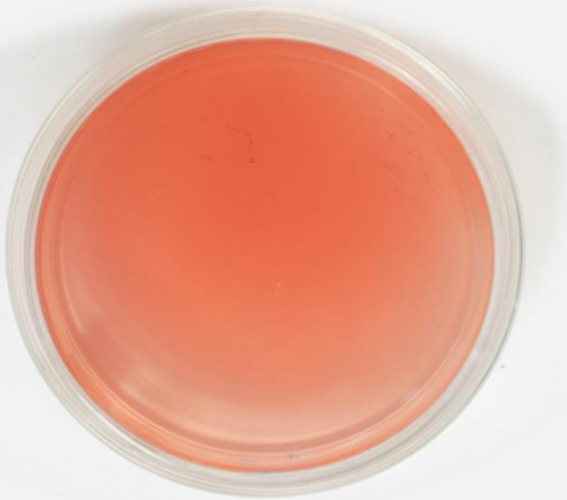
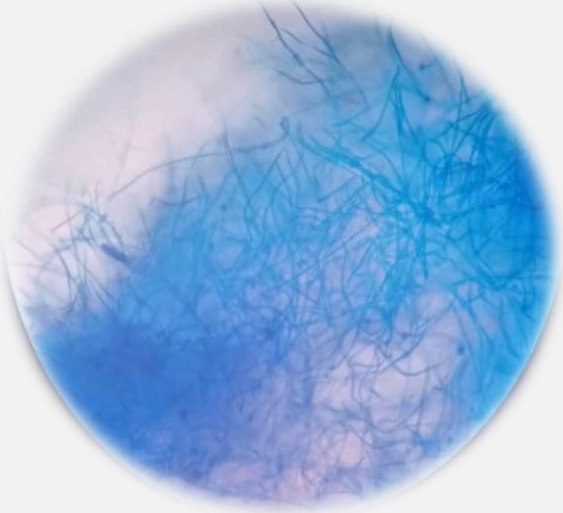
Mycetoma Grains Culture Technique





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

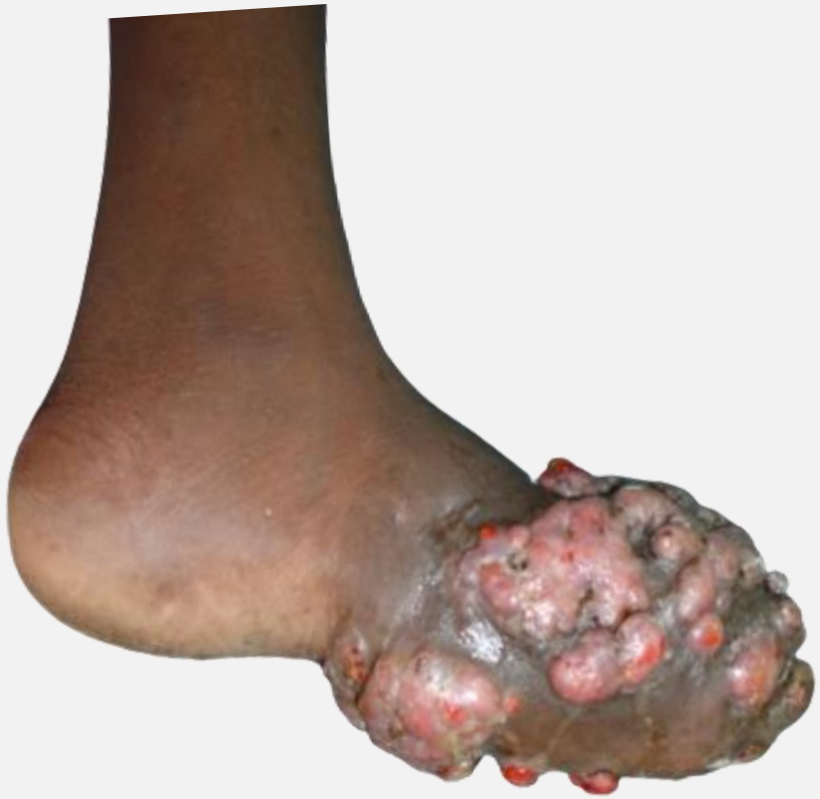
Nema Ahmed EL Faki
Ahmed Fahal
The Mycetoma Research Center

Introduction

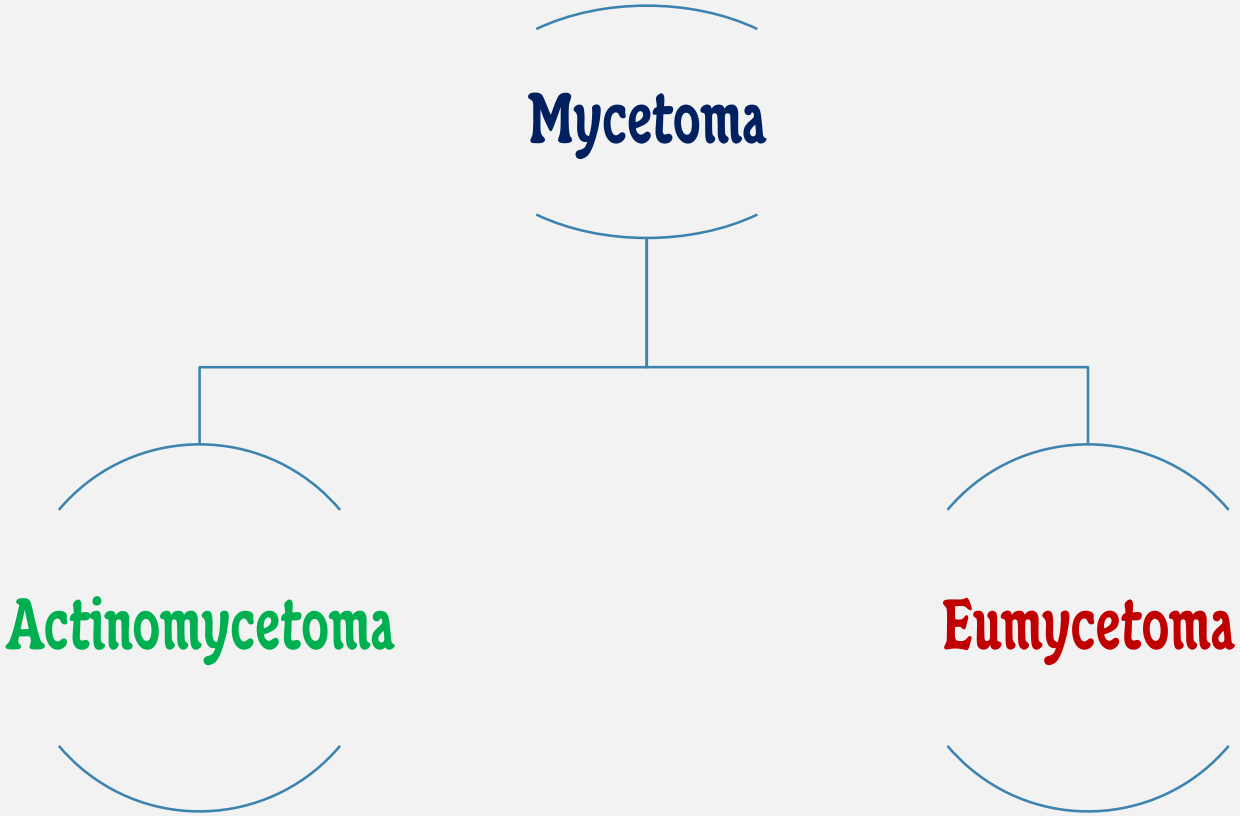
Mycetoma is chronic deep implantation mycosis characterised by

- Large painless subcutaneous swellings
- The formation of sinuses,
- Discharge that contains grains.

The frequently located are foot and hand.

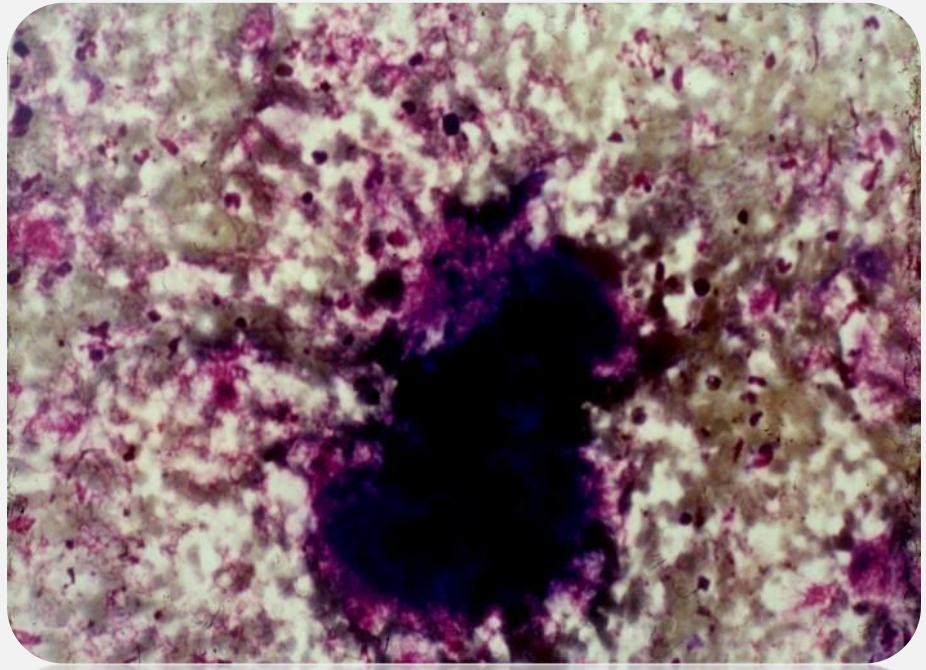


Mycetoma Classification



Actinomycetoma
Causative microorganisms

- *Actinomadura madurae*
- *Actinomadura pelletierii*
- *Nocardia brasiliensis*
- *Streptomyces somaliensis*

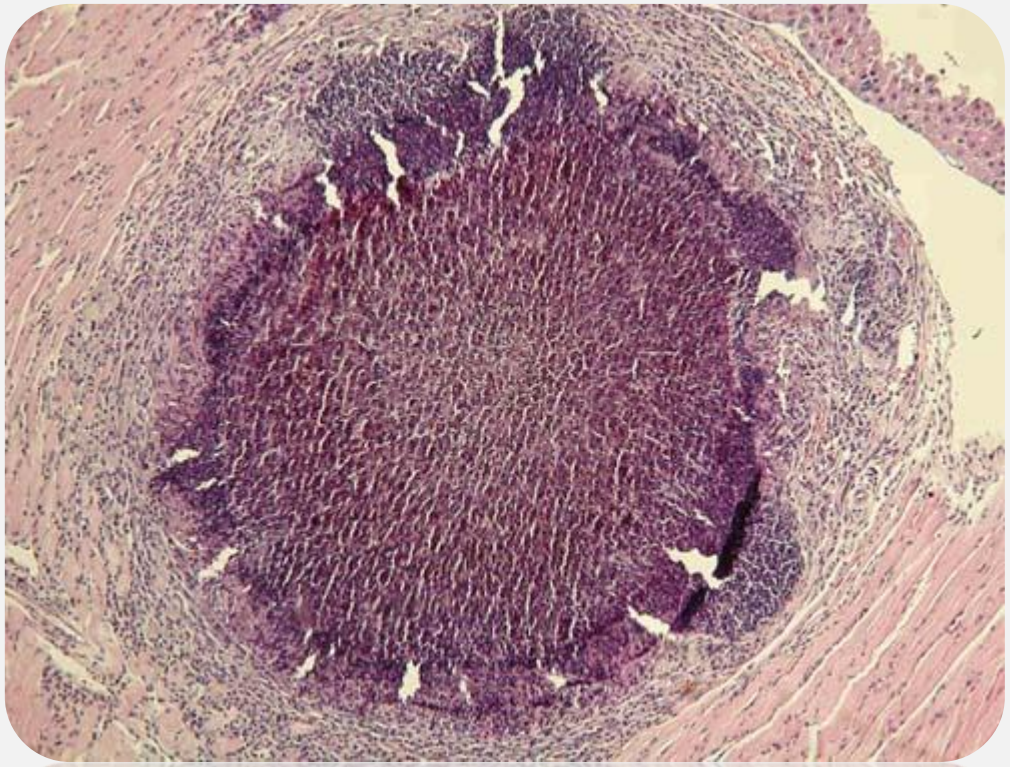


Actinomadura pelletierii

Eumycetoma
Causative microorganisms

The Common Causative Agents:

- *Madurella mycetomatis*
- *Trematosphaeria grisea*
- *Scedosporium spp*
- *Acremonium spp*
- *Fusarium spp*

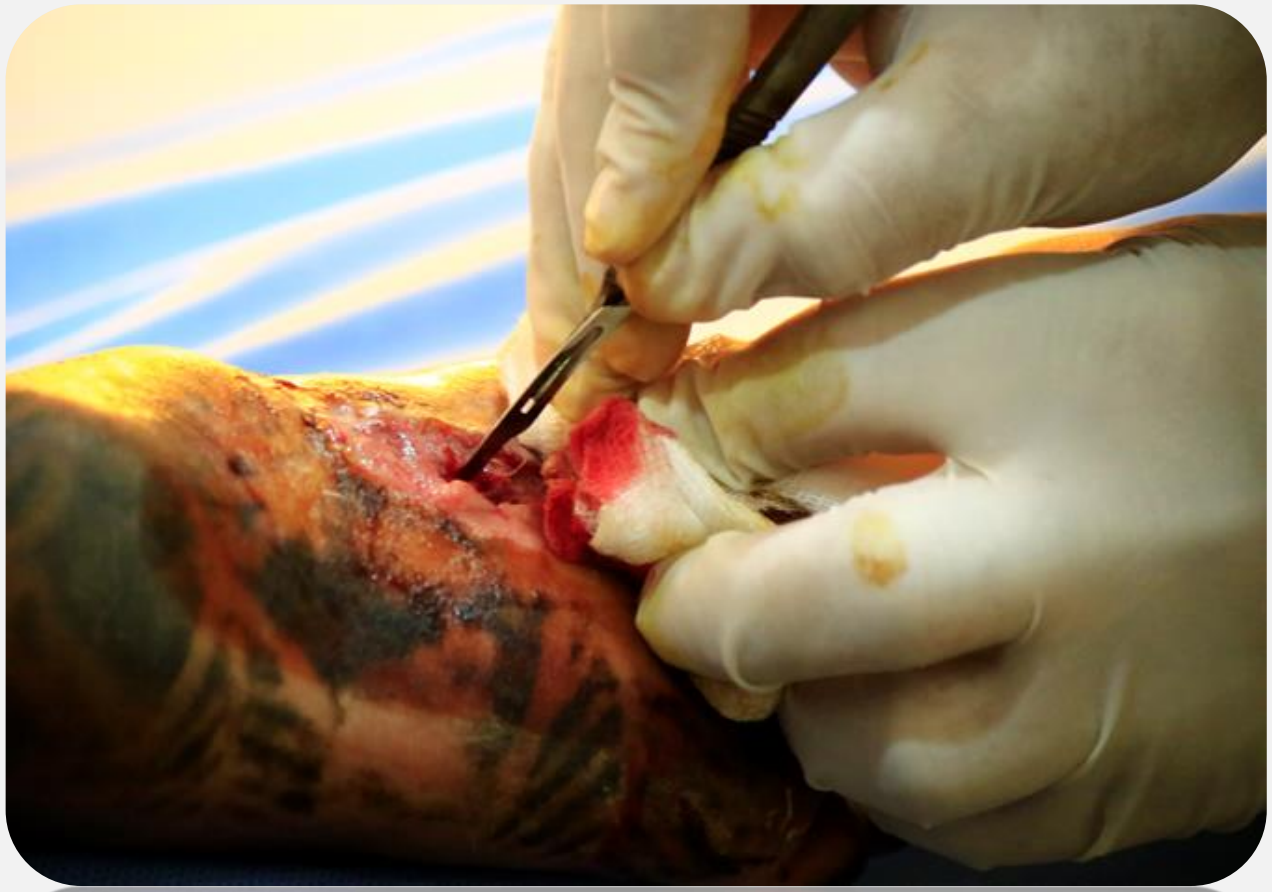


Madurella mycetomatis

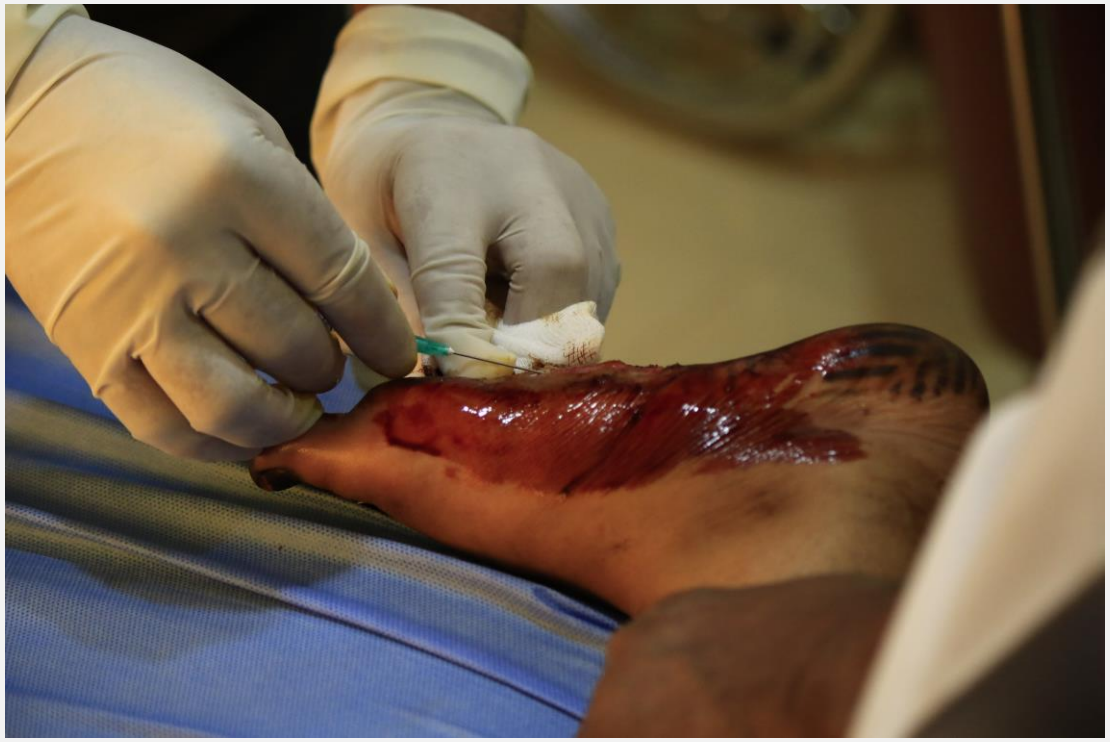
Samples
Collection & Preparation

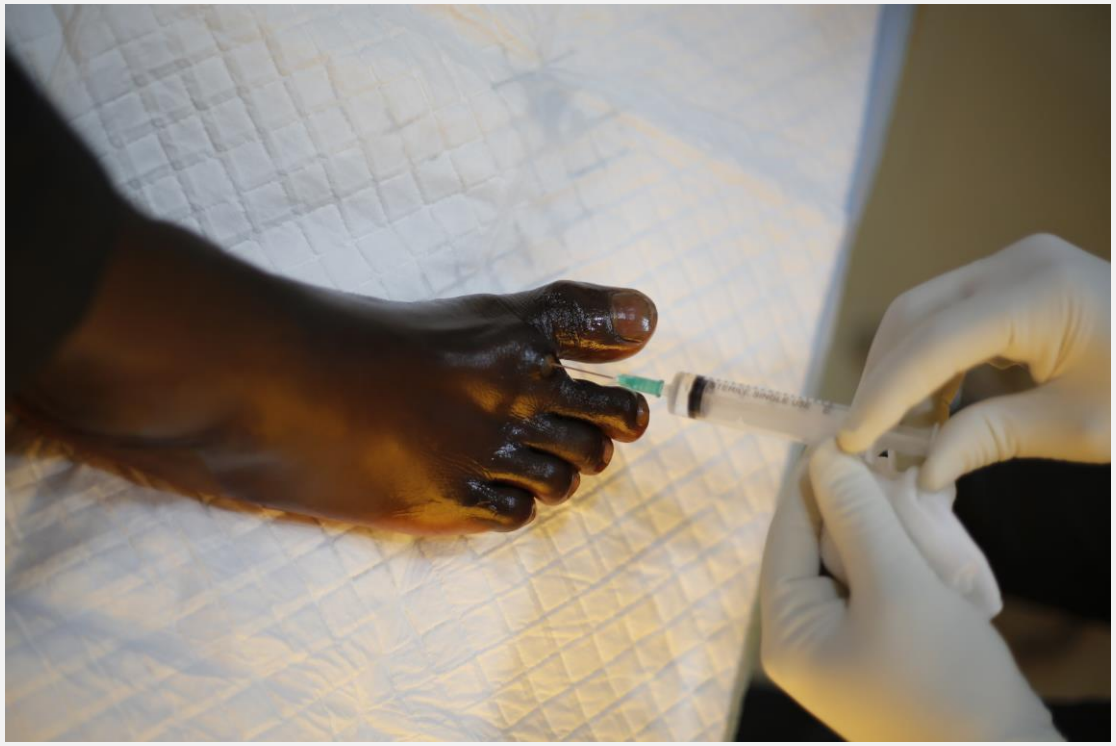
Grains from a deep surgical Biopsy must be collected

Grains from open sinuses discharge should be avoided as they are frequently dead and infected.



Samples Collection & Preparation

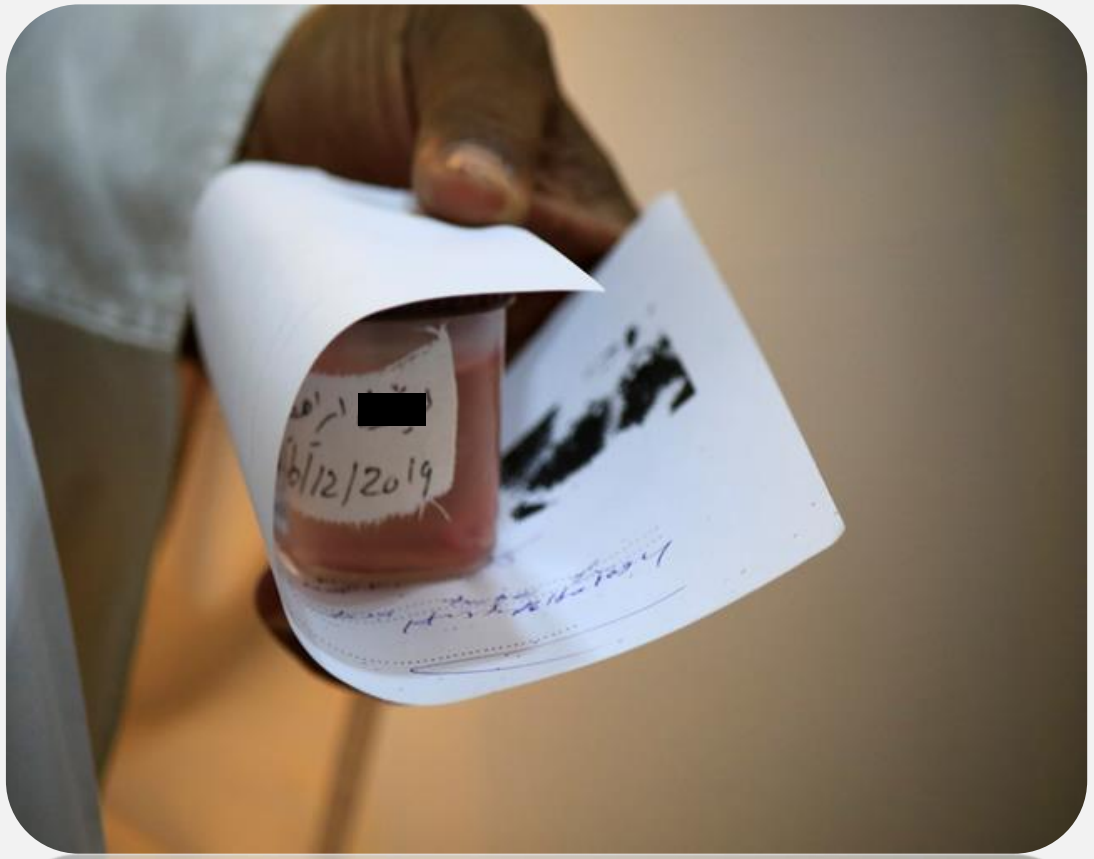




Grains from a deep surgical Biopsy must be collected

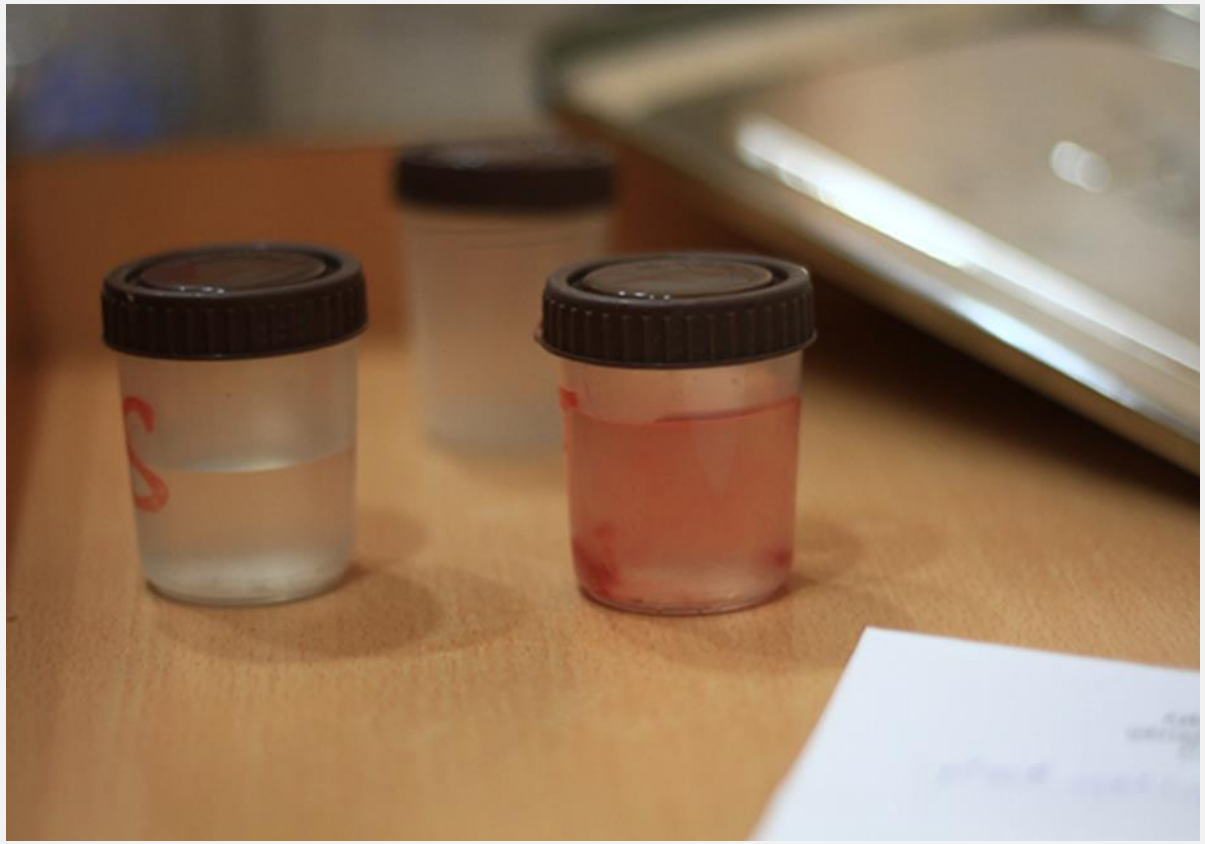
Samples
Collection & Preparation

Grains should be placed in a sterile container filled with normal saline and labelled with the patient identification and date of collection.



Samples
Collection & Preparation

Grains should be washed with normal saline until they appear clear and without any tissue debris.



Samples
Collection & Preparation

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

Samples
Collection & Preparation

Actinomycetoma grains usually appear as granules or aggregates with a chalky consistency and various colours ranging from yellow to red.

Culture Media

For Actinomycetes the Commonly used media are:

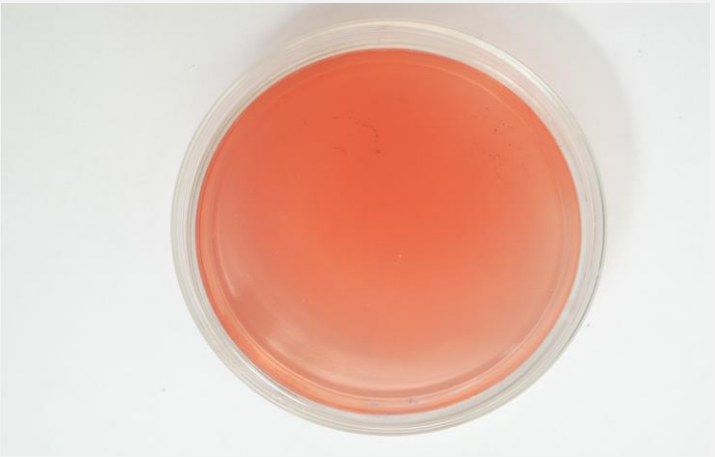
- **Blood agar:**

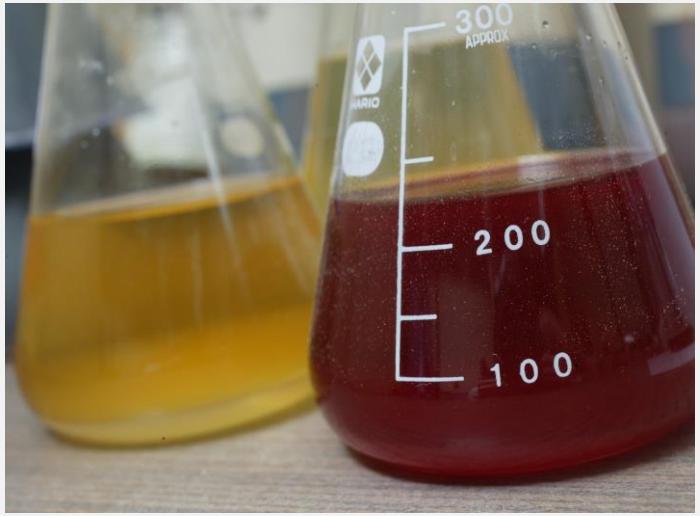
  To support the growth of most bacterial pathogens, including actinomycetes.

- **Yeast extract Agar:**

  To support the growth of slow-growing actinomycetes.

- **Lowenstein Jensen (LJ) Media**





Culture Media

For Eumycetoma, the Commonly used medium is :

Sabouraud Dextrose agar

supplemented with gentamycin to inhibit the growth of bacteria

Culture Technique

The microbiological sterile loop or syringe is used to inoculate the grains into Media at the center of the universal tube.

Culture is repeated in duplicate.

Culture Technique

In the absence of grains, the biopsy is fragmented, rinsed, and then inoculated into the universal tube as sections.

Culture Technique

Lable the universal tube with patient

identification and date of inoculation.

Culture Technique

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

Culture Technique

Incubate the cultured grains at 25-37°C for 7-10 days for both actinomycetoma and eumycetoma.

Culture Technique

Daily observation for growth is mandatory.

Actinomycetoma Macroscopical & Microscopical Examination

Upon positive growth being observed, macroscopic identification of colonial morphology must be done.

Microscopic examination using:

- Gram stain
- Modified Ziehl-Neelsen

. (Gram Stain & ZN Stain SOPs in MRC website)

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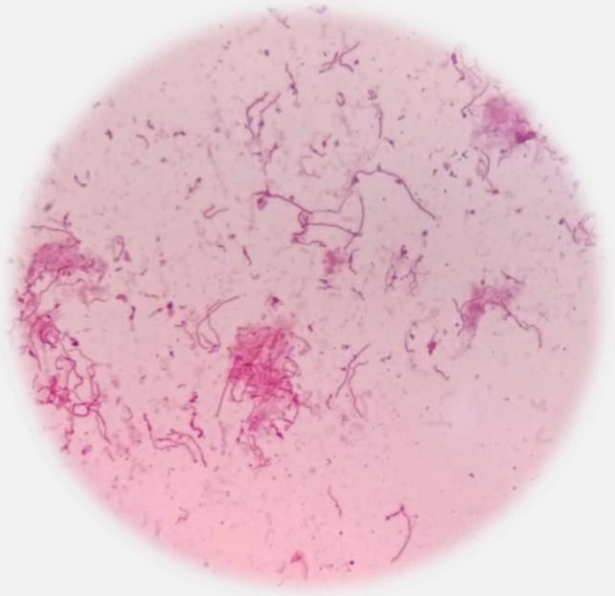


. (Gram Stain & ZN Stain SOPs in MRC website)

Eumycetoma Macroscopical & Microscopical Examination

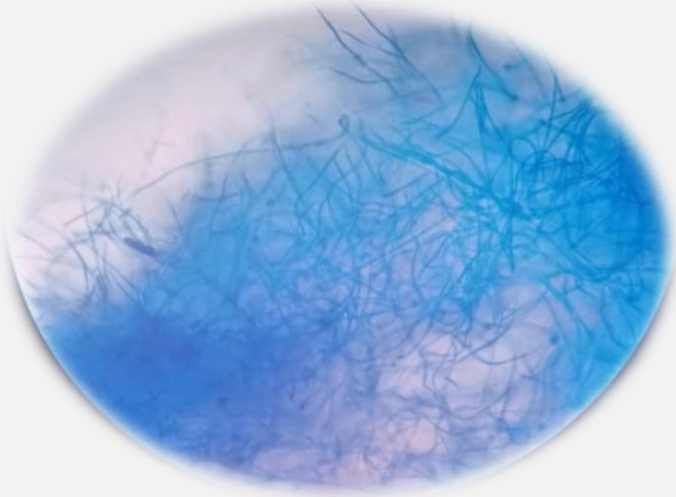
Once fungal colonies have developed, they should be examined macroscopically for characteristic features such as colour, texture, and growth pattern.

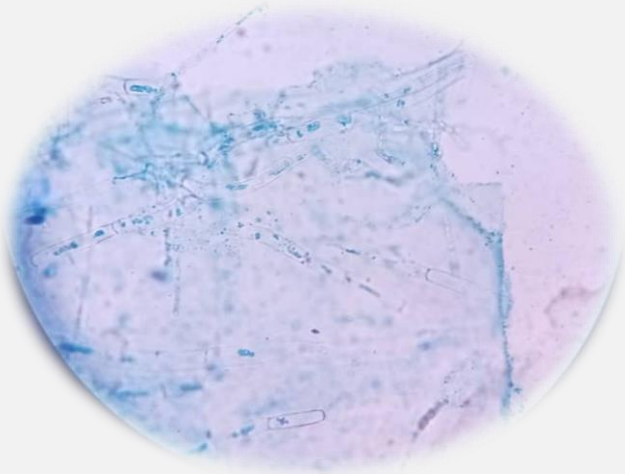
Microscopical Examination:

microscopic morphology of hyphae and spores are observed using LPCB Stain

(LPCB Stain SOPs in MRC website)

Eumycetoma Macroscopical & Microscopical Examination



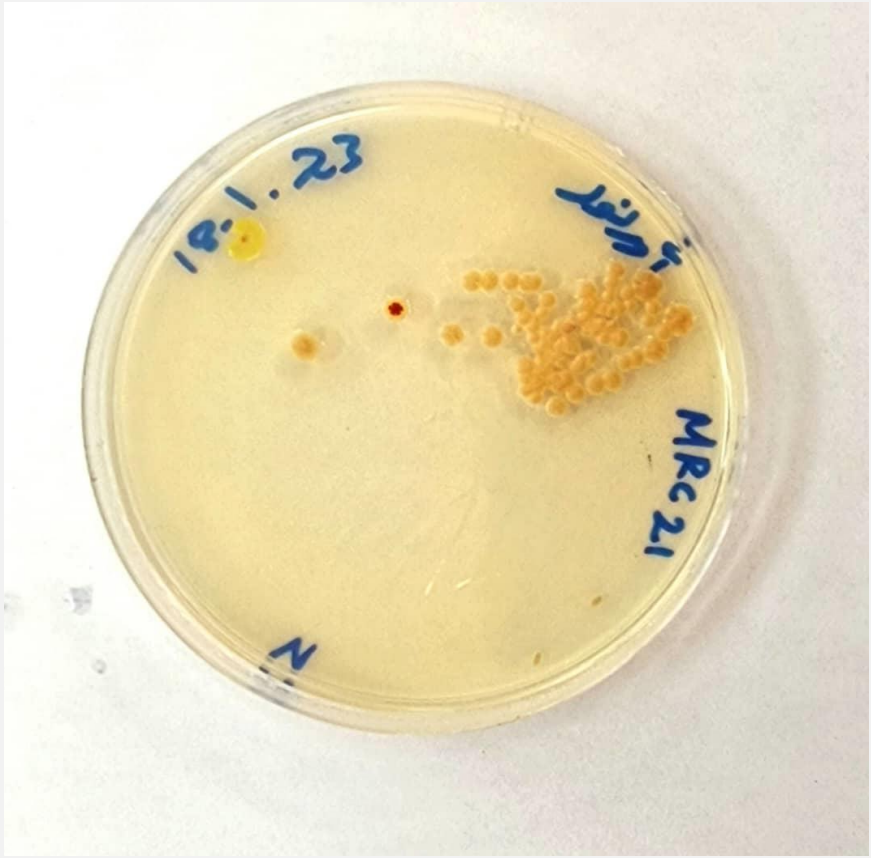


(LPCB Stain SOPs in MRC website)

Subculturing Technique

For actinomycetoma

1. Once colonies are observed,
2. Subculture them onto fresh media to obtain pure cultures for further characterisation and identification.
3. Using a sterile loop or syringe, gather multiple pieces of the growth and introduce them into yeast extract Agar plate and slope
4. Wrap the plate well to Avoid agar drying.

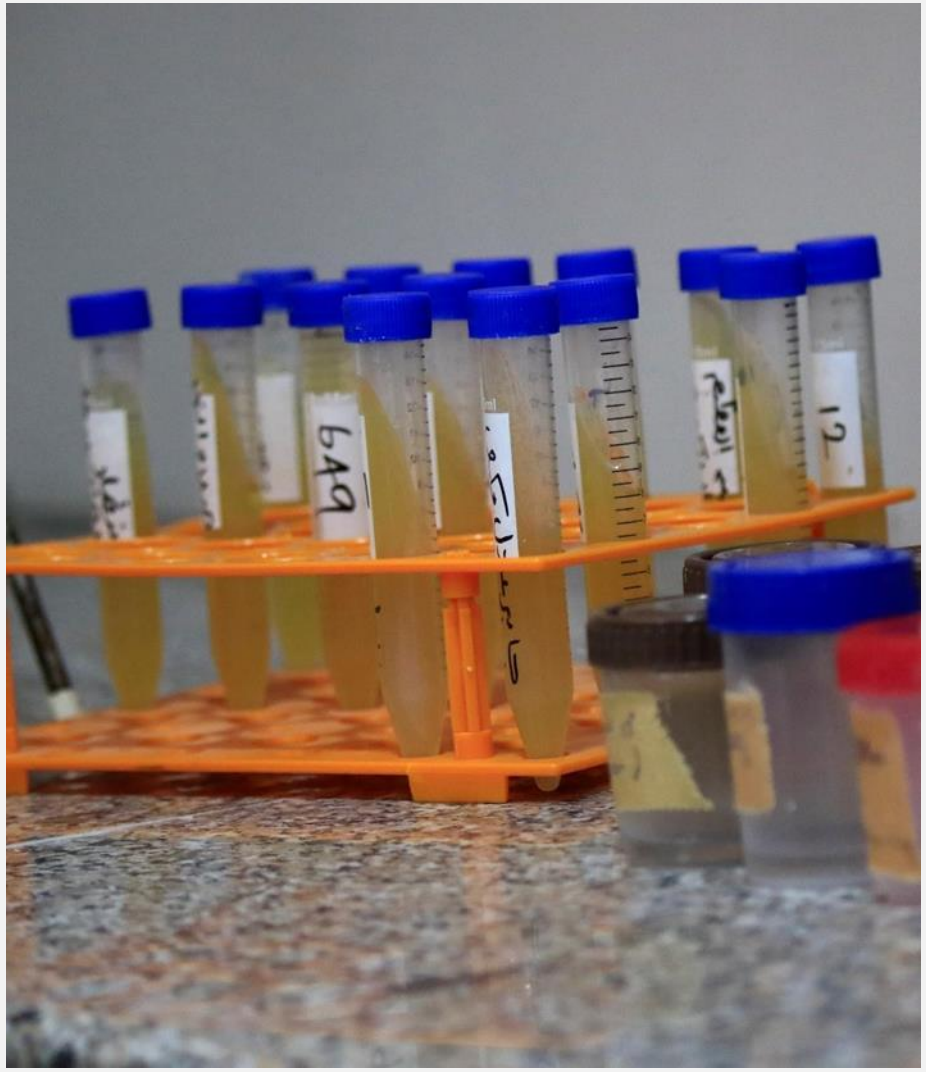


Subculturing Technique

For actinomycetoma

5. Incubate the tubes and plates at 37°C for 3-7 days.

6. Regularly monitor the plates for the development of characteristic colonies



Subculturing Technique

For Eumycetoma

1. Collect multiple pieces from growth using a mycological loop or syringe, inoculate into a Sabouraud dextrose plate, and slop.
2. Wrap the plate well to Avoid agar drying
3. Incubate the tubes at 37°C for 7-10 days.
4. Incubate the tubes and plates at 37°C for 3-7 days.
5. Regularly monitor the plates and tubes for the development of characteristic colonies.

Culture Preservation

1. Preserve pure cultures of the isolated strains for future reference and research in cryotube filled with 10% glycerol in the appropriate storage conditions, commonly in a -80C refrigerator.

2. For Actinomycetes isolates the cryotube filled with peptone water.

3. Culture and grains are stored at a -80C refrigerator for research and shipment purposes.

4. Eumycetoma culture can be stored in glycerol at room temperture

(preservation and shipment SOPS at MRC website)

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