Ministry of Health

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Acknowledgment

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Acronyms:

LPCB	Lactophenol cotton blue stain
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
CPHL	Central Public Health Laboratory

1. **Purpose**

This document describes the procedure for fungal microscopy using Lactophenol cotton blue (LPCB).

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 Lactophenol cotton blue stain: a mixture of methyle blue, histological stain and lactophenol (a solution of phenol, lactic acid and glycerol in water). It is used to visualize fungal structure.

4. **Procedure**

4.1. Clinical background:

Fungal infections are seen frequently in diagnostic laboratories. There are several ways to classify the fungal infections either on the involved body site or fungal morphology. Widely accepted classification divides the fungi into unicellular (yeast), multicellular (molds) or dimorphic. Fungal identification can be challenging from morphology examination alone. Advanced techniques are available.

4.2. Principle:

Lactophenol cotton blue (LPCB) stain used to stain molds (not yeast cells). It contains three components: the phenol which kills any live organism, lactic acid which preserves the structure of the fungus and the cotton blue which stains the fungus allowing better identification.

4.3. Pre – analytical stage:

4.3.1. Sample:

- Sample type: Skin scraping, fluids, tissues or fungal culture growth.
- Transportation media: Specimens should be collected and transported in a properly labelled, sealed, sterile container.
- Sample stability and storage requirements: most samples are stable even if processing is delayed for 24 hrs.
- If a delay in transport or processing is anticipated, store all specimens except hair, skin and nails at 4$_{o}$C. Hair, skin and nail specimens should be kept at room temperature.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
LPCB stain. 10% KOH Fungal plates	Microscopic slides Coverslips Sterile loops Scalp / blade/ needle Pipette	Microscope Safety cabinet class II Fridge

4.3.3. Safety precaution:

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.
- All work on filamentous fungus is carried out in biosafety cabinet type 2. Biosafety Level 2 procedures are recommended for personnel working with clinical specimens that may contain dimorphic fungi as well as other potential pathogenic fungi. Gloves should be worn for processing specimens and cultures.

4.3.4. Quality control:

- Check the expiry dates of all media, reagents and stains before use.
- All media, reagents, kits, and stains **MUST** be quality controlled before use.
- Identification tests should be run with appropriate controls.
- Record the quality control results in the appropriate QC sheet.

4.4.Analytical stage:

4.4.1. Skin scraping, Fluid exudate or Tissue staining procedure:

- Mix two drops of 10% KOH with the specimen whether a skin scraping, fluid exudate or tissue on a clean slide.
- Add 1-2 drops of the lactophenol cotton blue stain.

- Place a cover slip gently to make a thin mount avoiding air bubbles.
- Examine the slide under low power (x10) with reduced light.
- Switch to high power (x40) to check for the presence of suspected fungal structures.

4.4.2. Staining procedure for fungal growth:

Fungal cultures are examined directly by microscopic mount or adhesive tape method.

4.4.2.1. Direct microscopic mount

- Add 1 drop of lactophenol cotton blue stain to the microscope slide.
- Carefully take a small portion of the colony and place in the LPCB drop using a mounted needle / sterile loop.
- Place a coverslip, pressing gently to make a thin mount avoiding air bubbles.
- Examine the prepared slide under low power (x100) with reduced lighting.
- Switch to high power (x400) to examine the fungal structures in more detail.

4.4.2.2. Adhesive tape

This method is used to quickly examine the fungal colony under microscope and preserve the fungal morphology.

- Add 1-2 drops of lactophenol cotton blue stain to a clean glass microscope slide.
- Take a good appropriate length of clear adhesive tape (around 40mm) and gently place the sticky side on to the surface of the culture. Apply low pressure allowing fungal elements to become attached to the tape.
- Slowly lift the tape from the colony and place it on to the LPCB stain on the slide gently pressing down.
- Examine the prepared slide under low power (x100) with reduced lighting.
- Switch to high power (x400) to examine the presence of suspected fungal structures in more detail.

4.5.Post – analytical stage:

4.5.1. Interpretation / Results / Alerts:

Positive result: mycelia and fruiting structures stain a delicate blue colour while the background appears a faint, pale blue.

Negative result: The absence of fungal structures indicates a negative result.

4.5.2. Reporting:

Positive result: "Fungal hyphae seen"

Negative result: No Fungal elements seen.

4.5.3. For fungal identification, may refer to Atlas book, identification of pathogenic fungi, HPA.

4.5.4. Result communication: notify microbiologist / senior lab technologist regarding positive microscopy result.

4.5.5. Limitation:

The staining procedure does not always preserve the original position and structure of the conidia, spores, and other characterizing elements which are important for fungal identification

5. Responsibilities

5.1.Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

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
UK SMI staining procedures	HPA	2019	
Mount Sinai Technical Procedure Manual	Mount Sinai	2022	
Mount Sinai Mycology manual	Mount Sinai	2022	
Identification of pathogenic fungi, HPA, 1st edition	HPA	1996	