CRITERA FOR THE ACCREDITATION OF MICROBIOLOGY IN MEDICAL LABORATORIES

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Table of Contents

Page

1. PURPOSE AND SCOPE

The purpose of this document is to define the general, technical and specific requirements to be met by laboratories in the field of Medical Microbiology requiring accreditation to ISO 15189.

2. ABBREVIATIONS

AST	Antimicrobial Sensitivity Test
PPE	Personal Protective Equipment
BSC	Biological Safety Cabinet

3. GENERAL AND TECHNICAL REQUIREMENTS

3.1.1. The scope of activities of the laboratory shall indicate which of the following services are provided on site and which are not:

(i) Bacteriology
(ii) Mycobacteriology
(iii) Mycology
(iv) Parasitology
(v) Drug susceptibility testing

3.1.1 For the procedures carried out on site the laboratory shall indicate the extent to which such examination procedures are carried out:

(i) Direct examinations: Macroscopy /Microscopy
(ii) Culture: Aerobic, Anaerobic, Micro-aerophilic
(iii) Identification: limited or extensive
(iv) Screening methods
(v) Confirmatory methods

3.1.2 Where any samples are referred to another laboratory for complete or partial examination, such information shall be provided by the referring laboratory.

3.2 EXAMINATION PROCEDURES:

Detailed procedures shall be available for the direct examination, culture, identification and drug susceptibility testing for each species/ organism being tested for and shall be as extensive as required.

3.2.1 BACTERIOLOGY

3.2.1.1 Cerebrospinal Fluid (CSF)

(i) CSF samples shall be processed immediately on receipt.
(ii) The macroscopic appearance of the neat sample and supernatant shall be recorded.
(iii) Perform a differential cell count.
(iv) Centrifuge the CSF sample and perform Gram stain on the deposit.
(v) Routine culture of the sample shall allow for the recovery of organisms associated with meningeal disease, especially those organisms requiring fastidious growth requirements (e.g., *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae).*
(vi) All isolates shall be identified.
(vii) Bacterial antigens may be detected using rapid testing such as Latex agglutination tests.

3.2.1.2 Blood Cultures:

(i) Blood culture media type shall be selected as appropriate for the patient and volume of blood to be used e.g., Paediatric, Resin or standard blood culture bottles.
(ii) Blood culture medium should be at room temperature before inoculation.
(iii) Blood cultures should not be refrigerated for transportation, and should be incubated at 37°C immediately to aid recovery of:
- Aerobic organisms
- Anaerobic organisms (when indicated)
- CO_2 dependant organism
- Fastidious organisms.

(iv) Manual system blood cultures:
- Bottles shall be examined daily for signs of growth for the required number of days' incubation.
- Bottles should be agitated during the first 24 hours of incubation for optimal recovery of fastidious organisms.
- Negative cultures shall be sub cultured onto appropriate media within 24 hours, after 48 hrs and a terminal subculture performed.
- All isolates should be identified.

(v) Automated blood culture systems should follow the manufacturers recommendation for inoculation and incubation.

3.2.1.3 Aspirates, wound cultures, tissue and pus.

(i) Whole pus or tissue is preferred over the use of swabs for the collection of samples.
(ii) Special procedures shall be in place to prevent the loss of anaerobic organisms, e.g., use of appropriate transport medium.

(iii) Gram stains shall be performed routinely and reported on.
(iv) Culture media for the recovery of aerobic and anaerobic organisms shall be used routinely.
(v) Where appropriate, selective media and methods shall be used to recover strict anaerobes.

3.2.1.4 **Anaerobic Isolation**

(i) Specimens for anaerobic examination shall have adequate arrangements for preventing or minimising exposure to oxygen.
(ii) All specimens for anaerobes shall be transported in suitable transport medium.
(iii) The method used to obtain an anaerobic culture environment, shall be specified e.g. anaerobic jar.
(iv) Cultures on solid media must be placed into an anaerobic environment immediately.
(v) The liquid media used for anaerobes shall be specified, i.e., thioglycolate broth, Cooked meat media.
(vi) The method used to quality control the anaerobic environment shall be stated, (Control organism, commercial anaerobic indicators).
(vii) Disc diffusion susceptibility testing is not normally done as there are no CLSI guidelines available, alternate methods e.g., E-Test, broth dilution or agar dilution methods should be used.
(viii) Control organisms shall be tested with each batch of susceptibility tests performed.

3.2.1.5 Respiratory Samples

(i) When performing sputum cultures, all steps shall be taken to obtain a "true" sputum sample.
(ii) Sputum samples should be assessed before culture.
(iii) Procedures shall be available for the identification of Streptococcus pneumoniae and Haemophilus influenzae.
(iv) Where indicated other gram-negative bacilli shall be identified.
(v) Appropriate procedures shall be available for the isolation and identification of other pathogens i.e., *Staphylococcus aureus, Cryptococcus species, Candida albicans.*

3.2.1.6 Urine Samples

(i) Where possible urine samples should be examined within 2 hours of voiding or refrigerated if this is not possible.
(ii) Quantitative or qualitative cell counts, culture and colony counts should be performed.
(iii) Culture media used should allow for the isolation and the identification of gram positive and negative organisms.
(iv) Where appropriate urine samples with large amounts of red blood cells should be examined for the presence of Bilharzia ova (*Schistosoma haematobium*).

3.2.1.6.1 Faecal samples and Rectal swabs

(i) Routine procedures shall be used that allow for the rapid isolation and identification of enteric pathogens in patients with diarrhoea and from asymptomatic carriers
(ii) Enrichment and selective media shall allow for the recovery of small numbers of enteric pathogens.
(iii) Where indicated, procedures shall be available for the recovery of the following bacteria:
- *Vibrio cholera*
- *Yersinia enterocolitica*
- *Campylobacter species*. (microaerophilic culture conditions)
- *Aeromonas species*

3.2.1.6.1 Swab cultures for Neisseria *gonorrhoeae*:

(i) Cervical, Urethral or other appropriate sample types shall be inoculated directly onto appropriate selective media or must be submitted in an appropriate transport medium that will allow for optimum recovery of the organisms.
(ii) The culture media and the means of inoculation and incubation shall be optimum for the isolation of Neisseria.

3.2.2 PARASITOLOGY:

(i) A procedure manual shall be available in the work area and shall include instructions for proper sample collection, methods used and the criteria for ova and parasite identification.
(ii) Samples shall be examined in a fresh state, especially for the presence of trophozoites.
(iii) Where samples cannot be examined immediately, steps shall be taken to preserve the sample e.g.
  a. Direct Wet preparation with or without Iodine staining.
  b. A recognised concentration procedure.
  c. Permanent stained preparation (e.g. Haemotoxilin stain)
  d. Modified Ziehl Neelsen stain for Cryptosporidia.
(iv) If available the use of a calibrated ocular micrometre may be used for determining the size of ova, larva, cysts or trophozoites.
(v) Reference material should be available at the work bench and should include
  a. Printed atlas of parasite and ova.
  b. Similar illustrations and descriptions
  c. Permanent mounts and slides
(vi) The following shall apply to blood films for Plasmodium:
  a. Both thick and thin films shall be made and examined
  b. Films shall be prepared immediately from anti coagulated venous blood.

c. An appropriates staining method shall be used, e.g. Wrights, Giemsa stains.
d. The pH of the buffer used for the stains must be checked i.e 7.2.
e. For thick films at least 100 fields shall be examined before a no parasite seen report is given.
f. The % parasitaemia should be established.

3.2.2.5 MYCOLOGY

Isolation and identification techniques.

(i) Laboratory shall perform preliminary screening procedures such as direct wet preparations and stains.
(ii) The use of suitable selective culture media (e.g. DTM or Mycosel) shall be used for the growth and isolation of Dermatophytes and Systemic mycoses.
(iii) Antibiotics shall be added to the culture media to suppress the growth of bacterial and fungal contaminants.
(iv) The temperature ranges for the growth, isolation and differential tests for dermatophytes and systemic mycoses shall be defined in the culture methods.
(v) Appropriate procedures for the differential tests used for fungal identification shall be used in respect of the type of work undertaken by the lab and should include:
    a. Biochemical tests, manual and/or automated
    b. Chlamydiospore formation
    c. Specific temperature growth requirements
    d. Slide/block culture

3.2.4 MYCOBACTERIOLOGY:

Diagnostic Procedures**.**

a) Screening of samples may include TB microscopy, GeneXpert testing or Line Probe Assay (LPA).
b) Appropriate staining methods such as Ziehl Neelsen, Fluorescence or Kinyoun shall be used.
c) Positive and negative controls shall be included in all batches when performing microscopy, culture or drug susceptibility testing.
d) A decontamination procedure shall be used to prepare specimens for TB culture. The type of decontaminant to be used shall be stated in the procedure.
e) The concentration method used for the microscopy and culture of Mycobacteria in sputum and non-sputum samples shall be stated.
f) The use of specific culture medium for primary isolation shall be stated (for manual or automated systems)
g) The length of incubation of cultures before a negative report is issued shall be clearly stated.
h) All suspected mycobacterial isolates shall be checked for acid fastness.
i) Species identification shall be done and the specific method(s) used stated.

j) Reference strains shall be maintained as controls for identification and susceptibility tests.
k) Sensitivity tests shall be carried out on all relevant isolates of MTB, using an agreed method.

3.2.5 ANTIBIOTIC SUSCEPTIBILITY TESTING (AST):

a) Types of susceptibility testing:
   I. Disc susceptibility testing i.e., Kirby Bauer method.
   II. Minimum inhibitory concentration (MIC) i.e. Broth microdilution, ETEST©.
   III. Tests to predict Oxacillin resistance (mecA mediated resistance) for Staphylococcus shall be carried out as per a specified method.

b) Quality control of AST:
   I. Recognised Quality Control strains of known susceptibility shall be tested at least weekly as per the CLSI guidelines (or other internationally recognised organisations e.g. EUCAST).
   II. A standardised inoculum shall be used.
   III. For Disc AST the specific zone sizes of the control organism shall be measured and recorded and used to determine the antimicrobial agent's sensitivity or resistance.
   IV. For broth dilution testing, the concentration and identity of all diluted drugs shall be enforced.
   V. Any AST that has not passed quality control shall not be reported for patient results until it has been shown to pass QC over a period of at least 5 consecutive days.

4.0 REPORTING OF RESULTS:

4.1 Bacteriology

a) Preliminary reports shall be issued when final results are not available within 48hours.
b) Final negative results shall normally be available within 48-72 hours (specimen type dependant)
c) Drug susceptibility results shall be reported as sensitive or resistant.
d) MIC testing values shall be reported as well as the interpretation.

4.2 **Mycology:**

a) Preliminary results of the microscopic examinations shall be made available to requesting clinicians.
b) Final results shall be available within 4 to 6 weeks.

4.3 **Mycobacteriology:**

a) AFB microscopic examination of specimens shall indicate the quantity of AFB's in the smear as per IUAT guidelines.
b) Where appropriate the report shall make reference to specimens that are inadequate in quantity and quality

c) Microscopy and GeneXpert reports shall be issued within 24 hours of receipt of sample.
d) Interim results shall be issued for culture positive specimens.

5.0 LABORATORY EQUIPMENT AND REAGENTS

5.1. Equipment

a. The laboratory shall be furnished with all equipment needed for provision of services.
b. Equipment shall be verified upon installation and before use.
c. Equipment shall be uniquely labelled.
d. Operators' manual or SOP shall be available, and shall be operated by trained/ authorised personnel.
e. Records for each item of equipment shall be maintained as per the ISO standard.
f. The laboratory shall have a documented procedure for equipment calibration (SADCAS TR 09: Criteria for performing calibration and Intermediate checks on equipment used in accredited facilities)
g. Laboratories performing TB culture testing shall have a biological safety cabinet (BSC).
h. Laboratories that prepare in house reagents and culture media shall have the following equipment available:
    - Balance
    - pH meter and buffers.
    - Autoclave (size to be appropriate for the workload)

5.2. Reagents /Consumables

a) The laboratory shall have a documented procedure for the reception, storage, acceptance testing and inventory management of reagents and consumables.
b) Records shall be maintained for each reagent /consumable that contributes to performance of testing.
c) Where the laboratory prepares reagents or culture media in house, the records shall include the person/s responsible for the preparation, date of preparation, expiry date as well as the acceptance testing.

6.0 LABORATORY SAFETY AND ENVIRONMENTAL CONDITIONS:

a) Personal protective equipment (PPE) shall be available and appropriate to the class of organism being handled. At minimum shall include gloves, masks, goggles and laboratory coats.
b) Specimens and culture techniques involving Class III and IV organisms e.g. Mycobacteria, systemic fungal pathogens i.e. Histoplasma shall always be handled in an appropriate BSC.
c) Centrifuges must be capable of containing biohazardous aerosols and spillage i.e. centrifuge buckets with lids.
d) Appropriate disinfectants must be available in the work area.

e) Laboratories should have sufficient space to allow work to be carried out safely and competently.
f) BSC must be checked daily /weekly or when in use to ensure proper functioning.
g) There should be adequate separation of activities when performing TB testing in the laboratory environment.

7.0 REFERENCES

- ISO 15189: Medical Laboratories – Particular requirements for quality and competence
- SADCAS TR 09: Criteria for performing calibration and Intermediate checks on equipment used in accredited facilities.

APPENDIX - AMENDMENT RECORD

Revision Status	Change			Approved by	Effective Date
	Page	Clause/ Subclause	Description of Change		
Issue 1	-	-	-	CEO	2023-02-28