Eumycetoma Causative Organisms
Culture Technique
Standard Operating Procedures

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Standard Operating Procedures (SOPs)

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WHO Collaborating Center
on Mycetoma & Skin NTDs

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Standard Operating Procedure for

Eumycetoma Causative Microorganism Culture Technique

Background

Eumycetoma caused by more than 70 fungal microorganisms spanning various genera and species, with *Maderulla mycetomatis* being the predominant microorganism globally. Adhering to rigorous laboratory safety protocols is crucial to minimise the potential exposure to infectious agents. Moreover, the implementation of quality control measures is imperative to uphold the precision and dependability of the findings.

Purpose

- These standard Operating Procedures (SOP) provide a systematic guideline for the culture technique of eumycetoma-causative microorganisms.

Scope

- The procedures (SOP) provide a systematic guideline for the culture technique of eumycetoma-causative microorganisms cultured from clinical specimens for diagnostic purposes to the laboratory personnel involved in this technique.

Responsibilities

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

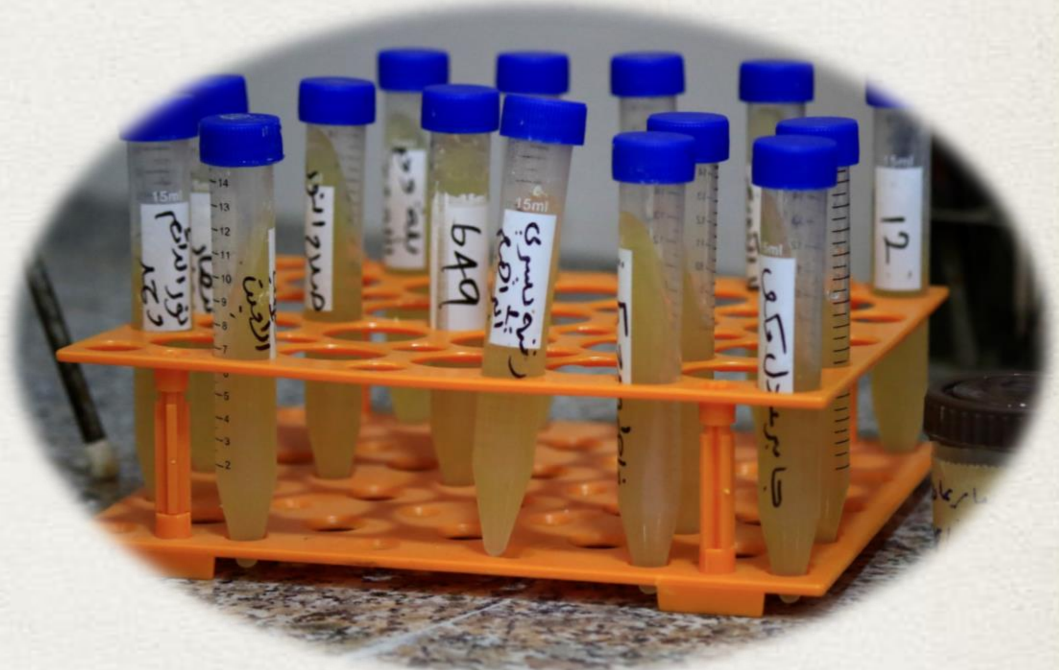
Safety Precautions

The microorganisms that cause eumycetoma exhibit mild infectivity. Although there have been no documented instances of hospital-acquired infections, it's crucial to exercise caution and follow safety protocols. It's advisable to:

- Store these microorganisms in appropriate facilities.
- Wear appropriate personal protective equipment (PPE), including gloves, laboratory coat, and safety goggles.
- Work in a Biosafety Level 2 (BSL-2) laboratory.
- Handle potentially infectious materials with care.
- Follow the established biohazard waste disposal procedures.

Materials and Equipment

- Incubator.
- PPE
- Autoclave.
- Safety cabinet.
- Refrigerator.
- Culture worksheet.
- Culture equipment.



Commonly used media

These include:

- **Sabroud dextroses agar**: To support the growth of fungi.
- **Malt Extract Agar:** To support the growth and sporulation of fungi.

The Procedure

Preparation before the culture

- To cultivate effectively, the specimen must be a deep surgical biopsy containing multiple grains.
- Avoid grains collected from open sinuses discharge as they are frequently dead and infected.
- The collected specimen is split into two parts: one for immediate direct microscopy using 25% potassium hydroxide solution and the other for culture.
- Employ a sterile container filled with normal saline to house the grains.

Transport

- The grains should be promptly transferred from the operating theatre to the laboratory immediately or using appropriate transport media to maintain viability and quality and prevent contamination.

The Culture Technique

The Culture Technique

1. Add chloramphenicol antibiotic with normal saline to a sterile container and wash the grains three times.

2. Label containers with the patient identification and the dates of inoculation.

3. Use a microbiological loop or a mycological needle to inoculate the grain into the Sabouraud dextrose agar or the Malt Extract Agar at the center of the culture medium.

4. Culture each sample in duplicate.

5. Incubate the cultured grains at 37° - 25° C for 7-10 days.

6. **The growth daily observation is mandatory.**

7. Record the specimen identification and date of inoculation on the documentation log.

8. The next step for a positive growth culture is identification macroscopically. This is done by observing the rate of growth and colonial morphology, and the conidia production is observed microscopically using the lactophenol cotton blue (LPCB) standard operation procedure.

9. Preparation of LPCB: Take a tiny part of the fungal colony in a clean slide, then add a drop of LPCB Stain. Hold the coverglass between your forefinger and thumb, touch the stain mountant from one side, and lower gently, avoiding air bubbles. Observe the fungal structure under a Microscope using ×40 lens.

For negative growth culture, more incubation at 37° C is recommended.

Sub-culturing technique

Collect multiple pieces from growth using a mycological loop or syringe and inoculate into Sabaraud dextrose media, and incubate the tubes at 37° C for 7-10 days.

Observation

Once fungal colonies have developed, they are examined macroscopically and microscopically for characteristic features such as colour, texture, growth pattern, and microscopic morphology of hyphae and spores.

Eumycetoma grains usually appear as granules or aggregates with a chalky consistency and various colours ranging from brown to black.

If growth is observed after 3-7 days:

Collect multiple pieces from the culture and, put them in 10% glycerol, and store them.

Add sterile paraffin oil to fill the tube.

Store the tube at room temperature and subculture after every month.

In case the subculture does not grow, start the culture again from the grains stored in the lab.

Identification

- Each causative microorganism has distinct macroscopic and microscopic appearance .
- Use species-specific PCR to identify the common fungal causative species.
- Use ITS2 sequencing for the identification of less common and unknown species.

Antifungal Susceptibility Testing

Perform susceptibility testing to determine the most effective antifungal agents to suggest treatment.

Reporting

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

Storage

Preserve pure cultures of the isolated strains for future reference and research purposes in the appropriate storage conditions using 10% glycerol at room temperature.

Quality Control Measures

- Sterile culture media undergo quality control by being left part at room temperature and another part at 37° C incubator 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.

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References

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Revision History

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

Distribution

- Copies of these SOPs shall be distributed to all personnel involved in the culture technique.
- 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 these SOPs' 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 SOPs are reviewed by staff directly implementing the SOPs.

The laboratory manager or designee oversees the review process.

Attachments

None.

The culture organism in media

Approval

These Standard Operating Procedure have been prepared, reviewed and approved by:

Miss Nema Ahmd Alfaki	Technologist	Nema
Mrs Lubna Sulayman Elnour	Technologist	Lubna
Mrs Ribab Mohammed	Senior Technologist	Rihab
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On May 4, 2024.

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