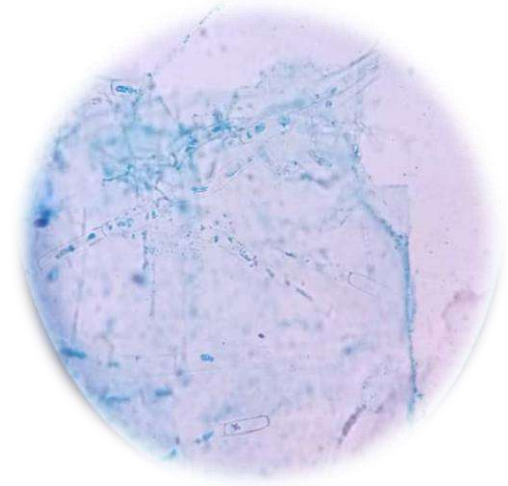
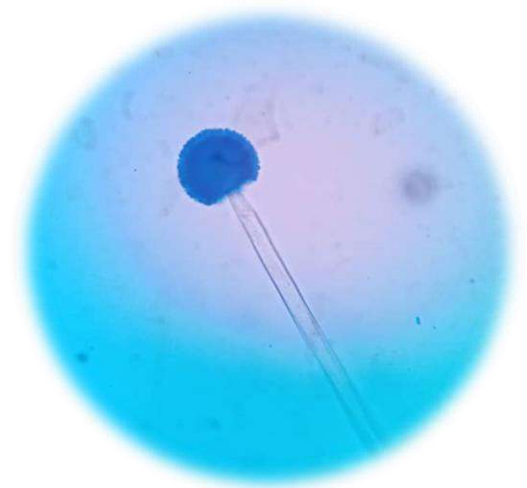


Standard Operating Procedures for Lactophenol Cotton Blue Staining Technique



The Mycetoma Research Center,
University of Khartoum
WHO Collaborating Center
on Mycetoma & Skin NTDs

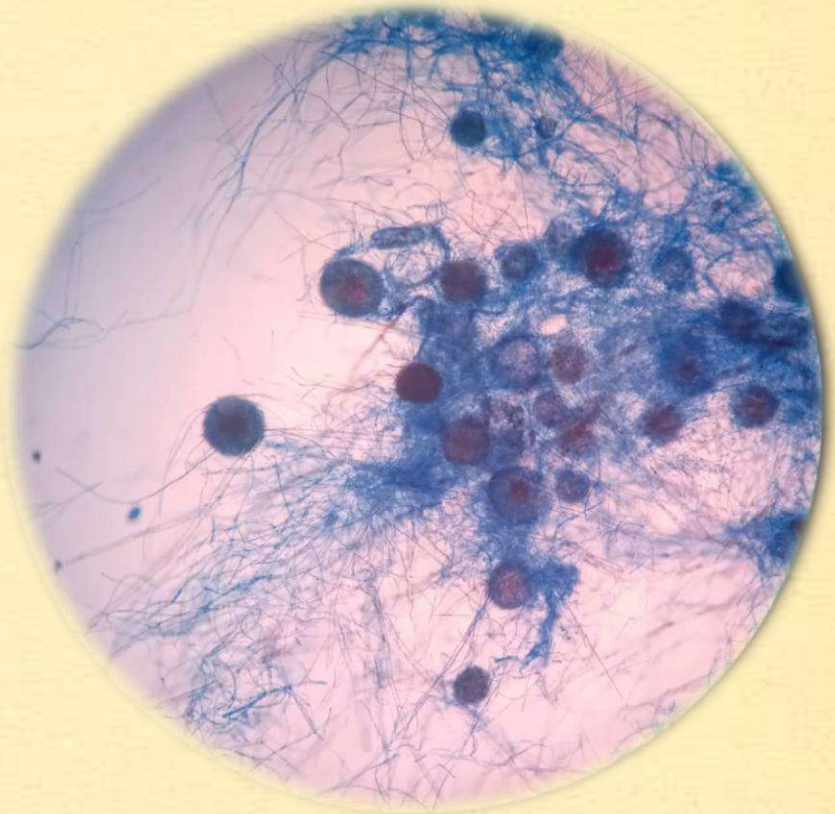


**Standard Operating Procedures
for
Lactophenol Cotton Blue
Staining Technique**



**The Mycetoma Research Center,
University of Khartoum**

**WHO Collaborating Center
on Mycetoma & Skin NTDs**



Number: 007/MRC/2024

Date: May 03 2024

Standard Operating Procedures for Lactophenol Cotton Blue Staining Technique

Purpose

These SOPs provide guidelines for preparing and using Lactophenol Cotton Blue Staining for the microscopic examination of fungal cultures.

Scope

These SOPs are to be used by the laboratory personnel involved in the Lactophenol Cotton Blue Staining technique for the microscopic examination of fungal cultures.

Responsibilities

- **Laboratory Technicians:** Responsible for performing the Lactophenol Cotton Blue Stain according to this SOP.
- **Laboratory Supervisor/Manager:** Responsible for overseeing the implementation of this SOP and ensuring compliance.
- **Quality Assurance Officer:** Responsible for monitoring and evaluating the quality control measures outlined in this SOP.

Safety Precautions

- Work in a Biosafety Level 2 (BSL-2) laboratory.
- Wear appropriate personal protective equipment (PPE), including lab coats, gloves, and safety glasses.
- Handle phenol with extreme caution, as it is toxic and can cause severe burns.
- Handle potentially infectious materials with care.
- Follow established biohazard waste disposal procedures.
- Dispose of waste materials according to laboratory safety protocols and regulations.

Materials and Equipment

- Glass slide
- Wooden stick
- Cover class
- Reagent lactophenol cotton blue stain.
- Microscope slides
- Fungal specimens
- Glass droppers
- Microscope
- Personal protective equipment (lab coat, gloves, safety glasses)



Precaution:

- The stain should be stored at room temperature.
- Protect from light.
- Avoid the eye contact.



Procedure:

Preparation of Lactophenol Cotton Blue Stain Solution

- Ensure a clean and sterile working environment.
- In a clean glass container, mix the following components in the specified proportions:
 - Lactic acid: 20 ml
 - Phenol: 20 g
 - Glycerol crystals: 40 ml
 - Distilled water: 20 ml
 - Cotton blue (Aniline Blue): 0.05g
- Dissolve the phenol crystals in the distilled water. Phenol is caustic, so handle it with care, preferably under a fume hood while wearing gloves and eye protection.
- Add the lactic acid to the phenol solution. Lactic acid serves as a clearing agent to enhance the visibility of fungal structures.
- Add the glycerol to the mixture. Glycerol acts as a mounting fluid, preventing the specimen from drying out.
- Add the Cotton Blue dye to the solution. Mix until the dye is completely dissolved. Cotton Blue stains the chitin in fungal cell walls, making the structures more visible under a microscope.
- Store the prepared Lactophenol Cotton Blue stain in an amber-colored bottle to protect it from light. The stain is now ready for use.

Preparation of Smear

- Using a dropper, place a small drop of Lactophenol Cotton Blue stain in the center of a clean glass slide.
- Using a sterilised inoculating loop, small spatula, or forceps, gently pick up a small portion of the fungal culture (e.g., mycelium, conidia, or spore).
- Carefully place the fungal specimen in the drop of LPCB stain on the slide. Make sure that the specimen is well-submerged in the stain to avoid drying out and to ensure thorough staining.
- If the fungal structure is dense or large, use a sterile needle or forceps to gently tease apart the specimen in the stain. This step helps to spread out the mycelium or spores for better visualisation under the microscope.



Place drop



Mix



Examine

Preparation of Smear

- Gently place a coverslip over the stained specimen. Start by touching one edge of the coverslip to the edge of the drop and then lower it slowly to avoid trapping air bubbles.
- The slide is now ready for microscopic examination. Place it on the microscope stage.
- Begin with a low-power objective lens (e.g., 10x) to locate the fungal structures, then switch to a higher-power lens (e.g., 40x or 100x) for more detailed observation.
- Look for characteristic fungal structures such as hyphae, conidia, spores, and other identifying features.
- Record observations and capture images if necessary.
- After examination, dispose of the slide in a biohazard.

Quality Control

- Periodically check the quality of the Lactophenol cotton blue stain solution for any signs of contamination or deterioration.
- Internal quality control of the Lactophenol Blue Solution is done regularly on known organisms to ensure the efficacy of the stain.
- Verify the staining results by comparing them with known standards or controls.
- Check the stain a shelf life, and it is about 52 weeks from the date of manufacture.
- Lactophenol cotton blue solution should be Ink blue in colour.
- It should be clear and free of insoluble particles.
- After staining the fungal cell with Lactophenol cotton blue, fungal spores and hyphae are viewed under a microscope with a high power (40X) objective lens.
- Fungal spores and hyphae will be pale to dark blue in colour.

Documentation

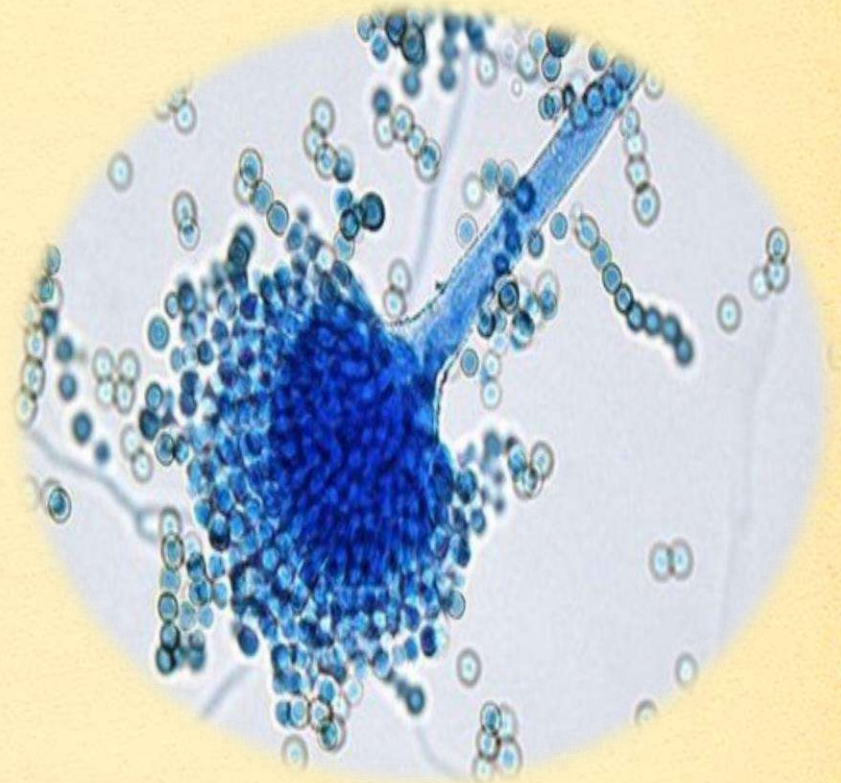
Record the preparation and utilisation of Lactophenol cotton blue stain in a laboratory notebook or electronic record, including dates, quantities, and observations.

Reporting

Compile the findings into a comprehensive report, including the identification of the causative agent and any other relevant information.

Storage

The stained slides should be stored in appropriate storage conditions according to the institutional regulations.



Quality Control Measures

Sterile culture media undergo quality control by being left at room temperature for three days. If no growth occurs, the media is stored in the refrigerator for future use.

Employ suitable positive and negative controls to verify the absence of contamination in the growth medium and equipment.

Regularly observe and record the appearance and growth attributes of the cultures.

Document Control

All personnel must record the details of the culture procedures and any deviations from the standard protocol and document the results for future reference in the laboratory logbook.

Any revisions or updates to this SOP must be documented and communicated to relevant personnel.

References

- Emmons CW, Binford CH, Utz JP, KwonChung KJ. Medical mycology. 4th ed. Philadelphia: Lea & Febiger, 1977.
- McInnis MR. Laboratory handbook of medical mycology. New York: Academic Press, 1980.
- Baron EJ, Finegold SM. Bailey & Scott's diagnostic microbiology. St. Louis: Mosby Company, 1990.
- Larone DH. Medically important fungi: a guide to identification. Washington DC: ASM Press, 1995.
- Murray PR, Baron E, Pfaller M, Tenover F, Tenover FC, Eds. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.
- lactophenol cotton blue staining preparation for fungi. <https://www.scribd.com/doc/113187014/Lactophenol-Cotton-Blue-Staining-Preparation-for-Fungi>

Revision History

- Version 1.0: [2017] - Initial SOP created.
- Version 1.1: [2019] - Minor revisions for clarity and accuracy.

Distribution

- Copies of this SOP shall be distributed to all personnel involved in grain culture.
- Ensure that all personnel are trained and familiarised with the procedures outlined in this SOP before performing the grains culture technique.

**Document Control Section:
SOP tracking review log:**

Purpose:

The log records this SOP's review dates and the status of the review.

The Tracking Changes and Version Control Log are completed to detail the status of the review.

When:

The SOP is reviewed every two years or more often when necessary.

By whom:

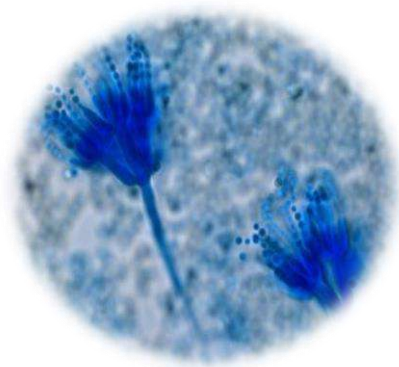
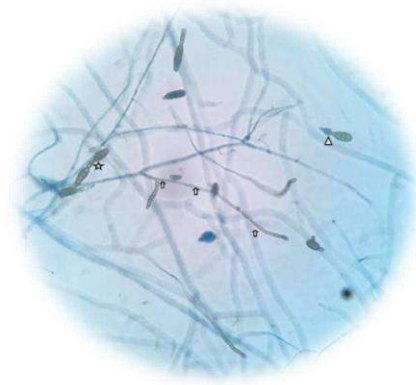
The SOP is reviewed by staff directly implementing the SOP.

The laboratory manager or designee oversees the review process.

Attachments

None.

Fungal isolates stained by Lactophenol Cotton Blue Staining



Approval

This Standard Operating Procedure has been prepared, reviewed and approved by:

Mrs Lubna Sulayman Elnour	Technologist	<i>Lubna</i>
Miss Nema EL Shibly	Technologist	Nema
Dr Abdella Osaman	Senior Researcher	<i>Abdulla</i>
Prof Ahmed Fahal	Center Director	<i>Fahal</i>

On May 3 2024.



The Mycetoma Research Center,
University of Khartoum
WHO Collaborating Center
on Mycetoma & Skin NTDs

www.mycetoma.edu.sd