretation of results.

8. **Assuring Quality of Examination Procedures –**

- 8.1 Quality Control and Proficiency Testing
Refer to Quality control and proficiency testing in **General Technical Note: Medical - 001**. In addition to that the following is applicable to microbiology laboratory.
- 8.2 Results of control tests shall be reviewed before reporting patient results.
- 8.3 There shall be records of at least weekly review of quality control results by the head or designee.
- 8.4 External quality assurance (proficiency testing) specimens shall be tested in the same manner as patient specimens. Follow up action, where applicable, should be documented.
- 8.5 Corrective actions taken when errors or unacceptable results are detected or when tolerance limits are exceeded shall be documented. Any impact on test methods or reporting procedure should be addressed.

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

8.7 Reagents / Stains / Media / Kits / Antimicrobials

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

8.7.2 They should be labelled, 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.

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

8.7.4 The use of commercial reagents and controls shall comply with manufacturer's instructions.

8.7.5 The laboratory shall 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.

8.7.6 The laboratory shall perform and record results with positive and negative controls at specified periodic intervals. Recording of qualitative observations e.g. colour change, should be unambiguous with respect to the expected result.

8.8 Media

8.8.1 The laboratory shall 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.

8.8.2 For purchased media, the manufacturer shall document to the user that their quality control activities meet the criteria described as above, 8.9.1 The laboratory should test media that are known to show significant variability in performance e.g. chocolate agar (for *H. influenzae*), campylobacter agar, Thayer-Martin medium

- 8.8.3 The user shall visually examine each batch of media for breakage, contamination, appearance, or evidence of freezing or overheating.
- 8.8.4 All the quality control procedures that are carried out in the laboratory shall be documented.
- 8.8.5 A record should be kept of all lot numbers and expiration dates of the media received for the past two years.
- 8.8.6 Reference cultures and sera should be maintained for the proper control of stains, media, reagents, antimicrobial susceptibility tests and serological tests.
- 8.9 Bacteriology
- 8.9.1 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.
- 8.9.2 Guidelines shall be established for the number and type of antibiotics to be reported for organisms isolated from different sites of infection. They should be clinically relevant and include suppressing the results of selected antibiotics to encourage prudent antibiotic use.
- 8.9.3 Only single isolates or pure cultures shall 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.
- 8.9.4 Inoculum density 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.
- 8.9.5 Written criteria shall be available for interpretation of the end point or zone size.
- 8.10 Mycology
- 8.10.1 All stains shall 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).
- 8.10.2 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.

8.11 Mycobacteriology

8.11.1 *Stains*

- 8.11.1.1 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)

8.11.2 *Identification*

- 8.11.2.1 A known strain of *M. tuberculosis* should be run whenever identification of *M. tuberculosis* complex is performed.
- 8.11.2.2 Biochemical tests used for identification should be checked each day of use with appropriate positive controls.
- 8.11.2.3 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.
- 8.11.2.4 Temperature growth requirements and photo-reactivity studies shall be done when appropriate if complete identification of mycobacterial organisms cultured is performed by conventional methods. Alternative methods for species identification e.g. HPLC, Maldi-TOF, DNA sequencing, should have been verified for accuracy.

8.11.3 *Susceptibility Testing*

- 8.11.3.1 A control strain of *M. tuberculosis*, which is sensitive to all antimycobacterial agents, should be included with each run.

8.12 Parasitology

- 8.12.1 Quality control checks of the specific gravity of concentrating solution (e.g. Zinc sulphate) should be done periodically.
- 8.12.2 All permanent stains shall 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) shall be checked with appropriate control organisms each time the stain is used.

8.13 Virology

- 8.13.1 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 "MOLECULAR PATHOLOGY" section).

- 8.13.2 Sterility of all culture media shall be ensured following the addition of ingredients post sterilization.
- 8.13.3 All cell cultures shall 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.
- 8.13.4 Animal sera for use in culture media shall be tested to exclude toxicity to cells.
- 8.13.5 Appropriate cell lines should be available to support the services offered by the laboratory.
- 8.13.6 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.
- 8.13.7 Media and diluents shall be checked for sterility and pH.
- 8.13.8 There shall be documentation of cell types, source, passages and media used in their propagation.
- 8.13.9 Worksheets or records shall indicate titres, when known, of reagents and control sera.
- 8.13.10 There shall be known positive and negative controls with each run of test specimens for all direct antigen tests on patient specimens.

9. Post-examination Procedures

9.1 Sample Storage

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

9.2 Serology for Infectious Agents

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

10. Reporting of Results

- 10.1 Reports with abnormal results shall be reviewed by senior personnel and evaluated for conformity with the clinical information available
- 10.2 Reports should be available in a timely manner, and preliminary reports issued if specimens take a longer time to work up.

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