Ministry of Health

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Acknowledgment

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Acronyms:

BA	Blood agar
BSC	Biosafety cabinet
CLSI	Clinical laboratory standards institute
CA	Chocolate agar
MAC	MaConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
IP	Infection preventions
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant *Staph. Aureus*
SOP	Standard operating procedure
AST	Antimicrobial susceptibility testing
QC	Quality control

1. **Purpose**

   This SOP describes the processing and investigation of tissues and biopsies from deep-seated sites and organs for bacteria and fungi.

2. **Scope:**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions:**

   3.1 Biopsy: A portion of tissue removed from body for further examination.

   3.2 Biohazard organism: biological organism that pose threat to human.

   3.3 Tissue Homogenization: preparation of a uniform dilution of a known quantity of tissue suspended within a known quantity of a suitable diluents and uniformly macerated or crushed in such a manner as to disperse minute fragments of tissue evenly throughout the mixture.

   3.4 Biosafety cabinet: an enclosed, ventilated laboratory workspace for safely working with materials contaminated with pathogens requiring a defined biosafety level.

4. **Procedure**

   4.1. Clinical background:

   With the increasing sophistication of clinical imaging and sampling devices there are few organs in the human body that cannot be biopsied. Tissue obtained at operation is particularly precious as the sampling procedure may not be repeatable. Ideally these specimens should be discussed with the laboratory prior to sampling to ensure that transport and processing are timely and appropriate tests are performed. Biopsies may be taken from chronically infected tissues and so, in addition to investigation for bacterial infection, they may also require investigation for fungi, Mycobacterium species and parasites. Histological investigation will often inform the decision to investigate for particular classes of infection.

   4.2. Principle:

   Different body tissues can be infected with different types of organisms (bacteria, fungi, viruses and parasites). To identify the causative organisms, a piece of infected tissue (sign of necrosis, abscess borders, gangrenous tissues, changed colors of tissue, tissues with signs of inflammation) should be collected and sent to microbiology laboratory for microscopy and culture.

4.3. Pre – analytical stage:

4.3.1. Sample:

4.3.1.1 Sample type: Tissue, biopsy.

4.3.1.2 Aseptic technique must be used for sample collection. Collect specimens before antimicrobial therapy where possible.

4.3.1.3 Appropriate amount must be collected that allow all investigations to be done (microscopy and cultures) with residual amount can be reserved if further investigation would be needed e.g., mycobacterial cultures or 16S rDNA PCR. Small sample size will limit the number of investigations.

4.3.1.4 . Multiple samples recommended to be collected depending on the site and extent of infection, in which case proper labeling of each sample shall be ensured. Repeat collection will depend on clinical condition.

4.3.1.5 Sample transported in leak proof container placed in sealed plastic bag, with or without normal saline. (For tiny piece of tissue, it is recommended to use normal saline or water to prevent desiccation). Note: Specimens received in formalin are not suitable for culture.

4.3.1.6 Specimens should be transported and processed as soon as possible. In case of delay, store the sample in 2-8 C.

4.3.1.7 Tissues and biopsies are not easily repeatable specimens thus prolonged storage after examination (up to 1 month) of residual specimens shall be considered or as per local protocol depending on availability of facilities for prolonged storage, 2-8 C.

4.3.2. Material:

Reagents	**Consumables/Supplies**	**Equipment**
MacConkey plate Blood Agar plate Chocolate plate SAB agar Gram stain reagent	Microscopic slides Sterile loops MTZ discs Petri dish	Microscope Slide dryer Safety cabinet class II Incubators Sterile scalpel *Grinder , Sterile Scissor

*Place the grinder in hycolin for sterilization overnight before autoclaving.

4.3.3. Safety precaution:

4.3.3.1 All specimens need to be treated as potentially infectious.

4.3.3.2 Where infection with a Hazard Group 3 organism eg Mycobacterium tuberculosis, Brucella spp, dimorphic fungi, is suspected, all specimens must be processed in a biosafety cabinet (BSC II).

4.3.3.3 Grinding and homogenization of all specimens must be undertaken in a microbiological safety cabinet. Wherever possible, the use of sterile scissors is recommended in preference to a scalpel blade.

4.3.4. Quality control:

4.3.4.1 Check the expiry dates of all media, reagents and stains before use.

4.3.4.2 All media, reagents, kits, and stains **MUST** be quality controlled before use.

4.3.4.3 Identification tests should be run with appropriate controls.

4.3.4.4 Record the quality control results in the appropriate QC sheet.

4.4. Analytical stage:

4.4.1 Pre-treatment:

4.4.1.1 Grind or homogenize specimen using a sterile tissue grinder, a sterile scalpel or sterile scissors and petri dish. Aseptic procedure must be followed to avoid contamination.

4.4.1.2 The addition of a small volume (approximately 0.5mL) of sterile, filtered water, saline, peptone, or broth will aid the homogenization process.

4.4.1.3 Select a portion of the tissue biopsy sample that is bordering and within the area of infection (i.e., necrotic tissue is usually at the center of infected tissue areas).

4.4.1.4 The process should be performed in a Class II BSC.

4.4.1.5 Specimens for fungal culture should be cut (finely sliced) rather than homogenized in order to maintain the structure and morphology of the fungus.

4.4.2 Microscopy :

4.4.2.1 For homogenized sample: Place one drop of specimen on to a clean microscope slide with a sterile pipette. Spread this with a sterile loop to make a thin smear for Gram staining.

4.4.2.2 For non-homogenized sample: touch the sides of one or more pieces of the specimen to a clean microscope slide for Gram staining.

4.4.2.3 For fungal culture: Place a small portion of tissue in a sterile tube and add equal proportions of 10-30% KOH. Add Calcofluor white (0.1%) or lactophenol stain. Leave to digest for 20 min at room temperature. After digestion, the tissue should be squashed to produce a single layer of cells.

4.4.2.4 Using a sterile pipette, place the digested tissue on a glass slide with cover slip, and examine under microscope.

4.4.3 Culture

4.4.3.1 Using sterile loop, inoculate each agar plate and enrichment broth (cooked meat, or BHI) with the specimen, see Table 1.

4.4.4 Antimicrobial Susceptibility testing (AST) : As appropriate; to test and report as per current CLSI standard.

Table 1 culture media, incubation condition and target organisms

Clinical details/ conditions	Media	Incubation			Target organism(s)
		Temp. °C	Atmos.	Time	
All clinical conditions	Blood agar	35-37	5-10% CO2	40- 48hr	Any organism
	Chocolate agar	35-37	5-10% CO2	40- 48hr	
	CLED/ MacConkey agar	35-37	Air	18- 24hr	
	Selective anaerobic agar	35-37	Anaerobic	40- 48hr	Anaerobes

	Enriched broth. Subculture if evidence of growth (turbidity) or at day 5 to above media	35-37 35-37	Air As above	Up to 5d As above	Any organism
Immunocompromised , or suspected fungal infection	Add Sabouraud agar	35-37 and 28-30C	Air	7days	Yeasts Molds
Actinomycosis	Blood agar supplemented with metronidazole and Nalidixic acid. (Many Actinomyces species may be inhibited by neomycin).	35-37	Anaerobic	10d	Actinomyces species

4.5. Post – analytical stage:

4.5.1. Microscopy: Report on WBCs and organisms detected. Report fungal structure

4.5.2. Culture :

4.5.2.1 Inform microbiologist when significant organism (ex.staph aureus, B heamlytic strept, pseudomonads, yeast) is detected or skin flora isolated from invasively collected specimens.

4.5.2.2 The microbiologist should decide about the extent of workup, ID and reporting when more than one pathogenic bacteria isolated depending on the site, sterility during collection and clinical background.

4.5.2.3 Notify IP staff when MRSA or MDRO isolated.

4.5.2.4 Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected. The following results should be reported:

- Clinically significant organisms isolated.
- Other growth with appropriate comment, eg No significant growth.
- absence of growth

4.5.3. Report AST as clinically indicated according to local or national protocol.

5. Responsibilities

5.1. Responsible (supervisor, incharge..etc) staff:

- To ensure the adherence to critical result communication procedure.
- To facilitate the alternative channels once needed.
- To communicate with microbiologist.

5.2. Quality manager /officer

- To follow up the implementation of the procedure.
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended.
- To report test failures or incident.

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
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