Actinomycetoma Causative Micro-organisms Culture Technique Standard Operating Procedures

The Mycetoma Research Center,
University of Khartoum
WHO Collaborating Center

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on Mycetoma & Skin NTDs

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Standard Operating Procedure for Actinomycetoma Causative Micro-organisms Culture Technique

Background

Culture techniques for isolating and identifying the causative microorganisms of actinomycetoma entail specific procedures. These microorganisms, which are filamentous bacteria, belong to the genera Actinomyces, Nocardia, and Streptomyces. It's essential to adhere to strict laboratory safety protocols throughout the process to minimise the risk of exposure to infectious agents. Additionally, quality control measures should be implemented to ensure the accuracy and reliability of the results.

Purpose

- These standard Operating Procedures (SOP) provide a systematic guideline for the Actinomycetoma Causal Microorganisms Culture Technique.

Scope

- These SOPs are to be used by the laboratory personnel involved in the actinomycetoma causative microorganism culture from clinical specimens for diagnostic purposes.

Responsibilities

- **Laboratory Technicians:** Responsible for performing the culturing procedure according to these SOPs.
- **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 these SOPs.

Safety Precautions

The microorganisms responsible for actinomycetoma are of low-grade infectivity. While there haven't been any reported cases of hospital cross-infection, it's essential to take precautions and adhere to safety measures:

- Proper storage facilities should be utilised for these microorganisms.
- 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:

- **Blood agar:** To support the growth of most bacterial pathogens, including actinomycetes.
- **Yeast extract Agar:** To support the growth of slow-growing actinomycetes.
- **Brain heart infusion agar**: Riched medium suitable for fastidious organisms.

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 microscopy and the other for culture.
- Employ a sterile container filled with 10% 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
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The Culture Technique

1. Place the sample in a sterile container and rinse it with 10% normal saline then wash until grains appear clear without any tissue debris.

2. Label the container with patient identification, date of collection and date of culture.

3. Use a microbiological loop or sterile needle to introduce the grain into culture media at the center of the universal tube.

4. Culturing in duplicate is essential.

5. Incubate the inoculated plates at appropriate conditions. Actinomycetes typically grow slowly and may require prolonged incubation periods (up to several weeks) at a temperature around 37° C.

6. Monitor daily signs of growth.

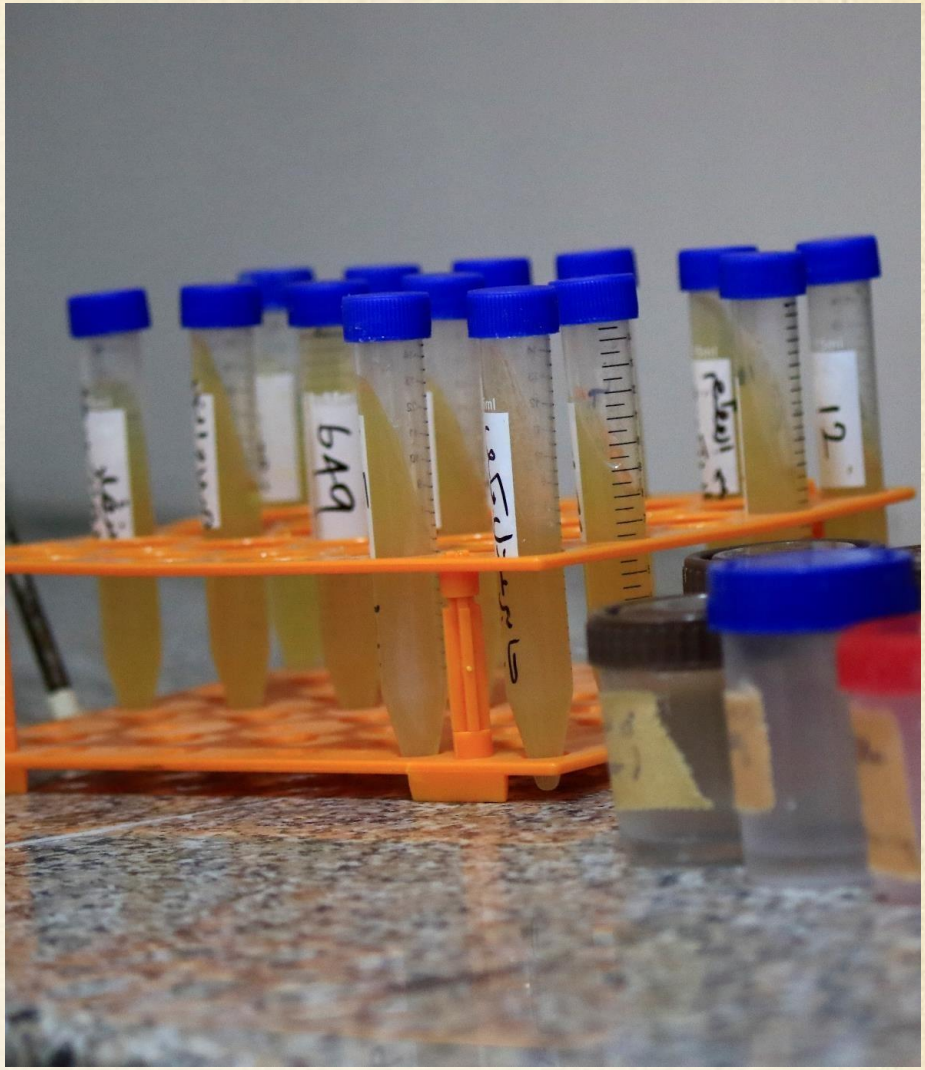
7. Document the specimen identification and date of inoculation in the documentation log.

8. Upon positive growth in culture, the subsequent step involves macroscopic identification through observation of colonial morphology, followed by microscopic examination using Gram stain and modified Ziehl-Neelsen standard operating procedure.

In case of negative growth in culture, further incubation at 37° C is advisable.

Sub-culturing Technique

- Once colonies are observed, subculture them onto fresh media to obtain pure cultures for further characterisation and identification.
- Using a sterile loop or sterile needle gather multiple pieces of the growth and inoculate it into the Agar plates and slopes and wrap the plate well to avoid agar drying.
- Incubate the tubes and plates at 37° C for 7 - 10 days.



Observation

Regularly monitor the plates for the development of characteristic colonies. Actinomycetoma grains usually appear as granules or aggregates with a chalky consistency and various colours ranging from yellow to red.

If growth is observed after 3-7 days:

Scrape the growth using a sterile bacteriology loop and place it into an Eppendorf tube containing glycerol peptone water, then store it at -80° C.

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 and biochemical properties.
- Perform biochemical tests and molecular techniques (such as PCR)to identify the isolated strains at the species level.

Antimicrobial Susceptibility Testing

Perform susceptibility testing to determine the most effective antimicrobial agents for 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, commonly in peptone water at 80° C refrigerator.

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 in the laboratory logbook and document the results for future reference.
- Any revisions or updates to this SOP must be documented and communicated to relevant personnel.

References

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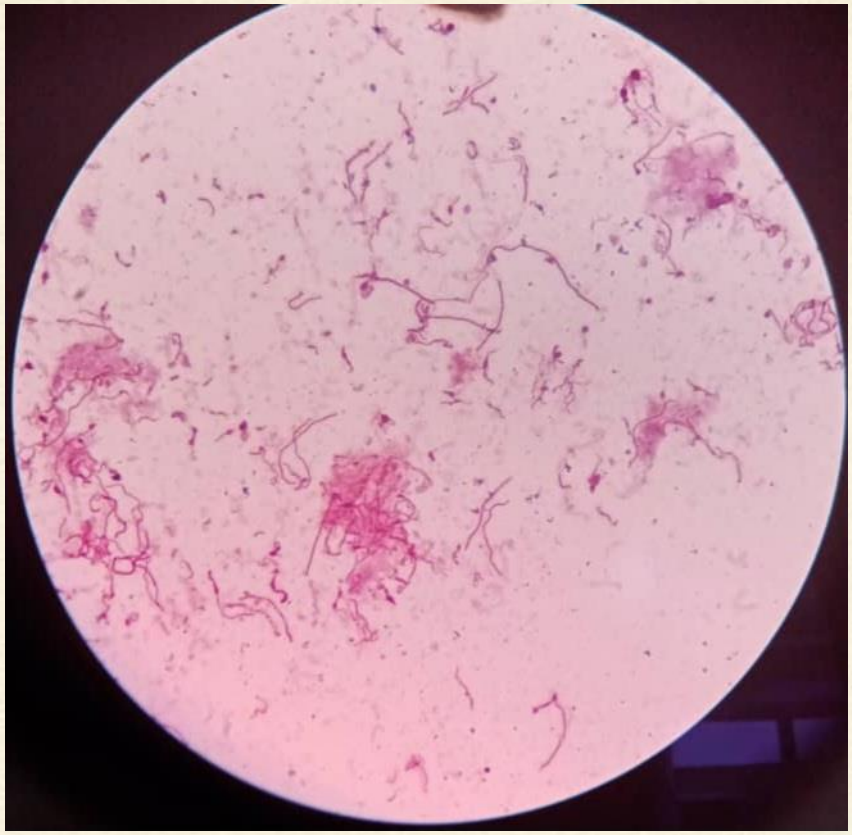
Revision History

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

Distribution

- Copies of 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.



Microscopical examination of Actinomadura isolate using Gram stain, ×100

Different microorganisms cultured in different media

Approval

These Standard Operation Procedures were prepared, reviewed and approved by

Mrs Lubna Sulayman Elnour .	Technologist	***Lubna Sulayman***
Miss Nema Ahmed Alfaki	Technologist	**Nema**
Mrs Rahab Mohamed	Senior Technologist	*Rihab*
Dr Abdulla Osman Ahmed	Senior Scientist	**Abdula**
Prof Ahmed Fahal	Center Director	*Fahal*

On May 3 2024.

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