



# **MYCETOMA LABORATORY DIAGNOSIS**

## **An Advanced Laboratory Training Manual**

### **for Healthcare Providers**

**Version 1.0**

**September 2026**

**Compiled with reference to current peer-reviewed literature and WHO technical guidance**



## Authors

Nadir Musa Khalil<sup>1,2</sup>, Abdalla Ahmed<sup>1,2</sup>, Ahmed EL Hadi<sup>1,2</sup>, Eiman Siddig Ahmed<sup>1</sup>, Osama EL Hadi<sup>1</sup>, Lamis Ahmed Fahal<sup>1</sup>, Bouthina Mamoum<sup>1</sup>, M.Aljaali<sup>2</sup>, Elmogtaba Nasreldin<sup>1,2</sup>, Zainab Kamal Mahjoob<sup>1,2</sup>, Samah Kamal Abuzaid<sup>1,2</sup>, Nusiyya Mahjoub Babiker Fadul Almoula<sup>1,2</sup>, Mohammed E. Mohammed<sup>1,2</sup>, Mohamed N Elnour<sup>1</sup>, Saifeldin Ali Elbasheir<sup>1</sup>, Onoor Ibrahim Musa Idris<sup>1</sup>, Mawahib Abdel Moneim I. Ismail<sup>1</sup>, Mahmoud Eisa Abdalla Yahia<sup>1</sup>, Alkhair Adam Khalil Mohamed<sup>1,2</sup>, Marwa Salah S. Osman<sup>1,2</sup>, Ali Awadallah Saeed<sup>1,2</sup>, Samira Mohamed Hussein<sup>1,2</sup>, Ahmed Hassan Fahal<sup>1,2</sup>

1: The Mycetoma Research Center, University of Khartoum, Khartoum, Sudan

2: National University - Sudan, Khartoum, Sudan

# TABLE OF CONTENTS

|                             |                                                                                                   |
|-----------------------------|---------------------------------------------------------------------------------------------------|
| <b>Authors</b>              | Authors                                                                                           |
| <b>Acknowledgments</b>      | Acknowledgments                                                                                   |
| <b>Abbreviations</b>        | Abbreviations                                                                                     |
| <b>Foreword</b>             | Foreword                                                                                          |
| <b>The Document Control</b> | The Document Control                                                                              |
| <b>Section I</b>            | Mycetoma Epidemiology, Clinical Presentation and Treatment                                        |
| <b>Section II</b>           | Laboratory Biosafety Standards and Decontamination                                                |
| <b>Section III</b>          | Clinical Specimen Collection & Transportation                                                     |
| <b>Section IV</b>           | Long-Term Biobank Storage Standard Operating Procedures                                           |
| <b>Section V</b>            | Grain Macroscopic Examination, Frontline Preparation & Direct Microscopy (KOH, Gram, Modified ZN) |
| <b>Section VI</b>           | Cytopathological Techniques (FNAC & Cell Block)                                                   |
| <b>Section VII</b>          | Histopathological Examination of Mycetoma Lesions                                                 |
| <b>Section VIII</b>         | Mycetoma Causative Organisms: Isolation & Culture                                                 |
| <b>Section IX</b>           | Molecular Diagnostics: DNA Extraction, PCR, Real-Time PCR, MALDI-TOF & LAMP                       |
| <b>Section X</b>            | Antimicrobial Susceptibility Testing (AST)                                                        |
| <b>Section XI</b>           | Patient Monitoring: Haematology & Biochemistry                                                    |
| <b>Section XII</b>          | Comprehensive Laboratory Equipment & Reagent Specifications                                       |
| <b>Section XIII</b>         | Laboratory Signpost                                                                               |
| <b>Section XIV</b>          | Standard Diagnostic Reporting Templates                                                           |
| <b>Further Reading</b>      | Key References                                                                                    |



## Foreword

The World Health Assembly formally recognised mycetoma on 28th May 2016 as a Neglected Tropical Disease (NTD), the 18th disease added to the WHO's official NTD list, following years of advocacy led by mycetoma experts, including the Mycetoma Research Centre.

This chronic, progressive, and destructive subcutaneous infection remains one of the most neglected clinical conditions worldwide, disproportionately afflicting impoverished, agricultural, and rural communities across low-resource tropical and subtropical regions, particularly within the so-called 'mycetoma belt' between latitudes 15°S and 30°N.

Since its establishment in 1991, the Mycetoma Research Center (MRC) at the University of Khartoum has served at the forefront of global efforts to combat this disease. Drawing upon more than three decades of frontline clinical experience, the MRC provides holistic patient care spanning early diagnosis, complex medical and surgical management, physical rehabilitation, and socio-economic reintegration for recovered individuals. Designated as a WHO Collaborating Centre on Mycetoma and Skin NTDs, the MRC contributes to the international research agenda on disease pathogenesis, translational diagnostics, novel therapeutic protocols, and public health surveillance.

This technical laboratory manual synthesises institutional expertise and current published evidence into a unified diagnostic framework intended to standardise laboratory operations from peripheral primary-care clinics to national reference centres. By setting out step-by-step Standard Operating Procedures (SOPs) spanning biosafety, specimen collection, microscopic identification, culture, and advanced molecular diagnostics, this manual aims to reduce diagnostic latency, prevent iatrogenic harm and unnecessary limb amputations, and support evidence-based care for mycetoma patients.

The Authoring Committee

August 2026



## Acknowledgments

The development of Mycetoma Laboratory Diagnosis: An Advanced Laboratory Training Manual for Healthcare Providers has been a collaborative endeavour driven by a shared commitment to advancing patient care, early point-of-care diagnosis, and clinical research in neglected tropical diseases. We extend our deepest gratitude to the experts, partner institutions, and laboratory teams whose dedication and steadfast support made this manual possible.

First and foremost, we express our profound appreciation to the patients, their families, and the endemic communities whose resilience in the face of mycetoma continues to inspire our research, diagnostic innovations, and field outreach efforts. Their endurance underscores the urgent imperative to advance diagnostic precision and ensure equitable access to quality healthcare.

Finally, we honour the global health advocates, laboratory technicians, and frontline healthcare workers serving in remote health centers across the Mycetoma Belt. Their relentless dedication to applying these laboratory and diagnostic protocols at the bench and bedside transforms scientific research into life-saving clinical practice.



## ABBREVIATIONS

| Term                                                       | Abbreviation |
|------------------------------------------------------------|--------------|
| Acid-Fast Bacilli                                          | AFB          |
| Antimicrobial Susceptibility Testing                       | AST          |
| Biosafety Cabinet                                          | BSC          |
| Biosafety Level 2                                          | BSL-2        |
| Brain Heart Infusion agar                                  | BHI          |
| Cation-Adjusted Mueller-Hinton Broth                       | CAMHB        |
| Clinical and Laboratory Standards Institute                | CLSI         |
| Cryoprotective Agent                                       | CPA          |
| Formalin-Fixed Paraffin-Embedded                           | FFPE         |
| Fine Needle Aspiration Cytology                            | FNAC         |
| Grocott's Methenamine Silver stain                         | GMS          |
| Haematoxylin and Eosin stain                               | H&E          |
| Internal Transcribed Spacer                                | ITS          |
| Laboratory Information Management System                   | LIMS         |
| Laboratory Safety Officer                                  | LSO          |
| Loop-mediated Isothermal Amplification                     | LAMP         |
| Matrix-Assisted Laser Desorption/Ionization Time-of-Flight | MALDI-TOF    |
| Minimum Inhibitory Concentration                           | MIC          |
| Multilocus Sequence Typing                                 | MLST         |
| Neglected Tropical Disease                                 | NTD          |
| Neutral Buffered Formalin                                  | NBF          |
| Periodic Acid-Schiff stain                                 | PAS          |
| Personal Protective Equipment                              | PPE          |
| Polymerase Chain Reaction                                  | PCR          |
| Sabouraud Dextrose Agar                                    | SDA          |
| Standard Operating Procedure                               | SOP          |
| Ultra-Low Temperature (freezer)                            | ULT          |
| Whole Genome Sequencing                                    | WGS          |
| World Health Organization                                  | WHO          |
| Ziehl-Neelsen stain                                        | ZN           |



# The Document Control

This technical manual serves as an operational reference for healthcare professionals, clinical microbiologists, pathologists, and laboratory scientists conducting mycetoma diagnostics. It is intended for use at peripheral, district, and reference-laboratory levels within endemic settings. Read it alongside institutional biosafety policy, national diagnostic guidelines, and manufacturer instructions for specific reagents and equipment.

Mycetoma remains one of the world’s most neglected and debilitating chronic inflammatory diseases. These standardised protocols were compiled in line with the WHO mandate for the MRC and are intended to synthesise clinical experience, translational research, and field-tested diagnostic methodologies into a single framework.

The manual bridges the gap between high-resource laboratory innovation and the realities of low-resource, field-level healthcare settings, covering biosafety, deep-tissue biopsy collection, microscopic grain identification, cytology, histopathology, culture, molecular profiling, and antimicrobial susceptibility testing to support evidence-based, actionable diagnostic practice.

| Field          | Detail                                                                 |
|----------------|------------------------------------------------------------------------|
| Document Title | Mycetoma Laboratory Diagnosis — An Advanced Laboratory Training Manual |
| Document Owner | Mycetoma Research Center (MRC), University of Khartoum                 |
| Version        | 1.0                                                                    |
| Effective Date | September 2026                                                         |
| Review Cycle   | Every 2 years, or upon major diagnostic/technical change               |
| Distribution   | Reference laboratories, district hospitals, training centres           |

## Section I

### Mycetoma Epidemiology, Clinical Presentation and Treatment

Mycetoma is a chronic, progressive, granulomatous inflammatory disease affecting the subcutaneous tissues, skin, underlying fascia, and bone. It follows an indolent clinical course, classically presenting as a triad of painless subcutaneous swelling, multiple discharging sinus tracts, and purulent exudate containing characteristic grains. The infection is endemic in tropical and subtropical regions within the global 'mycetoma belt' (approximately 15°S to 30°N), with the largest documented case series from Sudan, Mexico, and India, and substantial burdens also reported in Somalia, Ethiopia, Chad, Senegal, Mauritania, Yemen, and Venezuela. Because population-level surveillance is limited, true global incidence and prevalence remain unknown; despite this, and despite its severe physical, social, and economic consequences, the World Health Assembly recognised mycetoma as a neglected tropical disease (NTD) on 28th May 2016.



**Massive foot eumycetoma**

## Aetiological Classification

The disease is broadly classified into two aetiological groups based on the causative microorganism:

- **Eumycetoma:** caused by true fungi, most frequently *Madurella (M.) mycetomatis*, the single most common global cause, together with the related species *M. tropicana*, *M. fahalii*, and *M. pseudomycetomatis*, as well as *Falciformispora senegalensis*, *Trematosphaeria grisea*, and *Scedosporium (Pseudallescheria) apiospermum*.
- **Actinomycetoma:** caused by aerobic actinomycetes (filamentous bacteria), primarily *Actinomadura madurae*, *Actinomadura pelletierii*, *Streptomyces somaliensis*, *Nocardia brasiliensis*, and members of the *Nocardia abscessus* species complex.

Definitive laboratory diagnosis is a prerequisite before initiating therapy, since treatment and outcomes differ fundamentally between the two forms. Eumycetoma requires prolonged antifungal therapy, usually combined with surgical excision, whereas actinomycetoma is managed with combination, prolonged antibacterial chemotherapy. Misdiagnosis leads to inappropriate treatment, disease progression, unnecessary amputation, and avoidable drug toxicity.

## Clinical Presentation and Differential Diagnosis

The classical presentation is a firm, painless subcutaneous swelling that gradually expands over months to years. As the disease advances, it breaches deep fascial planes, creating multiple interconnected sinus tracts that intermittently discharge purulent or serosanguineous fluid containing coloured grains.

| Clinical Feature    | Eumycetoma                          | Actinomycetoma                        |
|---------------------|-------------------------------------|---------------------------------------|
| Disease progression | Slow, well encapsulated, localised  | Rapid, aggressive, extensive tracking |
| Sinus tracts        | Few, localised, late breakdown      | Multiple, active, inflammatory        |
| Bone involvement    | Few, large, well-sclerotic cavities | Numerous small, ill-defined cavities  |
| Primary management  | Antifungals + surgical excision     | Combination antibacterial therapy     |
| Role of surgery     | Essential adjunct                   | Reserved for biopsy/complications     |

## Anatomical Distribution and Atypical Variants

Pedal involvement accounts for the majority of cases (commonly cited at over 70% in large African and Sudanese series) due to direct inoculation via thorn pricks or minor trauma during barefoot agricultural work, but mycetoma may affect any anatomical site. Extra-pedal involvement of the hands, knees, buttocks, chest wall, head, and spine presents diagnostic challenges, particularly because discharging sinuses are often absent early on, leading to misdiagnosis as a benign soft-tissue tumour, osteosarcoma, cutaneous tuberculosis, or other deep fungal infections such as chromoblastomycosis.



**Back mycetoma presented as a painless lump**

## The Case for Definitive Laboratory Confirmation

Medical management cannot be empirical. Administering an antibacterial regimen (e.g. amikacin or co-trimoxazole) to a patient with fungal disease caused by *Madurella mycetomatis* provides no benefit while exposing the patient to nephrotoxicity, ototoxicity, and haematological risk. Conversely, treating actinomycetoma with prolonged, costly antifungal therapy allows bacterial infection to progress unchecked through fascial planes into bone, risking irreversible skeletal destruction.

## **Surgery and Toxicity Considerations**

- In eumycetoma, wide local excision or debulking is an established adjunct to antifungal therapy to reduce fungal burden and remove encapsulated fibrous masses.
- In actinomycetoma, management is primarily conservative and chemotherapy-driven; surgery is generally reserved for diagnostic biopsy, emergency spinal decompression, or salvage procedures, since the disease is potentially curable with targeted antimicrobials.
- Cyclic intravenous amikacin used for actinomycetoma requires monitoring to prevent irreversible ototoxicity and acute kidney injury.
- Extended azole therapy for eumycetoma carries hepatotoxicity risk and a substantial financial burden for patients and the health system in low-resource, endemic settings.

## **Current Treatment Landscape**

Standard-of-care for eumycetoma remains oral itraconazole (typically 400 mg/day) for at least 12 months, combined with surgery, though itraconazole is estimated to be effective in only a minority of patients and requires twice-daily dosing with food, which limits adherence.

A phase 2 randomised, double-blind trial conducted at the Mycetoma Research Centre in Khartoum (published in *The Lancet Infectious Diseases*, 2024) compared once-weekly fosravuconazole (a repurposed triazole, at 200 mg or 300 mg) against daily itraconazole, both combined with surgery. Neither fosravuconazole dose was superior to itraconazole in complete-cure rate, but fosravuconazole showed no new safety signals and offers a markedly lower pill burden, fewer drug–drug interactions, and no food requirement, potentially improving adherence in field settings. Companion pharmacokinetic-pharmacodynamic modelling (2025) is being used to inform dose selection for a planned phase II/III superiority trial.

Actinomycetoma is treated with prolonged combination antibacterial regi-

mens (e.g. amikacin plus co-trimoxazole, the 'Welsh regimen', or other combinations guided by susceptibility results), typically for a minimum of 5–12 months depending on response.

### **Clinical note**

Treatment protocols evolve as new trial data emerge. Laboratories should coordinate with treating clinicians and refer to current national or WHO treatment guidance rather than relying solely on this manual for therapeutic decisions.

## Section II

### Laboratory Biosafety Standards and Decontamination

Given the chronic infectious nature of mycetoma agents and the potential for opportunistic secondary pathogens in tissue and grain specimens, process all clinical specimens using Biosafety Level 2 (BSL-2) practices. Neither *M. mycetomatis* nor the common actinomycetoma agents are classified as high-consequence pathogens, but aerosol-generating steps (vortexing, homogenisation, centrifugation) and sharps handling carry genuine occupational risk and must be controlled.



Part of the MRC laboratory setup

| BSL-2 Requirement | Detail                                                                         |
|-------------------|--------------------------------------------------------------------------------|
| Equipment         | Class II Biological Safety Cabinet (BSC) for all specimen manipulation.        |
| PPE               | Nitrile gloves, fluid-resistant lab coat, face shield or safety goggles.       |
| Key hazard        | Aerosol generation during vortexing, tissue homogenisation, or centrifugation. |
| Decontaminant     | Freshly prepared 10% sodium hypochlorite (0.5% free chlorine) or 70% ethanol.  |



### **Class II Biological Safety Cabinet**

#### **Aerosol Minimisation**

- All tissue mincing and vortexing must be performed inside a certified Class II Type A2 BSC.
- Centrifuge aspirates in sealed safety cups, loaded and unloaded exclusively within the BSC.

#### **Sharps and Waste Management**

- Handle scalpels and biopsy punches with forceps; never manipulate exposed blades by hand.
- Dispose of all sharps immediately into puncture-resistant biohazard



sharps containers; never recap needles.

- Autoclave solid biological waste (culture plates, contaminated tubes) at 121°C for 30 minutes before final disposal.

### Surface Decontamination

Decontaminate the BSC and bench surfaces before and after each shift: apply freshly prepared 10% household bleach (0.5% sodium hypochlorite) with a minimum 15-minute contact time, then wipe with 70% ethanol to remove corrosive chloride residues from stainless steel.

### Mandatory Biosafety Documentation

| Document / Log                  | Maintenance Frequency  | Responsible Personnel                 |
|---------------------------------|------------------------|---------------------------------------|
| BSC maintenance & certification | Annual / post-repair   | Certified biomedical engineer         |
| Autoclave sterilisation log     | Daily / per cycle      | Laboratory technician/quality officer |
| Staff training & competency     | Annual / onboarding    | Laboratory Safety Officer (LSO)       |
| Incident & exposure log         | Per event, within 24 h | Affected staff & LSO                  |
| Equipment calibration log       | Monthly / quarterly    | Quality manager                       |
| Waste disposal chain-of-custody | Weekly                 | Biohazardous waste management lead    |

### Incident & Exposure Reporting

- Immediately wash the affected skin area with water and antiseptic soap for at least 15 minutes.
- Flush eyes or mucous membranes at an eyewash station for 15 minutes.
- Notify the Laboratory Safety Officer (LSO) immediately.
- Complete a biohazard incident and exposure report within 24 hours of the event (see Section XVI form templates).



**Working within the Class II Biological Safety Cabinet**

---

---

## Section III

### Clinical Specimen Collection & Transportation

The accuracy of laboratory diagnosis depends fundamentally on the quality and integrity of the collected specimen. Grains obtained aseptically during surgical procedures or deep biopsy are the primary source material for identification; superficial material from open sinus tracts is frequently non-viable and heavily contaminated with secondary bacterial flora, and should be avoided as the sole diagnostic specimen wherever a deeper sample can be obtained.

#### Grain Extraction Principles

- Surgical biopsy: take grains from deep-seated tissue during standard surgical procedures or deep biopsy rather than from superficial sinus discharge.
- Aseptic technique: use sterile forceps, scalpels, or curettes to extract grains from clean tissue margins, avoiding contact with surrounding skin flora.
- Immediate stabilisation: place collected grains into a labelled sterile container with 1.0–2.0 mL of sterile physiological (0.9%) saline or PBS to prevent desiccation during transport.
- Transport promptly to the reference laboratory; if delayed, store at 4°C. Do not freeze microbiology specimens prior to culture; freezing damages fungal hyphae and delicate bacterial filaments and renders cultures non-viable.



**Grain extraction from an open deep sinus**

### **Deep Tissue Surgical Biopsy (Gold Standard)**

Indicated for closed masses, closed sinus tracts, or active deep lesions.

- Clean the skin with 70% isopropyl alcohol, then 2% chlorhexidine gluconate, and allow to air dry.
- Provide adequate anaesthesia (general, spinal, regional, or local 1–2% lidocaine with epinephrine where not contraindicated, e.g. avoided in distal digits).
- Obtain a deep surgical biopsy extending into subcutaneous tissue, capturing part of the granuloma containing grains.
- Bisect the specimen with a sterile scalpel inside the BSC and divide it into two portions:
  - » Place Portion A into 10% neutral buffered formalin (1:10 tissue-to-fixative ratio) for histopathology.
  - » Place Portion B into 1–2 mL sterile 0.9% saline for microbiological processing.
  - » Never allow tissue to dry out.



**Foot lesion for deep surgical biopsy**



**Surgical biopsy procedure**



**Specimen collection in normal saline  
and 10% neutral buffered formalin**

## **2. Deep Sinus Aspiration / Fine Needle Aspiration**

Indicated for fluctuant, closed subcutaneous lesions or deep open sinuses.

- Decontaminate overlying skin as above.
- Attach a 19–21 gauge needle to a 10 mL syringe containing 1 mL sterile normal saline.
- Insert the needle into the deep indurated base of the lesion, apply negative pressure, and gently fan through the lesion.
- Aspirate fluid and grains into the syringe barrel and express the contents into a sterile container for immediate macroscopic examination.



## Specimen Transport Matrix

| Specimen Type                                                                                                                                                        | Container                          | Transport Temp             | Max Delay — Microbiology | Max Delay — Histology |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|----------------------------|--------------------------|-----------------------|
| Fresh biopsy (microbiology)                                                                                                                                          | Sterile container with 0.9% saline | 4–8°C (ice pack)           | < 4 hours                | N/A                   |
| Biopsy (histopathology)                                                                                                                                              | 10% neutral buffered formalin      | Room temperature (20–25°C) | N/A                      | Stable > 72 hours     |
| Aspirate / expressed grains                                                                                                                                          | Sterile screw-cap vial with saline | 4–8°C                      | < 2 hours                | N/A                   |
| <b>Critical warning</b><br><br>Do not freeze microbiology specimens. Freezing damages fungal hyphae and delicate bacterial filaments, rendering cultures non-viable. |                                    |                            |                          |                       |

## Section IV

### Long-Term Biobank Storage Standard Operating Procedures

This SOP defines the requirements for collection, quality verification, processing, cryopreservation, database cataloguing, and long-term storage of clinical mycetoma biological specimens. A centralised, internationally compliant biobank supports downstream clinical trials, biomarker discovery, resistance monitoring, epidemiological surveillance, and multi-centre phylogenomic studies. Institutions establishing a biobank should align its governance with ISO 20387 (biobanking) requirements.



**Part of the MRC biobank**

### Safety and Compliance

- All handling of unfixed clinical tissue, fresh grains, and primary isolates must occur within a certified Class II BSC under BSL-2 containment.
- PPE: nitrile gloves, fluid-resistant lab coats, face shields/goggles, and cryogenic gloves/aprons when handling liquid nitrogen or accessing ultra-low temperature ( $-80^{\circ}\text{C}$ ) freezers.

- All activities should adhere to the Declaration of Helsinki, ISO 20387 biobanking standards, local institutional ethics (IRB) approval, and documented informed patient consent.

### Washing, Purity and Viability Assessment

- Wash biopsy-harvested grains 3–5 times in sterile 0.9% saline supplemented with penicillin (100 U/mL) and streptomycin (100 µg/mL) to remove tissue debris and skin flora, inside the BSC.
- Inspect washed grains microscopically to confirm purity, colour, integrity, and freedom from heavy contamination.
- Quantity: a minimum of ~50 µL packed grain volume (or ≥10 mg tissue/mycelial mass) per cryovial is recommended for downstream resuscitation.
- Viability spot-check: inoculate a representative fragment onto SDA or BHI to verify viability before archiving the primary batch.
- Macroscopic logging: record grain colour, texture, dimensions, and quantity in the biobank database.

### Cryoprotectant Selection

| Specimen Type              | Primary Pathogens                                                   | Recommended Cryoprotectant                                   |
|----------------------------|---------------------------------------------------------------------|--------------------------------------------------------------|
| Eumycetoma fungal isolates | <i>Madurella mycetomatis</i> ,<br><i>Scedosporium apiospermum</i>   | 10–15% sterile glycerol in Sabouraud Dextrose Broth (SDB)    |
| Actinomycetoma isolates    | <i>Actinomadura</i> , <i>Streptomyces</i> , <i>Nocardia</i> species | 10% glycerol or 10% DMSO in Brain Heart Infusion (BHI) broth |
| Serum & plasma             | Humoral IgG/biomarkers                                              | Native state; no additive required                           |
| Genomic DNA / RNA          | Pathogen / host nucleic acids                                       | Nuclease-free TE buffer                                      |

## Freezing and Storage Protocol

- Label internally-threaded, O-ring sealed 1.8–2.0 mL polypropylene cryovials rated for ultra-low temperature storage.
- Transfer 3–5 clean, washed grains (or 1.0 mL dense cell suspension) into each vial with 1.0 mL of prepared freezing medium.
- Equilibrate at 4°C for 30–45 minutes inside the BSC to allow cryoprotectant penetration without chemical toxicity.
- Freeze in a controlled-rate container (e.g., an isopropanol-based freezing container) at approximately  $-1^{\circ}\text{C}/\text{minute}$  to  $-80^{\circ}\text{C}$  to prevent osmotic shock and intracellular ice formation.
- Short-term projects (<1 year): store at  $-80^{\circ}\text{C}$ . Permanent/reference archiving (>1 year): transfer to liquid-nitrogen vapour phase ( $-150^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$ ) for indefinite preservation.



**Biobank storage**

## LIMS Integration and Tracking

No sample is considered archived until fully logged in the Laboratory Information Management System (LIMS).

| Field Category     | Required Identifiers                                                          |
|--------------------|-------------------------------------------------------------------------------|
| Sample identifiers | Unique barcode ID, patient registry ID, specimen matrix type                  |
| Clinical context   | Anatomical site, clinical stage, grain colour, preliminary pathology          |
| Biobank mapping    | Freezer ID, rack number, box number, row/column coordinates                   |
| QC data            | Collection date/time, freezing date, operator ID, passage number (if culture) |

- Barcoding: use chemical-resistant, cryogenic-grade 2D matrix barcode labels that withstand  $-196^{\circ}\text{C}$  and organic solvents.
- Anonymisation: pseudonymise patient identifiers per institutional bioethics guidance; restrict re-identification to authorised biorepository managers via an encrypted master key.
- Audit trail: log every retrieval, thaw cycle, aliquot use, or relocation in real time to maintain chain of custody.

## Sample Resuscitation (Thawing)

- Retrieve the cryovial using LIMS spatial mapping and transport on dry ice.
- Thaw rapidly in a  $37^{\circ}\text{C}$  water bath, agitating gently for 1–2 minutes until only a small ice sliver remains, to limit recrystallisation.
- Inside the BSC, transfer contents to a sterile centrifuge tube, add 5 mL warm nutrient broth dropwise, and centrifuge at  $3,000\times g$  for 5 minutes.
- Discard the cryoprotectant-containing supernatant and inoculate the pellet onto fresh SDA or BHI blood agar slants.

## Section V

### Grains Macroscopic Examination, Frontline Preparation & Direct Microscopy

Before chemical digestion or staining, carefully assess collected grains macroscopically. Colour, size, and consistency provide vital preliminary indicators of the aetiological agent and should always be interpreted alongside microscopy, culture, and (where available) molecular results. Grain colour alone is not diagnostic.

#### Macroscopic Grain Identification

Place the specimen in a sterile Petri dish over a white background, then a black background, and inspect it under a stereomicroscope or hand lens.

| Grain Colour          | Consistency              | Size Range | Suspected Agents                                                                                                                |
|-----------------------|--------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------|
| <b>Black</b>          | Hard, brittle, cementing | 0.5–2.0 mm | <i>Madurella mycetomatis</i> , <i>Trematosphaeria grisea</i> , <i>Exophiala jeanselmei</i>                                      |
| <b>White/yellow</b>   | Soft, chalky, granular   | 0.2–1.0 mm | <i>Scedosporium</i> ( <i>Pseudallescheria</i> ) <i>apiospermum</i> , <i>Acremonium falciforme</i> , <i>Aspergillus nidulans</i> |
| <b>Yellow-green</b>   | Soft, lobulated          | 1.0–3.0 mm | <i>Actinomadura madurae</i> (actinomycetoma)                                                                                    |
| <b>Red/pink</b>       | Hard, irregular          | 0.1–0.5 mm | <i>Actinomadura pelletieri</i> (actinomycetoma)                                                                                 |
| <b>Whitish-yellow</b> | Soft, micro-granular     | 0.1–0.5 mm | <i>Nocardia brasiliensis</i> , <i>Nocardia asteroides</i> , <i>Streptomyces somaliensis</i>                                     |

#### Interpretation notes

Black grains are essentially specific to eumycetoma. White/pale-yellow grains can represent either eumycetoma (e.g. *Scedosporium*) or actinomycetoma (e.g. *Nocardia*), so further testing is mandatory. Red or distinct yellow-green grains strongly suggest actinomycetoma. Hard, cementing grains typically contain a melanised matrix; soft grains are typically non-cemented mycelial or bacterial aggregates.

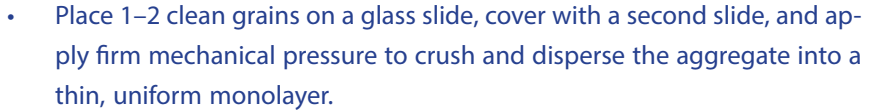


***Madurella mycetomatis* black grains collected at a surgical biopsy**

### **Frontline Specimen Washing & Mechanical Crushing**

Aspirates and surgical biopsies are routinely contaminated with secondary bacterial flora, blood, necrotic debris, and host proteins. Thorough sterile washing is mandatory before microscopy or DNA extraction.

- Transfer collected grains into a sterile 1.5 mL microcentrifuge tube using a sterile pipette.
- Add 1.0 mL sterile physiological saline or PBS and cap securely.
- Vortex for 10 seconds at moderate speed to separate host blood cells and debris from the grain matrix.
- Allow the tube to stand for 1–2 minutes so grains settle by gravity; aspirate and discard the contaminated supernatant.
- Repeat the wash-and-sedimentation cycle 2–3 times with fresh saline/PBS until the supernatant is clear.



## KOH Wet Mount Direct Microscopy

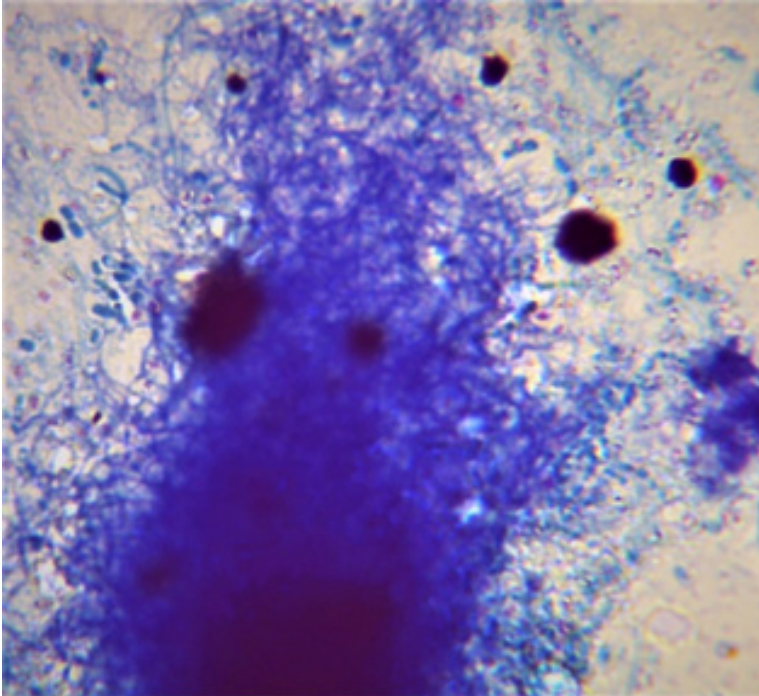
Potassium hydroxide (KOH) wet mount is the primary rapid diagnostic tool for immediate visualisation of fungal elements. KOH digests host protein, keratin, and cellular debris, leaving resistant fungal chitinous walls clearly visible under brightfield microscopy.

## Reagents

10–20% potassium hydroxide, with or without 40% dimethyl sulfoxide (DMSO) to speed clearing, or Calcofluor White for fluorescence enhancement.

## Procedure

- Pick 2–3 isolated grains using a sterile needle or micropipette and place the crushed grain monolayer on a slide.
- Dispense 1–2 drops of freshly prepared 10% KOH directly onto the specimen.
- Gently apply a coverslip, avoiding trapped air bubbles.
- Incubate at room temperature for 15–30 minutes (up to 45 minutes for dense black grains) to fully clear the host tissue.
- Examine under light microscopy, beginning at low power (10x) to locate grain fields, then high dry (40x) for structural detail.



### Microscopic Interpretation

| Feature            | Eumycetoma Grains                                                                            | Actinomycetoma Grains                                                        |
|--------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Element size       | Broad, septate fungal hyphae, 3–6 $\mu\text{m}$ diameter                                     | Extremely thin, delicate branching filaments, 0.5–1.0 $\mu\text{m}$ diameter |
| Structure          | Swollen chlamydospores (up to ~15 $\mu\text{m}$ ) embedded in a cement matrix (black grains) | Amorphous, fine-textured mass; individual hyphae not resolvable at 10–40x    |
| Associated finding | Cement matrix pigmentation                                                                   | Peripheral clubbing (Splendore-Hoepli phenomenon)                            |



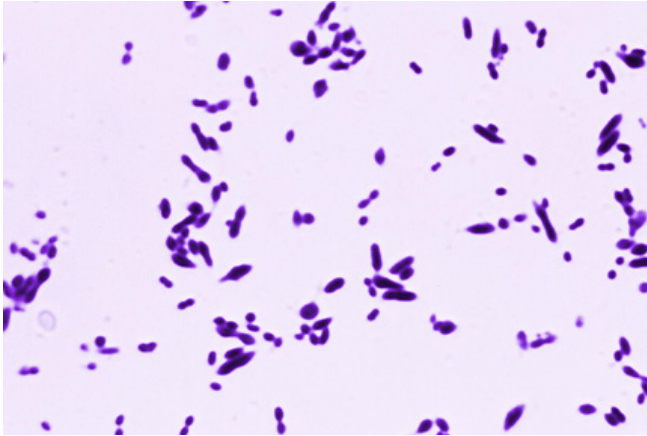
## Gram Staining Protocol for Crushed Grains

Gram staining is the standard first-line method for classifying actinomycetoma causative agents by peptidoglycan cell-wall structure.

- Smear preparation: prepare a thin smear of crushed grains, air-dry, and heat-fix by passing through a flame 3 times.
- Primary stain: flood with Crystal Violet for 1 minute; rinse with slow-running distilled water.
- Mordant: flood with Gram's Iodine for 1 minute; rinse.
- Decolourise: apply 95% ethanol or acetone-alcohol for 5–10 seconds; rinse immediately to stop decolourisation.
- Counterstain: flood with Safranin for 30 seconds; rinse, blot, and air dry.
- Examine at 100x oil immersion for Gram-positive (purple/blue), branching bacterial filaments (0.5–1  $\mu\text{m}$ ) or radiating clubs (Splendore-Hoepli phenomenon) typical of *Actinomadura* or *Streptomyces*.

### Interpretation

Gram-positive thin filaments (0.5–1.0  $\mu\text{m}$ ) support actinomycetoma (*Actinomadura*, *Streptomyces*, *Nocardia*). Broad fungal elements (3–6  $\mu\text{m}$ ), typically Gram-negative or irregularly Gram-positive, support eumycetoma.



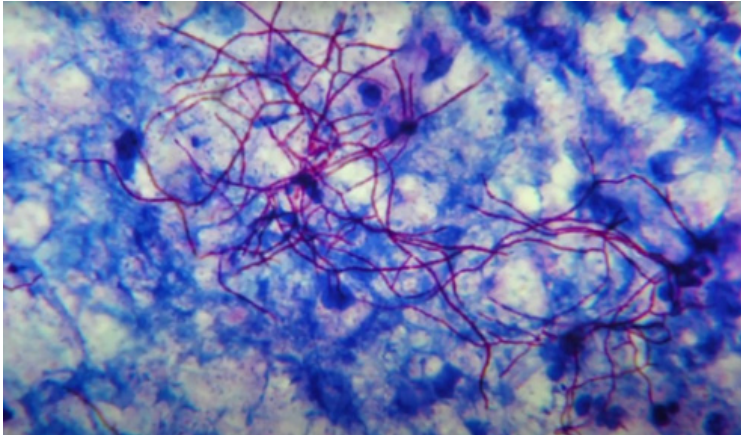
**Gram staining section**

### **Modified Ziehl-Neelsen (ZN) / Modified Kinyoun Staining**

This differential stain is essential for distinguishing partially acid-fast *Nocardia* species from non-acid-fast *Actinomyces* and *Streptomyces*, and from eumycetoma fungal elements.

- Prepare and heat-fix a thin smear as for Gram staining.
- Primary stain: flood with Carbol Fuchsin and gently heat the underside of the slide until steam rises (do not boil); maintain steaming for 5 minutes, replenishing stain as needed.
- Cool and rinse thoroughly with distilled water.
- Decolourise with a mild acid solution (1% aqueous sulfuric acid, or 1% acid-alcohol) for 1–2 minutes. This mild concentration is critical; standard 3% acid-alcohol will over-decolourise *Nocardia* and produce false negatives.
- Counterstain with Methylene Blue for 30 seconds; rinse and air-dry.

- Examine under 100x oil immersion. *Nocardia* species appear as bright pink/red, partially acid-fast, beaded branching filaments against a blue background. *Streptomyces somaliensis* and *Actinomadura* species stain blue (acid-fast negative).



**Modified Ziehl-Neelsen staining showing acid-fast filamentous bacteria**

## Section VI

### Cytopathological Techniques (FNAC & Cell Block)

Fine Needle Aspiration Cytology (FNAC) is a minimally invasive technique for obtaining diagnostic cellular material from closed, deep-seated mycetoma lesions, avoiding the morbidity of open surgical biopsy. It is particularly useful as a rapid triage tool at the district level, with material also directed to a cell block for higher diagnostic yield.

#### Technical SOP

- Aspiration: using a sterile 21–23 gauge needle on a 10 mL syringe, perform multidirectional passes into the subcutaneous lesion, aspirating cellular material and micro-granular grains.
- Smear cytology: expel a small drop onto a slide, prepare a thin smear, and fix immediately in 95% ethanol for Papanicolaou or H&E staining; air-dry a second slide for Giemsa staining.
- Cell block consolidation: transfer the remaining aspirate and micro-grains into 10% neutral buffered formalin and centrifuge at 3,000 rpm for 10 minutes to form a pellet.
- Tissue processing: Wrap the pellet in lens paper, place it in a tissue cassette, and process it as a routine histopathological specimen.
- Microscopic examination: H&E-stained cell block sections typically show polymorphic granulomatous inflammation with central fungal or bacterial grains surrounded by neutrophils and multinucleated giant cells.

#### Diagnostic role

FNAC/cell block cytology is a useful adjunct and triage tool but does not replace histopathology or culture for definitive species-level diagnosis; a negative FNAC does not exclude mycetoma.



**The Fine Needle Aspiration procedure as described above**



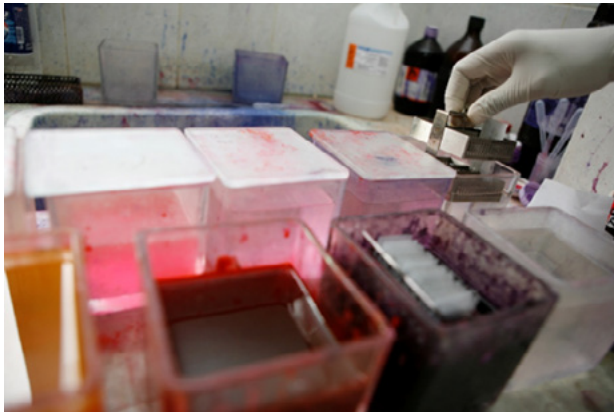
## Section VII

### Histopathological Examination of Mycetoma Lesions

Histopathological examination of surgical biopsies provides critical information on tissue architecture, host inflammatory response, and grain morphology, and remains a key confirmatory diagnostic pillar alongside microscopy, culture, and molecular testing.

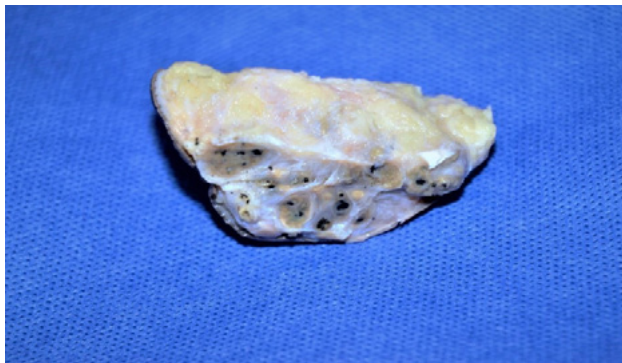
#### Biopsy Processing and Staining

- **Fixation:** immerse the biopsy in 10% neutral buffered formalin for 24–48 hours to prevent autolysis and preserve cellular architecture.
- **Dehydration & embedding:** process through graded alcohols, clear in xylene, and embed in paraffin wax.
- **Sectioning:** cut 4–5  $\mu\text{m}$  sections with a microtome and mount on clean glass slides.
- **Staining:** use H&E for general architecture; for eumycetoma, apply Grocott's Methenamine Silver (GMS) or Periodic Acid-Schiff (PAS) to highlight fungal walls.

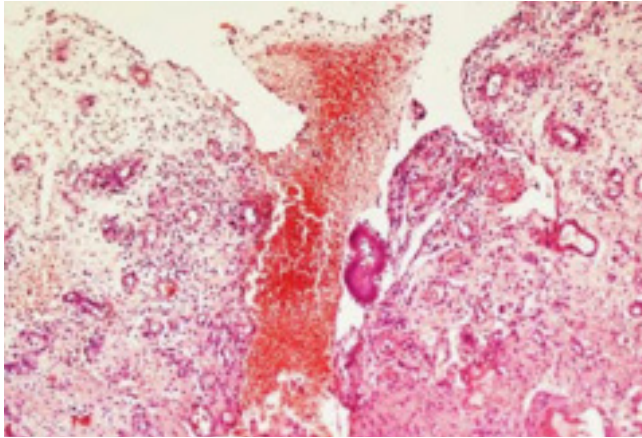


**Tissue processing, sectioning and staining at the Pathology Department, Soba University Hospital**

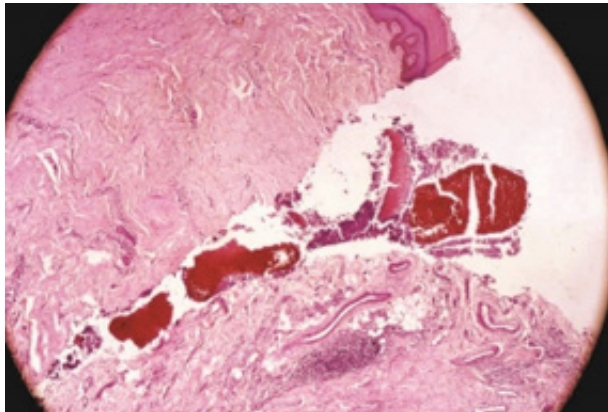
| Stain                                     | Diagnostic Use                                                                                                                         |
|-------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| <b>Haematoxylin &amp; Eosin (H&amp;E)</b> | Evaluates background tissue reaction (suppurative granulomas, lipid-laden macrophages, fibrosis) and overall grain architecture.       |
| <b>Grocott's Methenamine Silver (GMS)</b> | Stains fungal walls and actinomycete filaments jet black against a light-green background; essential for confirming fungal morphology. |
| <b>Periodic Acid-Schiff (PAS)</b>         | Stains fungal hyphae and chlamydospores bright magenta.                                                                                |
| <b>Ziehl-Neelsen (tissue)</b>             | Detects Nocardia in tissue sections; a xylene-peanut oil decolourisation step protects delicate cell walls.                            |



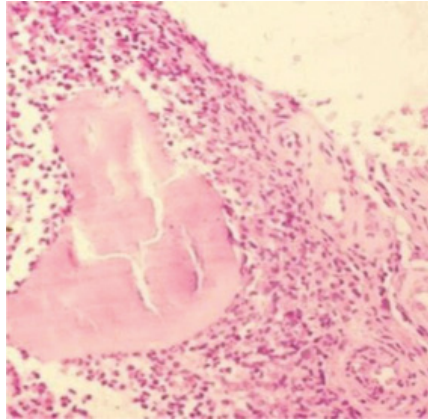
**Macroscopic appearance of surgical biopsy showing massive fibrosis and black grains**



***Photomicrograph showing *A. madurae* grains within the lumen of the intact blood vessel (H&E X 200)***



***Madurella mycetomatis* grain migrating through an open sinus (H&E X 200)**



***Streptomyces somaliensis* grain surrounded by intense inflammatory reaction (H&E X 200)**

### Microscopic Diagnostic Key

| Organisim                       | H&E Grain Morphology                                                                                         | Matrix & Special Stains                                                             |
|---------------------------------|--------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| <i>Madurella mycetomatis</i>    | Large, dark brown/black grain; round-to-oval with a vesicular/filamentous core and peripheral chlamydospores | Brown, dense cement matrix; GMS+, PAS+                                              |
| <i>Actinoadura madurae</i>      | Large, lobulated, ivory/yellow grains; dense deep-purple core with pale-pink periphery                       | Pink fringe with prominent Splendore-Hoepli phenomenon; Gram+, Fite-Faraco negative |
| <i>Actinoadura pelletieri</i>   | Small, dark red, round-to-crescent, intensely basophilic grains                                              | Dense, fragmented appearance; Gram+, Fite-Faraco negative                           |
| <i>Streptomyces somaliensis</i> | Round-to-oval, yellow/brown grains with longitudinal fractures                                               | Homogeneous with parallel cement cracks; Gram+, Fite-Faraco negative                |
| <i>Nocardia brasiliensis</i>    | Small, irregular, multi-lobular yellow/white micro-grains                                                    | Prominent Splendore-Hoepli fringes; Gram+, modified Fite-Faraco positive            |

## Section VIII

### Mycetoma Causative Organisms: Isolation & Culture

In vitro culture remains important for definitive species-level identification of the viable causative agent and for guiding antimicrobial susceptibility testing, even though sensitivity is limited by slow growth and prior antimicrobial exposure (see Section IX for comparative diagnostic performance).

#### Washing and Maceration

- Wash isolated grains 3–5 times in sterile physiological saline containing penicillin (100 U/mL) and streptomycin (100 µg/mL) inside a sterile tube to eliminate contaminating skin bacteria.
- Do not pulverise fungal grains aggressively; crush gently with a sterile glass pestle to preserve viable hyphal fragments.

#### Primary Isolation Media Battery

- Sabouraud Dextrose Agar (SDA), no antibiotics: one tube at 28–30°C and one at 37°C, incubated for up to 6 weeks.
- SDA with chloramphenicol (50 mg/L): inhibits bacterial contaminants; use for suspected eumycetoma only. Do not use cycloheximide/actidione, which inhibits *M. mycetomatis*.
- Brain Heart Infusion (BHI) agar with 5% sheep blood (2 tubes): incubate at 37°C aerobically for suspected actinomycetoma (*Nocardia*, *Actinomadura*, *Streptomyces*).
- Löwenstein-Jensen slants: incubate at 37°C for slow-growing *Actinomadura* and *Streptomyces*.

#### Inoculation and Growth

- Wash grains as above before plate inoculation.
- Fungal isolation: inoculate onto SDA with chloramphenicol, incubate at

28–37°C for 7–10 days initially (extend to 6 weeks before discarding as negative). Watch for brown-to-black, slow-growing colonies producing a diffusible brown pigment, characteristic of *M. mycetomatis*.

- Bacterial isolation: Inoculate onto blood agar or BHI agar and incubate aerobically at 37°C for 3–5 days (extend as needed for slow growers). Look for dry, folded, chalky colonies characteristic of *Actinomadura* or *Nocardia*.
- Examine colonies both macroscopically (colour, texture, pigment) and microscopically (hyphae, spore chains, arthrospores) using a lactophenol cotton blue or similar tape mount.



**Maintaining fungal culture collections**

## Cultural Characteristics

| Organism                        | Growth Rate          | Colony Morphology (SDA/BHI)                                                 | Microscopic Morphology                                                                 |
|---------------------------------|----------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| <i>Madurella mycetomatis</i>    | Slow, 7–21 days      | Dome-shaped white turning olivaceous-brown, brown diffusible pigment        | Septate hyphae, phialides with small conidia; sterile hyphae common                    |
| <i>Actinomadura madurae</i>     | Moderate, 10–14 days | Flat, glabrous, wrinkled; ivory, white, or pink-red; no aerial hyphae       | Thin branching filaments, short chain arthrospores, non-acid-fast                      |
| <i>Actinomadura pelletieri</i>  | Slow, 14–21 days     | Small, heap-like, wrinkled; bright crimson/coral-red, soft or cartilaginous | Fine branching filaments breaking into coccoid elements; non-acid-fast                 |
| <i>Streptomyces somaliensis</i> | Moderate, 7–14 days  | Cream to brownish-yellow, leathery, folded; chalky white aerial mycelium    | Aerial hyphae producing long, straight chains of smooth spores                         |
| <i>Nocardia brasiliensis</i>    | Rapid, 3–7 days      | Chalky-white to orange, wrinkled, dry, musty odour                          | Delicate branching filaments fragmenting into coccobacillary rods; partially acid-fast |

### Safety reminder

All culture manipulation, subculturing, and colony sampling must be performed inside a certified Class II BSC under BSL-2

## Section IX

### **Molecular Diagnostics: DNA Extraction, PCR, Real-Time PCR, MALDI-TOF & LAMP**

Direct microscopy and culture have well-recognised limitations: incubation periods of up to six weeks, culture negativity after prior antimicrobial exposure, failure to isolate fastidious or non-viable organisms from dense fibrous tissue, and morphologically indistinguishable species that lead to subjective misidentification.

Molecular and physical diagnostics, gene sequencing (ITS and 16S rRNA loci), species-specific loop-mediated isothermal amplification (LAMP), and MALDI-TOF mass spectrometry offer more rapid, objective, and often more sensitive alternatives, and increasingly complement rather than replace culture.



**Conventional and real-time PCR machines**

## Comparative Diagnostic Performance

| Method                             | Turnaround              | Sensitivity            | Specificity        | Field Feasibility       | Infrastructure Needs                                                   |
|------------------------------------|-------------------------|------------------------|--------------------|-------------------------|------------------------------------------------------------------------|
| <b>Direct microscopy</b>           | <2 hours                | Moderate (~60–75%)     | Low (~50–60%)      | Point-of-care           | Light microscope, KOH/Gram/ZN stains; low cost                         |
| <b>Tissue histopathology</b>       | 48–72 hours             | Moderate (~70–80%)     | Moderate (~75–85%) | District hospital       | Formalin fixation, microtome, histology suite, pathologist             |
| <b>Microbiological culture</b>     | 1–6 weeks               | Low–moderate (~40–60%) | High (~90–95%)     | Moderate/secondary care | BSL-2 facility, visualisation media, long incubation                   |
| <b>PCR &amp; Sanger sequencing</b> | 24–48 hours             | High (~90–95%)         | Very high (>99%)   | Reference laboratory    | Thermal cycler, gel electrophoresis, sequencer, bioinformatics         |
| <b>MALDI-TOF mass spectrometry</b> | <15 minutes from colony | High (~85–92%)         | High (~95–98%)     | Tertiary centre         | Mass spectrometer, spectral reference database, formic-acid extraction |
| <b>Isothermal LAMP assay</b>       | 45–60 minutes           | High (~92–97%)         | High (~95–99%)     | Field/peripheral        | Heat block or water bath, fluorometer or visual dye readout            |

### On reported sensitivities

Published performance figures for mycetoma diagnostics vary between studies and reference standards; the ranges above are indicative and should be interpreted as approximate rather than validated local performance data. Each laboratory should verify assay performance against its own case mix wherever feasible.

## Genomic DNA Extraction — Three-Tier Lysis SOP

Fungal cell walls contain complex chitin and  $\beta$ -glucan networks, so standard chemical lysis alone is often insufficient; a combined mechanical, enzymatic, and chemical lysis approach improves DNA yield from grains and culture material.

- **Mechanical lysis:** place washed grains in a 1.5 mL tube with 0.5 mm sterile glass beads and bead-beat for 3–5 minutes.
- **Enzymatic lysis:** suspend in 200  $\mu$ L lysis buffer with 20 mg/mL lysozyme (actinomycetes) or 100 U/mL lyticase (fungi); incubate at 37°C for 30–60 minutes.
- **Proteinase digestion:** add Proteinase K (20 mg/mL) and SDS to a final concentration of 1%; incubate at 56°C for 30 minutes.

- Chemical lysis & purification: apply chaotropic lysis buffer, load onto a silica-membrane spin column, wash to remove protein contaminants, and elute purified DNA using a standard commercial kit per manufacturer instructions.



**DNA extraction machine**

### **PCR Target Loci and Primers**

PCR-based species identification is a gold-standard confirmatory method that bypasses the delays of culture growth.

#### **Eumycetoma — Internal Transcribed Spacer (ITS) region of rDNA**

- ITS5 (forward, pan-fungal universal): 5'-GGAAGTAAAAGTCGTAACAAGG-3'
- ITS4 (reverse, pan-fungal universal): 5'-TCCTCCGCTTATTGATATGC-3'
- Cycling: initial denaturation at 95°C for 5 min; 35 cycles of (95°C 30 s, 55°C 30 s, 72°C 60 s); final extension at 72°C for 7 min.

Because the ITS4/ITS5 primer pair is pan-fungal (not *Madurella*-specific), amplicons require Sanger sequencing and BLAST/MycoBank comparison for species assignment. A *Madurella mycetomatis*-specific nested PCR (prim-

ers 26.1A/28.3A) is also used at reference centres, though this pair has been shown to cross-react with the more recently described species *M. pseudomycetomatis*; newly designed comparative-genomics-based primers (Mmy-Fw/Mmy-Rv) have been proposed to resolve this limitation and may be considered where available.

### **Actinomycetoma — 16S rRNA gene**

- 27F (forward, universal bacterial): 5'-AGAGTTTGATCMTGGCTCAG-3'
- 1492R (reverse, universal bacterial): 5'-TACGGYTACCTTGTACGACTT-3'
- Cycling: initial denaturation at 95°C for 5 min; 35 cycles of (95°C 45 s, 58°C 45 s, 72°C 90 s); final extension at 72°C for 10 min.

Sequence analysis: purify PCR products and sequence by Sanger sequencing. Compare resulting contigs against NCBI BLAST or MycoBank/EzBioCloud (for bacteria) databases; a threshold of >99% identity is generally required for species-level assignment, interpreted alongside colony and microscopic morphology.

### **Real-Time PCR**

Real-time PCR using fluorescent (e.g. TaqMan) probes provides high sensitivity and allows relative quantification of pathogen load directly from clinical samples, with the added advantage that reaction tubes remain closed throughout, reducing amplicon-contamination risk compared with conventional gel-based PCR.

### **Agarose Gel Electrophoresis**

For conventional PCR, visualise amplicons on a 1.5% agarose gel under UV (or blue-light) transillumination, and confirm band size against known positive control standards and a molecular-weight ladder.

### **MALDI-TOF Mass Spectrometry**

Matrix-Assisted Laser Desorption/Ionisation–Time of Flight mass spectrometry enables rapid protein-fingerprint identification from primary culture material, provided a validated mycetoma-specific spectral reference library is available (routine clinical MALDI-TOF databases have historically had limited coverage of filamentous fungal and actinomycete mycetoma agents so that local library expansion may be required).

- Transfer a loopful of fresh colony material into a tube with 300  $\mu\text{L}$  molecular-grade water.
- Add 900  $\mu\text{L}$  absolute ethanol, vortex, centrifuge at 13,000 rpm for 2 minutes, and discard the supernatant.
- Air-dry the pellet completely.
- Resuspend in 20–50  $\mu\text{L}$  of 70% formic acid, vortex, and incubate for 10 minutes at room temperature.
- Add an equal volume of 100% acetonitrile, incubate for 10 minutes, and centrifuge at 13,000 rpm for 2 minutes.
- Spot 1  $\mu\text{L}$  of supernatant onto the MALDI target plate, air-dry, and overlay with 1  $\mu\text{L}$  of  $\alpha$ -cyano-4-hydroxycinnamic acid (HCCA) matrix.
- Acquire spectra and compare against a dedicated filamentous fungal/actinomycetal reference library.

### **Loop-Mediated Isothermal Amplification (LAMP)**

LAMP assays provide rapid, field-adapted diagnostics by amplifying target DNA isothermally (60–65°C) without a thermal cycler, making them attractive for peripheral and rural endemic settings.

- Principle: 4–6 specialised primers target a species-specific locus (e.g. *M. mycetomatis* translation elongation factor 1-alpha, or intergenic spacer regions for *Actinomadura madurae*).
- Reaction: combine target DNA, primers, Bst DNA polymerase, dNTPs, and



reaction buffer; incubate in a heated water bath or block at ~63°C for 45 minutes.

- Visual readout: add SYBR Green I or Calcein dye. A positive reaction shows bright green fluorescence under ambient or UV light (or visible turbidity); a negative reaction remains orange/clear.

### Emerging methods

LAMP assays and short-tandem-repeat (STR) genotyping tools for *Madurella mycetomatis* are active areas of research and validation; laboratories adopting these methods should confirm analytical and clinical performance locally before relying on them for treatment decisions.

## Section X

### Antimicrobial Susceptibility Testing (AST)

Standardised AST supports regimen selection, helps prevent premature treatment default, and monitors emerging resistance. Testing should follow current Clinical and Laboratory Standards Institute (CLSI) methodology; laboratories should consult the latest published CLSI documents, as breakpoints and methods are periodically updated.

#### 1. Actinomycetoma AST — CLSI Broth Microdilution (Aerobic Actinomycetes)

Testing of aerobic actinomycetes (*Nocardia*, *Actinomadura*, *Streptomyces*) follows CLSI guidance for aerobic actinomycetes, historically published as CLSI document M24.

- Inoculum preparation: select 4–5 colonies from fresh growth on BHI agar, transfer to cation-adjusted Mueller-Hinton broth (CAMHB), homogenise to break up clumps, and adjust turbidity to a 0.5 McFarland standard ( $\sim 1.5 \times 10^8$  CFU/mL).
- Dilution: dilute 1:100 in CAMHB for a final test inoculum of approximately  $5 \times 10^5$  CFU/mL.
- Plate setup: inoculate 96-well IsuLab plates pre-loaded with two-fold serial dilutions of the target agents (e.g. amikacin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, linezolid, imipenem/meropenem, select panel per local prescribing practice and current CLSI recommendations).
- Incubation: incubate aerobically at 35–37°C; read visual endpoints at 48–72 hours (up to 5 days for slow-growing *Actinomadura*).

The Minimum Inhibitory Concentration (MIC) is the lowest drug concentration that completely inhibits visible growth compared with the drug-free growth control well.

## 2. Eumycetoma AST — Modified CLSI Broth Microdilution (Filamentous Fungi)

Antifungal susceptibility testing for *Madurella mycetomatis* and related fungi follows a modified version of the CLSI filamentous-fungi broth microdilution methodology, historically published as CLSI document M38.

- Inoculum: because *M. mycetomatis* rarely sporulates in culture, mycelial fragments are harvested and visualised in sterile saline; heavy particles are allowed to settle, and the upper suspension turbidity is adjusted spectrophotometrically (commonly to 68–70% transmittance at 530 nm).
- Medium: RPMI-1640 broth buffered with 0.165 M MOPS to pH 7.0.
- Antifungal panel: typically includes itraconazole, voriconazole, posaconazole, amphotericin B, and terbinafine, tested across a two-fold dilution series per current CLSI ranges.
- Incubation: 37°C for 7 days (longer for slow growers).

Endpoint reading at 7 days: for azoles/terbinafine, the MIC is the lowest concentration showing prominent (commonly  $\geq 80\%$ ) growth reduction relative to the control; for amphotericin B, the MIC is the lowest concentration showing complete (100%) growth inhibition.



**Antimicrobial Susceptibility Testing (AST)**

## Section XI

### Patient Monitoring: Haematology & Biochemistry

Long-term pharmacological treatment of mycetoma involves prolonged, potentially toxic antimicrobial regimens: azole antifungals for eumycetoma, or aminoglycoside/sulfonamide-based combinations for actinomycetoma. Meticulous clinical laboratory monitoring is essential to detect toxicity early and avoid treatment discontinuation due to preventable adverse events.

| Diagnostic Panel            | Critical Markers                          | Monitoring Frequency (indicative)                           | Significance of Deviations                                                                                                       |
|-----------------------------|-------------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| <b>Liver function (LFT)</b> | ALT, AST, bilirubin, alkaline phosphatase | Every 2–4 weeks initially, then less frequently once stable | Azoles can cause hepatotoxicity; discontinue or seek specialist review on significant (e.g. >3x upper limit of normal) elevation |
| <b>Renal function (RFT)</b> | Urea, creatinine, electrolytes            | Every 2–3 weeks during aminoglycoside therapy               | Aminoglycosides (amikacin, streptomycin) are nephrotoxic; monitor creatinine trend closely                                       |
| <b>Haematology (CBC)</b>    | WBC, haemoglobin, platelets               | Monthly throughout therapy                                  | Sulfonamides can cause bone marrow suppression, anaemia, or leukopenia                                                           |

The treating clinical team should set exact monitoring intervals and action thresholds based on the specific drug regimen, local formulary, and national guidance; the frequencies above are indicative starting points, not fixed rules. Audiometric assessment should also be considered periodically for patients receiving prolonged aminoglycoside therapy, given the risk of irreversible ototoxicity.



**Haematology section**

## Section XII

### Comprehensive Laboratory Equipment & Reagent Specifications

Establishing a dedicated mycetoma reference laboratory requires appropriate infrastructure, instrumentation, and reagents scaled to the intended level of service (peripheral triage vs full reference diagnostics).

#### 1. Essential Instrumentation

##### Biosafety Infrastructure

- Class II Type A2 biological safety cabinet (certified to relevant national/international standard).
- HEPA-filtered exhaust systems with annual certification.

##### Microscopy Suite

- Brightfield clinical microscope with 10x, 20x, 40x, and 100x (oil immersion) objectives.
- Fluorescence module with an appropriate UV filter cube for Calcofluor White visualisation, where available.
- Stereomicroscope with dual top/bottom illumination for grain harvesting and macroscopic examination.

##### Incubation & Culture Equipment

- Refrigerated microbiological incubator, temperature range approximately 20–45°C.
- CO<sub>2</sub>-controlled incubator for fastidious actinomycetes where required.
- Vortex mixers and a refrigerated high-speed microcentrifuge.

##### Molecular Diagnostics Suite

- Thermal cycler with gradient block capability.

- Real-time quantitative PCR system.
- Isothermal heat block or water bath for LAMP assays.

Horizontal gel electrophoresis unit with UV or blue-light transilluminator/gel documentation system.



## 2. Representative Media Formulations

### BHI Blood Agar with Antibiotics (Actinomycetoma Isolation)

- BHI agar powder: 52.0 g
- Distilled water: 1000 mL
- Defibrinated sheep blood: 50 mL (5% v/v)
- Colistin sulfate: ~10 mg/L (helps suppress Gram-negative bacterial overgrowth)

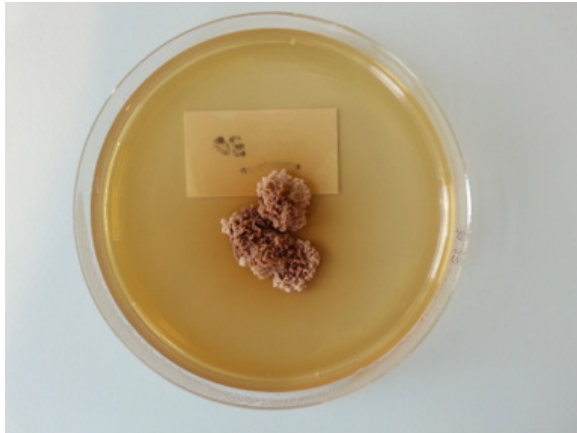
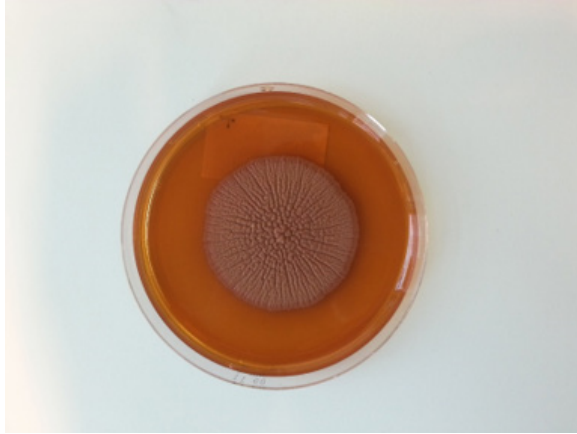
Preparation: autoclave BHI powder in water at 121°C for 15 minutes; cool to 48–50°C in a water bath; aseptically add defibrinated sheep blood and fil-



### Sabouraud Dextrose Agar with Chloramphenicol (Eumycetoma Isolation)

- Peptone: 10.0 g
- Dextrose (glucose): 40.0 g
- Agar: 15.0 g
- Chloramphenicol: 0.05 g
- Distilled water: 1000 mL

Preparation: heat with frequent agitation to dissolve completely; autoclave at 121°C for 15 minutes; final pH  $5.6 \pm 0.2$  at 25°C. Do not add cycloheximide.



**Madurella mycetomatis cultures**

## Section XIII

### Laboratory Signpost

#### Diagnostic Matrix

| Diagnostic Method             | Eumycetoma                                            | Actinomycetoma                                                             |
|-------------------------------|-------------------------------------------------------|----------------------------------------------------------------------------|
| <b>Direct microscopy</b>      | Broad, septate, branched fungal hyphae (2–6 µm)       | Fine, delicate, branching bacterial filaments (0.5–1 µm)                   |
| <b>Gram staining</b>          | Gram-negative or irregular staining of hyphae         | Gram-positive (purple/blue) filaments; radiating clubs                     |
| <b>Acid-fastness</b>          | Non-acid-fast (stains blue)                           | Nocardia: partially acid-fast.<br>Actinomadura/Streptomyces: non-acid-fast |
| <b>Molecular target genes</b> | ITS region, $\beta$ -tubulin, RPB2                    | 16S rRNA gene, gyrB, secA1                                                 |
| <b>Growth media</b>           | SDA with antibiotics; 28–37°C, 7–10 days initial read | Blood agar, BHI agar; 37°C, 3–5 days initial read                          |
| <b>Reagent controls</b>       | Standard fungal reference culture                     | S. aureus (Gram+) and E. coli (Gram-) controls                             |

#### Key Operational Benchmarks (Key Performance Indicators)

| Item                                   | Target Turnaround / Benchmark                                                |
|----------------------------------------|------------------------------------------------------------------------------|
| <b>Direct smear / KOH result</b>       | < 2 hours from receipt of specimen                                           |
| <b>Histopathology report</b>           | Within 72 hours of tissue receipt (fixation time permitting)                 |
| <b>Contamination rate for cultures</b> | Institution-defined threshold (commonly targeted at ≤5% of inoculated tubes) |
| <b>Internal QC compliance</b>          | 100% documentation of batch-level controls before releasing clinical results |

Institutions should set locally appropriate numeric KPI targets based on their own baseline performance, case volume, and resources, and review them periodically as part of the quality management system.



## Standard Diagnostic Reporting Templates

Standardised reporting ensures clinical teams receive actionable laboratory findings for timely therapeutic decision-making. Institutions should adapt the header fields below to their own patient registry and LIMS.

## Mycetoma Reference Laboratory

Form MD-01: Grains Examination Results

| Field               | Detail |
|---------------------|--------|
| ID No.              |        |
| Patient Name        |        |
| Age / Sex           |        |
| Attending Physician |        |
| Clinic / Hospital   |        |
| Anatomical Site     |        |
| Date/Time Collected |        |
| Date/Time Received  |        |

## Direct Macroscopic & Microscopic Examination

| Grain Present | Colour                                     | Consistency                             | Size (mm) |
|---------------|--------------------------------------------|-----------------------------------------|-----------|
| Yes / No      | Black/ Yellow/ White/<br>Red/ Brown/ Other | Hard-brittle/ Soft-mu-<br>coid/ Elastic |           |

| Direct Microscopy (KOH / Calcofluor White)        | Present (Y/N) |
|---------------------------------------------------|---------------|
| Broad septate hyphae (3–6 μm) with chlamydospores |               |
| Delicate branching filaments (0.5–1.0 μm)         |               |
| Splendore-Hoeppli phenomenon                      |               |



| Differential Staining  | Result                                                          |
|------------------------|-----------------------------------------------------------------|
| Gram stain             | Gram-positive filaments / Gram-negative / Broad fungal elements |
| Modified Ziehl-Neelsen | Partially acid-fast / Acid-fast negative                        |

Preliminary diagnostic impression (within 2 hours): ☐ Highly suggestive of eumycetoma ☐ Highly suggestive of actinomycetoma ☐ Inconclusive / re-sampling required



## Mycetoma Reference Laboratory

### Form MD-02: Histopathological Diagnostic Report

| Field               | Detail |
|---------------------|--------|
| ID No.              |        |
| Patient Name        |        |
| Age / Sex           |        |
| Attending Physician |        |
| Clinic / Hospital   |        |
| Anatomical Site     |        |
| Date/Time Collected |        |
| Date/Time Received  |        |

Stain(s) used (H&E / GMS / PAS): \_\_\_\_\_

Macroscopic examination: \_\_\_\_\_

Microscopic examination: \_\_\_\_\_

Tissue reaction type: \_\_\_\_\_

Grain description: \_\_\_\_\_

Histological diagnosis: \_\_\_\_\_

Pathologist name and signature: \_\_\_\_\_



Mycetoma Reference Laboratory

Form MD-03: Grains Culture Result

| Field               | Detail |
|---------------------|--------|
| ID No.              |        |
| Patient Name        |        |
| Age / Sex           |        |
| Attending Physician |        |
| Clinic / Hospital   |        |
| Anatomical Site     |        |
| Date/Time Collected |        |
| Date/Time Received  |        |

Incubation period: \_\_\_\_ days      Temperature: \_\_\_\_ °C      Media: \_\_\_\_\_

Macroscopic examination: \_\_\_\_\_

Microscopic examination: \_\_\_\_\_

Organism isolated: \_\_\_\_\_

Final diagnosis: \_\_\_\_\_

Microbiologist name and signature: \_\_\_\_\_



## Mycetoma Reference Laboratory

### Form MD-04: Molecular Examination Result

| Field               | Detail |
|---------------------|--------|
| ID No.              |        |
| Patient Name        |        |
| Age / Sex           |        |
| Attending Physician |        |
| Clinic / Hospital   |        |
| Anatomical Site     |        |
| Date/Time Collected |        |
| Date/Time Received  |        |

Specimen type: Grains / Isolate

Method: ☐ 16S rRNA PCR ☐ ITS PCR ☐ MALDI-TOF MS ☐ LAMP

Confirmed species: \_\_\_\_\_

Match score/identity: \_\_\_\_\_ %

Final diagnosis: \_\_\_\_\_

Microbiologist name and signature: \_\_\_\_\_



Mycetoma Reference Laboratory

**Form MD-05: Antimicrobial Susceptibility Testing Result (CLSI Methodology)**

| Field               |                                | Detail                 |
|---------------------|--------------------------------|------------------------|
| ID No.              |                                |                        |
| Patient Name        |                                |                        |
| Age / Sex           |                                |                        |
| Attending Physician |                                |                        |
| Clinic / Hospital   |                                |                        |
| Anatomical Site     |                                |                        |
| Date/Time Collected |                                |                        |
| Date/Time Received  |                                |                        |
| Tested Agent        | MIC Value ( $\mu\text{g/mL}$ ) | Interpretation (S/I/R) |
| Co-trimoxazole      |                                |                        |
| Linezolid           |                                |                        |
| Voriconazole        |                                |                        |
| Amikacin            |                                |                        |
| Itraconazole        |                                |                        |

Medical Microbiologist Signature: \_\_\_\_\_



Form BS-101 and BS-102 (Biosafety Logs)

Form BS-101: Daily BSL-2 Safety Checklist

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ Shift: [ ] Morning [ ] Afternoon Inspector:

-----

| Check Item                                                | Pass | Fail | Comments |
|-----------------------------------------------------------|------|------|----------|
| Class II BSC airflow/pressure differential normal         |      |      |          |
| Eyewash station inspected and flushed                     |      |      |          |
| Disinfectants freshly prepared (10% bleach / 70% ethanol) |      |      |          |
| PPE stock adequate (nitrile gloves, masks, face shields)  |      |      |          |
| Sharps containers below 3/4 capacity                      |      |      |          |
| Autoclave cycle temperature/pressure verified             |      |      |          |
| Biohazard waste bags labelled and sealed properly.        |      |      |          |
| Laboratory access restricted to authorised personnel      |      |      |          |

Supervisor Signature: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_



## Form BS-102: Biohazard Incident & Exposure Report

|                                                                                                                                                                                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Date of incident: ____/____/____                                                                                                                                                               |
| Time: ____:____ [ ] AM [ ] PM                                                                                                                                                                  |
| Location/Room:                                                                                                                                                                                 |
| Affected person:                                                                                                                                                                               |
| Role/Title:                                                                                                                                                                                    |
| Supervisor:                                                                                                                                                                                    |
| Incident category:<br><br>[ ] Percutaneous injury [ ] Mucous membrane exposure [ ] Non-intact skin exposure<br><br>[ ] Biological spill >10 mL [ ] Equipment malfunction / loss of containment |
| Description of the incident:                                                                                                                                                                   |
| Specimen/pathogen involved:                                                                                                                                                                    |
| Specimen ID:                                                                                                                                                                                   |
| Suspected agent: [ ] Eumycetoma [ ] Actinomycetoma                                                                                                                                             |
| First aid administered: [ ] Yes [ ] No                                                                                                                                                         |
| Disinfection/decontamination performed: [ ] Yes [ ] No                                                                                                                                         |
| Medical evaluation/follow-up scheduled: [ ] Yes [ ] No                                                                                                                                         |



Corrective action plan (to be completed by Safety Officer:

Affected individual signature:

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Laboratory Safety Officer signature:

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Laboratory Director signature:

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

## Further Reading

Key sources consulted and recommended for further study.

Readers should consult primary literature and current WHO/CLSI publications directly, as diagnostic and treatment evidence continues to evolve.

- The Mycetoma Research Center, Laboratory Standard Operating Procedure (SOP)  
[https://mycetoma.edu.sd/?page\\_id=%205896](https://mycetoma.edu.sd/?page_id=%205896)
- World Health Organization. Mycetoma — fact sheet and EMRO health topic pages (who.int; emro.who.int).
- van de Sande WWJ. Global burden of human mycetoma: a systematic review and meta-analysis. PLoS Negl Trop Dis, 2013.
- Fahal AH, van de Sande WWJ, et al. Mycetoma: the journey from neglect to recognition as a neglected tropical disease. PLoS Negl Trop Dis / related reviews, 2016–2021.
- van de Sande W, Fahal A, Ahmed SA, et al. Closing the mycetoma knowledge gap. Medical Mycology, 2018.
- Zijlstra EE, van de Sande WWJ, Welsh O, Mahgoub ES, Goodfellow M, Fahal AH. Mycetoma: a unique neglected tropical disease. Lancet Infectious Diseases, 2016.
- Mahgoub ES, Fahal AH, et al. Global sociodemographic, clinical, and epidemiological profiling of patients with mycetoma: a systematic review. 2025.
- Fahal AH, Ahmed ES, Bakhiet SM, et al. Two dose levels of once-weekly fosravuconazole versus daily itraconazole in combination with surgery in patients with eumycetoma in Sudan: a randomised, double-blind, phase 2, proof-of-concept superiority trial. Lancet Infectious Diseases, 2024.
- Chu WY, Fahal AH, Ahmed ES, et al. Pharmacokinetics and pharmaco-

dynamics of fosravuconazole, itraconazole, and hydroxyitraconazole in Sudanese patients with eumycetoma. *Journal of Infectious Diseases*, 2025.

- de Hoog GS, Adelmann D, Ahmed AO, van Belkum A. Phylogeny and typification of *Madurella mycetomatis*, with a comparison of other agents of eumycetoma. *Mycoses*, 2004; and subsequent *Madurella* species descriptions (*M. tropicana*, *M. fahalii*, *M. pseudomycetomatis*).
- Ahmed AO, van Leeuwen W, Fahal A, van de Sande W, Verbrugh H, van Belkum A. Mycetoma caused by *Madurella mycetomatis*: a neglected infectious burden. *Lancet Infectious Diseases*, 2004.
- Nyuykonge B, et al. Comparing the performance of commonly used eumycetoma diagnostic tests. *Mycoses*, 2023.
- Ahmed SA, de Hoog GS, Stevens DA, Fahal AH, van de Sande WWJ. In vitro antifungal susceptibility of coelomycete agents of black grain eumycetoma using CLSI and EUCAST methodology. *Medical Mycology and related CLSI M38 methodology papers*.
- Clinical and Laboratory Standards Institute (CLSI). Susceptibility Testing of *Mycobacteria*, *Nocardia* spp., and Other Aerobic Actinomycetes (current edition, historically M24); Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi (current edition, historically M38).
- International Organisation for Standardisation. ISO 20387: Biotechnology — Biobanking — General requirements for biobanking.
- Drugs for Neglected Diseases initiative (DNDi). Mycetoma programme updates and fosravuconazole portfolio page ([dndi.org](http://dndi.org)).
- Fahal AH, Hassan MA. Mycetoma. *Br J Surg*. 1992; 79(11): 1138-1141. PMID: 1467883.
- Fahal AH, Suliman SH, Gadir AFA, EL Hag IA, EL Amin FI, Gumaa SA, Mahgoub ES. Abdominal wall mycetoma: an unusual presentation. *Trans R Soc Trop Med Hyg*. 1994; 88(1):78-80. PMID: 8154012.

- Fahal AH, Suliman SH. Clinical presentation of mycetoma. Sudan Med J. 1994; 32: 46-66.
- Fahal AH, El Hassan AM, Abdelalla AO, Sheikh HE, Cystic mycetoma: an unusual clinical presentation of *Madurella mycetomatis*. Trans R Soc Trop Med Hyg. 1998; 92:6-67.
- Fahal AH, EL Toum EA, EL Hassan AM, Gumaa SA, Mahgoub ES. A preliminary study on the ultrastructure of *Actinomyces pelletieri* and its host tissue reaction. J Med Vet Mycol. 1994; 32: 343-348. PMID: 7844700.
- EL Hassan AM, Fahal AH, EL Hag IA, Khalil EAG. The pathology of mycetoma: Light microscopic and ultrastructural features. Sudan Med J. 1994; 32: 23-45.
- Fahal AH, Hassan MA, Sanhoury M. Surgical treatment of mycetoma. Sudan Med J. 1994; 32: 98-104.
- Fahal AH, EL Toum EA, EL Hassan AM, Gumaa SA, Mahgoub ES. Host tissue reaction to *Madurella mycetomatis*: New classification. J Med Vet Mycol. 1995; 33: 15-17.
- EL Hag IA, Fahal AH, Khalil EAG. Fine needle aspiration cytology of mycetoma. Acta Cytologica. 1996; 40(3): 461-46.
- Fahal AH, Omer SM, El Razig SA, Ali ABE, Mahdi EMA, Mahgoub ES. Thyroid function in mycetoma patients. East Afr Med J. 1995; 72(7): 454-456. PMID: 7498029.
- Fahal AH, Azziz KAA, Suliman SH, Galib HV, Mahgoub ES. Dual infection with mycetoma and tuberculosis. East Afr Med J. 1995; 72(11): 749-750. PMID: 8904072.
- Mohamed ARO, Fahal AH, Venge V. Immunoglobulin and inflammatory markers profile in mycetoma. East Afr Med J. 1996; 73(4): 212 (Letter). PMID: 8698027.
- Fahal AH, Yagi HI, EL Hassan AM. Mycetoma induced palatal deficiency and pharyngeal plexus dysfunction. Trans R Soc Trop Med Hyg. 1996;

90: 676-677. PMID: 9015514

- Fahal AH, Sheikh HE, EL Hassan AM. Pathological fracture in mycetoma. *Trans R Soc Trop Med Hyg.* 1996; 90: 675-676. PMID: 9015513.
- Fahal AH, Sharfi AR, Sheikh HE, EL Hassan AM. Internal fistula formation: an unusual complication of mycetoma. *Trans R Soc Trop Med Hyg.* 1996; 89: 550-552.
- Fahal AH, Sadig ME, Suliman SH, EL Razig SA, Mahgoub ES. Lack of association between ABO blood groups and Rh factor and the tendency to develop mycetoma. *East Afr Med J.* 1996; 11: 769. (Letter). PMID: 8997873
- Fahal AH, EL Hag IA, Gadir AFA, EL Lider AR, Baraka OZ, EL Hassan AM. The blood supply and vasculature in mycetoma. *J Med Vet Mycol.* 1997; 35: 101-106. PMID: 9147269
- Fahal AH, Sheikh HE, EL Lider MA, Homeida MA, EL Arabi YE, Mahgoub ES. Ultrasonic imaging in mycetoma. *Br J Surg.* 1997; 78: 765-766. PMID: 9278658
- Ahmed AOA, Fahal AH, Abugroun EAM, Zijlstra E, Belkum A van, Verburgh HA. Unexpected high prevalence of secondary bacterial infection in mycetoma. *J Clin Microbiol.* 1998; 36(3): 850-851.
- Fahal AH, Sharfy ARA. Vulval mycetoma: a rare cause of bladder outlet obstruction. *Trans R Soc Trop Med Hyg.* 1998; 92: 652-653. PMID: 10326112.
- Ahmed AO, Mukhtar MM, Kools-Sijmons M, Fahal AH, de Hoog S, van den Ende BG, Zijstara, ED, Verburgh H, Abugroun ESA, EL Hassan AM, van Beklum A. Development of a species-specific PCR RFLP procedure for the identification of *Madurella mycetomatis*. *J Clin Microbiol.* 1999; 37(10):3175-8. PMID: 10488173.
- Fahal AH. The management of mycetoma. *Post-Grad -Caribbean.* 2000;16(4):229-232.
- EL Hassan AM, Fahal AH, Ahmed AO, Ismail A, Veress B. The immuno-

pathology of actinomycetoma lesions caused by *Streptomyces somaliensis*. *Trans R Soc Trop Med Hyg.* 2001; 95(1); 89-92. PMID: 11280076

- Ahmed AOA, Adelmann D, Fahal AH, Verbrugh HA, Van Belkum A, De Hoog S. Environmental occurrence of *Madurella mycetomatis*, major agent of human eumycetoma in Sudan. *J Clin Microbiol.* 2002 40(3): 1031-1036. PMID: 11880433.
- Ahmed AOA, van Vianen W, ten Kate M, van de Sande WWJ, Van Belkum A, Fahal AH, Verbrugh HA, Bakker-Woudenberg IAJM. A murine model of *Madurella mycetomatis* eumycetoma. *FEMS Immunol Med Microbiol.* 2003; 37: 29-36. PMID: 12770757..
- Ahmed AOA, van de Sande W, Van Belkum A, Verbrugh HA, Fahal AH, van Belkum A. *Madurella mycetomatis* strains from mycetoma lesions in Sudanese patients are clonal. *J Clin Microbiol.* 2003; 41(10):4537-41 PMID: 14532179.
- Abd Bagi ME, Fahal AH, Sheikh HE, Abdul Wahab O, Taifoor MK, Osman EM. High incidence of pathological fractures in mycetoma. *Trans R Soc Trop Med Hyg.* 2003; 97(5):582-4. PMID: 15307432
- Fahal AH. Mycetoma thorn on the flesh: Review article. *Trans R Soc Trop Med Hyg.* 2004; 98(1):3-11. Review.
- Ahmed AO, van de Sande WW, van Vianen W, van Belkum A, Fahal AH, Verbrugh HA, Bakker-Woudenberg IA. In vitro susceptibilities of *Madurella mycetomatis* to itraconazole and amphotericin B assessed by a modified NCCLS method and a viability-based 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) assay. *Antimicrob Agents Chemother.* 2004; 48(7):2742-6. PMID: 15215141.
- Ahmed AO, van Leeuwen W, Fahal A, van de Sande W, Verbrugh H, van Belkum A. Mycetoma caused by *Madurella mycetomatis*: a neglected infectious burden. *Lancet Infect Dis.* 2004; 4(9):566-74. Review. PMID: 15336224
- EL Hassan AM, Fahal AH, Veress B. Cell phenotypes, immunoglobulins,

and complement in lesion of eumycetoma caused by *Madurella mycetomatis*. *Sud J Dermato* 2006; 4 (1):2-5.

- van de Sande WW, Fahal A, Verbrugh H, van Belkum A. Polymorphisms in genes involved in innate immunity predispose toward mycetoma susceptibility. *J Immunol.* 2007; 179(5):3065-74. PMID: 17709521.
- van de Sande WW, de Kat J, Coppens J, Ahmed AO, Fahal A, Verbrugh H, van Belkum A. Melanin biosynthesis in *Madurella mycetomatis* and its effect on susceptibility to itraconazole and ketoconazole. *Microbes Infect.* 2007; 9: 1114-1123. PMID: 17644456.
- van de Sande WW, Fahal A, Verbrugh H, van Belkum A. *Madurella mycetomatis* compounds cross-reactive with galactomannan are detectable in culture supernatant but not in serum. *J Med Microbiol.* 2007; 56: 869-70. PMID: 17510278.
- Ahmed AA, van de Sande WW, Fahal A, Bakker-Woudenberg I, Verbrugh H, van Belkum A. Management of mycetoma: major challenge in tropical mycoses with limited international recognition. *Curr Opin Infect Dis.* 2007; 20(2):146-51. PMID: 17496572.
- van de Sande WW, Fahal AH, Riley TV, Verbrugh H, van Belkum A. In vitro susceptibility of *Madurella mycetomatis*, prime agent of Madura foot, to tea tree oil and artemisinin. *J Antimicrob Chemother.* 2007; 59(3):553-5 PMID: 17324961. .
- Hussein A, Ahmed AEM, Fahal AH, Mahmoud A, Sidig A, Awad K, Ahmed EM. Cervical cord compression secondary to mycetoma infection. *Sud J Pub Health.* 2007; 2(2): 112-115.
- Quintana ET, Wierzbicka K, Mackiewicz P, Osman A, Fahal AH, Hamid ME, Zakrzewska-Czerwinska J, Maldonado LA, Goodfellow M. *Streptomyces sudanensis* sp. nov., a new pathogen isolated from patients with actinomycetoma. *Antonie Van Leeuwenhoek.* 2008; 93(3):305-13. PMID: 18157699.
- Dejan Stojković, Marina Soković, Ana Džamić, Mihailo Ristić, Ahmed Fahal, Silvana Petrović, Ivana Djujić, Sami Khalid. In vitro Susceptibility

of *Actinomadura madurae* Clinical Isolates to Turpentine Oil of *Pinus pinaster* and  $\alpha$ -Pinene. Arch. Biol. Sci., Belgrade. 2008; 60 (4): 697-701.

- Abd El-Bagi MEB, Fahal AH. Mycetoma revisited. Incidence of various radiographic signs. Saudi Med J. 2009;30(4):529-33. PMID: 19370281.
- Yousif BM, Fahal AH, Yahia MY. The Cell Block Technique: A New Diagnostic Tool for Mycetoma. Trans R Soc Trop Med Hyg. 2010; 104(1):6-9.
- Fahal AH, Abu Sabaa. AH. Mycetoma in Children. Trans R Soc Trop Med Hyg. 2010; 104: 117–12. PMID: 19716573.
- Fahal, AH. Management of mycetoma. Expert Rev Dermatol. 2010; 5(1), 87–93.
- van de Sande WWJ, Fahal AH, Tavakol M, van Belkum A. Polymorphisms in catechol-O-methyltransferase and cytochrome p450 sub-family 19 genes predispose towards *Madurella mycetomatis*-induced mycetoma susceptibility. Med Mycol. 26.2.2010; 48(7):959-68. PMID: 20184498
- van de Sande WW, Fahal AH, Bakker-Woudenberg IA, van Belkum A. *Madurella Mycetomatis* is not susceptible to the Echinocandin class of antifungal agents. Antimicrob Agents Chemother. 2010 June, 54(6):2738-2740. PMID: 20350944
- Fahal AH. Continuing Professional Development in Medical & Health Professions. International J Sud Research, 2010; 1(1): 29-34.
- Fahal AH, Rahman I A, El-Hassan AM, EL Rahman ME, Zijlstra EE. The efficacy of itraconazole in the treatment of patients with eumycetoma due to *Madurella mycetomatis*. Trans R Soc Trop Med Hyg. 2011; 105(3):127-32.
- Alex van Belkum, Ahmed H. Fahal, Wendy W.J van de Sande. In Vitro Susceptibility of *Madurella Mycetomatis* to Posaconazole and Terbinafine. Antimicrob Agents Chemother. 2011; 55(4):1771-3.
- Fahal AH, EL Sheik H, El Hassan AM. Venous Varicosity in Mycetoma. Sudan Med J. 2011; 47(1): 20-24.

- Fahal AH. Mycetoma. Review article, Khartoum Med J. 2011; 4(1): 514-523.
- de Klerk N, de Vogel C, Fahal AH, van Belkum A, van de Sande W. Fructose-bisphosphate aldolase and pyruvate kinase, two novel immunogens in madurella mycetomatis. Med Mycol. 2012; 50(2):143-51.
- Kirby R, Sangal V, Tucker NP, Zakrzewska-Czerwinska J, Wierzbicka K, Herron PR, Chu CJ, Chandra G, Fahal AH, Goodfellow M, Hoskisson PA. Draft genome sequence of the human pathogen *Streptomyces somaliensis*, a significant cause of actinomycetoma. J Bacteriol. 2012 Jul;194(13):3544-5. doi: 10.1128/JB.00534-12. PMID: 22689234; PMCID: PMC3434723.
- Zein HAM, Fahal AH, Mahgoub ES, EL Hassan T, Abdel Rahman ME. The Predictors of Cure, Amputation & Follow-up dropout among Mycetoma Patients as seen at The Mycetoma Research Centre, University of Khartoum. Trans R Soc Trop Med Hyg. 2012; 106(11):639-44.
- Mhmoud NA, Ahmed SA, Fahal AH, de Hoog GS, Gerrits van den Ende AH, van de Sande WW. *Pleurostomophora ochracea*, a novel agent of human eumycetoma with yellow grains. J Clin Microbiol. 2012; 50(9):2987-94
- Mhmoud NA, Fahal AH, van de Sande WW. CD4+ T-lymphocytopenia in HIV-negative tuberculosis patients in Sudan. J Infect. 2012; 65(4):370-2.
- de Hoog GS, van Diepeningen AD, Mahgoub el-S, van de Sande WW. New species of *Madurella*, causative agents of black-grain mycetoma. J Clin Microbiol. 2012; 50(3):988-94.
- Fahal AH, Arbab MAR, EL Hassan AM. Aggressive Clinical Presentation of Mycetoma due to *Actinomyces pelletierii*. Khartoum Med J. 2012; 5(1): 699-702.
- Mohamed, EW, Suleiman HS, Fadella, AI, Fahal, AH. Aggressive Perineal and Pelvic Eumycetoma: An unusual and challenging problem to treat. Khartoum Med J. 2012; (5)2:771-774.

- Kloezen W, Meis JF, Curfs-Breuker I, Fahal AH, van de Sande WW. In vitro antifungal activity of isavuconazole against *Madurella mycetomatis*. *Antimicrob Agents Chemother*. 2012 Nov;56(11):6054-6. doi: 10.1128/AAC.01170-12. Epub 2012 Sep 10. PMID: 22964246; PMCID: PMC3486573.
- Mohamed EW, Mohamed, ENA, Yousif, BM, Fahal, AH. Tongue Actinomycetoma due to *Actinomyces madurae*: A rare clinical presentation. *J Oral Maxillofac Surg*. 2012; 70(11):e622-4.
- El Shamy, ME, Fahal AH, Shakir MY, Homedia MMA. New MRI Grading System for the Diagnosis and Management of Mycetoma. *Trans R Soc Trop Med Hyg*. 2012; 106(12):738-42.
- Fahal AH, van de Sande WW. The Epidemiology of mycetoma. *Current Fungal Infection Reports*. 2012; 6( 4): 320-326
- Mhmoud NA, Fahal AH, van de Sande WW. The association between the interleukin-10 cytokine and CC chemokine ligand 5 polymorphisms and mycetoma granuloma formation. *Med Mycol*. 2013; 51(5):527-33. doi: 10.3109/13693786.2012.745201
- Fahal, AH, The Mycetoma Research Centre, University of Khartoum, Sudan: a success story that needs support. *IJSR*. 2013 3(1): 1-13.
- Ibrahim AI, El Hassan AM, Fahal A, van de Sande WW. A histopathological exploration of the *Madurella mycetomatis* grain. *PLoS One*. 2013; 8(3):e57774. doi: 10.1371/journal.pone.0057774.
- Suleiman AM, Fahal AH. Oral cavity eumycetoma: a rare and unusual condition. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2013; 115(4): e23-5.
- Ehab AM Elagab, Maowia M Mukhtar, Ahmed H Fahal, Wendy WJ van de Sande. Peripheral Blood Mononuclear Cells of Mycetoma Patients React Differently to *Madurella mycetomatis* Antigens than Healthy Endemic Controls. *PLoS Negl Trop Dis*. 2013; 7(4): e2081. doi: 10.1371/journal.pntd.0002081.

- Suleiman HS, EL Hassan LAM, Fahal AH. Long-Standing Gluteal Mass: An Uncommon Presentation of Tuberculosis. *Khartoum Med J* 2013; 6(1): 864–867.
- Osman AH, Ezaldeen EA, Ali BM, Jacoub AO, Suleiman SH, Fahal AH. Poor Infusion Therapy Knowledge among Young Doctors in Two Tertiary Teaching Hospitals in Khartoum State. *Khartoum Med J* 2013; 6(1): 854–858.
- van Hellemond JJ, Vonk AG, de Vogel C, Koelewijn R, Vaessen N, Fahal AH, van Belkum A, van de Sande WW. Association of eumycetoma and schistosomiasis. *PLoS Negl Trop Dis*. 2013 May 23;7(5):e2241. doi: 10.1371/journal.pntd.0002241.
- van Belkum A, Fahal A, van de Sande WW. Mycetoma caused by *Madurella mycetomatis*: a completely neglected medico-social dilemma. *Adv Exp Med Biol*. 2013; 764:179-89.
- Fahal AH, Elkhawad AO. Managing mycetoma: guidelines for best practice. *Expert Rev Dermatol* 2013; 8(3): 301-307.
- GS de Hoog, S. Abdalla Ahmed, MJ Najafzadeh, DA Sutton, M. Saradeghi Keisari, AH Fahal, U. Eberhart, B. Stielow, GJ Verkley, Lian Xin, WWJ van de Sande. Phylogenetic findings suggest possible new habitats and routes of infection of human eumycetoma. *PLoS Negl Trop Dis*. 2013 May 16; 7(5):e2229. doi: 10.1371/journal.pntd.0002229.
- Najwa A. Mhmoud, Ahmed H. Fahal, Wendy W. J. Van De Sande. The association between the interleukin-10 cytokine and CC chemokine ligand 5 polymorphisms and mycetoma granuloma formation. *Med Mycol* 2013, 51, 527–533.
- Mhmoud N, Fahal A, Wendy van de Sande WJ. Polymorphisms of Interleukin-10 and CCL5 Genes in Tuberculosis. *Trop Med Int Health*. 2013; 18(9): 1119-1127. doi:10.1111/tmi.12141
- Ezaldeen EA, Fahal AH, Osman A. Mycetoma herbal treatment: the Mycetoma Research Centre, Sudan experience. *PLoS Negl Trop Dis*. 2013 Aug 22;7(8):e2400. doi: 10.1371/journal.pntd.0002400. e Collec-

tion 2013.

- Khamis, AAG, Ali BM, Jacoub AO, Suleiman SH, Fahal AH, Knowledge, Attitudes and Practices of Surgical Staff at Khartoum State towards the Hepatitis B virus infection. *Khartoum Med J* 2014;7(1): 932- 940.
- Fahal AH, Shaheen S. Jones DH. Orthopaedic Aspects of Mycetoma: Instructional Review. *Bone Joint J* 2014; 96-B: 420–5. doi: 10.1302/0301-620X.96B3.31421.
- Al Dawi AF, Mustafa MI, Fahal AH, EL Hassan AM, Bakhiet SM, Mahgoub ES, Mergani A, Mukhtar MM. The Association of HLA-DRB1 & HLA-DQB1 and the occurrence of Eumycetoma. *Khartoum Med J.* 2013; 6(3): 923 - 929
- Geneugelij K, Kloezen W, Fahal AH, van de Sande WWJ. Active matrix metalloprotease-9 is associated with the collagen capsule surrounding the *M. mycetomatis* grain in mycetoma. *PLoS Negl Trop Dis* Published: March 27, 2014 DOI: 10.1371/journal.pntd.0002754.
- Wendy W. J. van de Sande, El Sheikh Maghoub, Ahmed H. Fahal, Michael Goodfellow, Oliverio Welsh, Ed Zijlstra. The Mycetoma Knowledge Gap: Identification of Research Priorities. *PLoS Negl Trop Dis* Published: March 27, 2014 DOI: 10.1371/journal.pntd.0002667.
- Fahal AH, Mahgoub ES, EL Hassan ESM, EL Tayeb E, EL Hassan AM. Eye Eumycetoma: The Mycetoma Research Centre, Sudan Case Series & Literature Review. *Infectious Diseases in Clinical Practice* 6 June 2014, doi: 10.1097/IPC.0000000000000157.
- Sarah Abdalla Ahmed, Wendy Kloezen, Frederick Duncanson, Ed Zijlstra, G. Sybren de Hoog, Ahmed H. Fahal, Wendy WJ van de Sande. *Madurella mycetomatis* is highly susceptible towards Ravuconazole. *PLoS Negl Trop Dis* June 19, 2014 DOI: 10.1371/journal.pntd.0002942
- Najwa A Mhmoud, Ahmed H Fahal, El Sheikh Mahgoub, Wendy W J van de Sande. The combination of Amoxicillin-clavulanic acid and ketoconazole proved to be effective and safe for treatment of *Madurella mycetomatis* eumycetoma patients with secondary *Staphylococcus*

aureus infection. PLoS Negl Trop Dis June 19, 2014 DOI: 10.1371/journal.pntd.0002959.

- Wendy W. J. van de Sande, Ahmed H. Fahal, Michael Goodfellow, El Sheikh Maghoub, Oliverio Welsh, Ed Zijlstra. The merits and pitfalls of the currently used diagnostic tools in mycetoma. PLoS Negl Trop Dis July 03, 2014 DOI: 10.1371/journal.pntd.0002918
- Ahmed SA, van de Sande WJ, Stevens DA , Fahal AH, van Diepeningen A, Menken SBJ, and de Hoog GS. Revision of agents of black-grain eumycetoma in the order Pleosporales. Persoonia 2014; 33: 141–154.
- Samy AM, van de Sande WWJ, Fahal AH, Peterson AT. Mapping the Potential Risk of Mycetoma Infection in Sudan and South Sudan Using Ecological Niche Modeling. PLoS Negl Trop Dis 2014; 8(10): e3250. doi:10.1371/journal.pntd.0003250
- Welsh O, Al-Abdely HM, Salinas-Carmona MC, Fahal AH (2014) Mycetoma Medical Therapy. PLoS Negl Trop Dis 8(10): e3218. doi:10.1371/journal.pntd.0003218. <http://www.plosntds.org/article/info:doi/10.1371/journal.pntd.0003218>
- Fahal A, Mahgoub ES, EL Hassan AM, Abdel-Rahman ME, Alshambaty Y, Ed E Zijlstra. A New Model for Management of Mycetoma in the Sudan. PLoS Negl Trop Dis 2014, 8(10): e3271. doi:10.1371/journal.pntd.0003271
- Fahal AH. Mycetoma Comm Dermato J. 2014; 10: 15-17.
- Sarah A. Ahmed, Bert Gerrits van den Ende, Ahmed H. Fahal, Wendy W. J. van de Sande, GS. de Hoog. Rapid Identification of Black Grain Eumycetoma causative agents Using Rolling Circle Amplification. PLoS Negl Trop Dis. 2014 Dec 4;8(12):e3368. doi: 10.1371/journal.pntd.0003368. eCollection 2014 Dec.
- Ahmed AK, SH Suleiman, Fahal AH. Aggressive Uncontrolled Gluteal Eumycetoma Invading the Pelvic Cavity and the Spinal Cord: Unusual Clinical Presentation. Khartoum Med J. 2014; 7(2): 1005–1007.

- Kimberly Eadie, Ahmed Fahal, Wendy WJ van de Sande. In vitro activity of antiseptic solutions against *M. mycetomatis*, implications on eumycetoma management. *Br J Dermatol*. 2015;172(6):1657-9. doi: 10.1111/bjd.13570
- Ahmed SA, Kloezen W, Fahal AH, de Hoog GS, van de Sande WW. In Vitro Interaction of Currently Used Azoles with Terbinafine Against *Madurella mycetomatis*. *Antimicrob Agents Chemother*. 2015 Feb;59(2):1373-4. doi: 10.1128/AAC.04487-
- Ahmed SA, de Hoog GS, Stevens DA, Fahal AH and van de Sande WJ. "In Vitro Antifungal Susceptibility of Coelomycete Agents of Black Grain Eumycetoma to Eight Antifungals. *Med Mycol*. 2015; 53(3):295-301. doi: 10.1093/mmy/myu098
- Hay RJ, Fahal AH. Mycetoma: an old and still neglected tropical disease. *Trans R Soc Trop Med Hyg*. 2015; 109(3):169-70. doi:10.1093/trstmh/trv003.
- Ahmed SA, van de Sande WW, Stevens DA, Fahal A, van Diepeningen AD, Menken SB, de Hoog GS. Revision of agents of black-grain eumycetoma in the order Pleosporales. *Persoonia*. 2014 Dec; 33:141-54. doi: 10.3767/003158514X684744.
- Nenoff P, van de Sande WW, Fahal AH, Reinelt D, Schöfer H. Eumycetoma and actinomycetoma - an update on causative agents, epidemiology, pathogenesis, diagnostics and therapy. *J Eur Acad Dermatol Venereol*. 2015; 29(10):1873-83. doi: 10.1111/jdv.13008
- Ahmed SA, de Hoog GS, Stevens DA, Fahal AH, van de Sande WW. In vitro antifungal susceptibility of coelomycete agents of black grain eumycetoma to eight antifungals. *Med Mycol*. 2015; 53(3):295-301. doi: 10.1093/mmy/myu098.
- Ahmed SA, Kloezen W, Fahal AH, de Hoog GS, van de Sande WW. In Vitro Interaction of Currently Used Azoles with Terbinafine against *Madurella mycetomatis*. *Antimicrob Agents Chemother*. 2015 Feb; 59(2):1373-4. doi: 10.1128/AAC.04487-14.

- Fahal A, Mahgoub ES, EL Hassan AM, Jacoub AO, Hassan D. Head and Neck Mycetoma: The Mycetoma Research Centre Experience. *PLoS Negl Trop Dis* (2015) 9(3): e0003587. doi:10.1371/journal.pntd.0003587
- Elfadil H, Fahal A, Kloezen W, Ahmed EM, van de Sande W. The In Vitro Antifungal Activity of Sudanese Medicinal Plants against *Madurella mycetomatis*, the *Eumycetoma* Major Causative Agent. *PLoS Negl Trop Dis* (2015) 9(3): e0003488. doi:10.1371/journal.pntd.0003488
- Fahal AH, EL Hassan AM, Mahgoub ES, Rahman ME, Mycetoma in the Sudan: The Mycetoma Research Centre Update. *PLoS Negl Trop Dis*. 2015 Mar 27; 9(3):e0003679. doi: 10.1371/journal.pntd.0003679. eCollection 2015 Mar.
- Hala Taha, Ahmed Fahal, Wendy W. J. van de Sande. Mycetoma: Epidemiology, Treatment Challenges and Progress. *Mycetoma: epidemiology, treatment challenges and progress*. *Res Rep Trop Med*. 2015;6 31–36
- Wendy WJ van de Sande, Marian ten Kate, Wim van Vianen, Ahmed Fahal, Irma Bakker-Woudenberg. Amphotericin B but not itraconazole is able to prevent grain formation in experimental *M. mycetomatis* mycetoma in mice. *Br J Dermatol*. 2015 Jul 6. doi: 10.1111/bjd.14025.
- Kloezen W, van Helvert-van Poppel M, Fahal AH, van de Sande WWJ. A *Madurella mycetomatis* Grain Model in *Galleria mellonella* Larvae. *PLoS Negl Trop Dis* 2015; 9(7): e0003926. doi:10.1371/journal.pntd.0003926
- Eshraga A. Ezaldeen, Raif Mohamed Ahmed, EL Sammani Wadella, Nadia EL Dawi, Ahmed Hassan Fahal. Actinomycetoma induced Cervical Spinal Cord Compression: A rare and serious complication. *JMM Case Reports*. 2015; DOI 10.1099/jmmcr. 0.000074.
- Ahmed SA, van de Sande WW, Desnos-Ollivier M, Fahal AH, Mhmoud NA, de Hoog GS. Application of Isothermal Amplification Techniques for the Identification of *Madurella mycetomatis*, the Prevalent Agent of Human Mycetoma. *J Clin Microbiol*. 2015; 53(10):3280-5. doi: 10.1128/

JCM.01544-15.

- Verwer PEB, Notenboom CC, Eadie K, Fahal AH, Verbrugh HA, van de Sande WWJ. A polymorphism in the chitotriosidase gene associated with risk of mycetoma due to *Madurella mycetomatis* - a retrospective study. *PLoS Negl Trop Dis*. 2015 Sep 2;9(9):e0004061. doi: 10.1371/journal.pntd.0004061. eCollection 2015.
- Najwa MA, Badr M Yousif, Ahmed Fahal, Wendy van de Sande. The nature of the *M. mycetomatis* mycetoma granuloma tissue pigments. *Khartoum Med J*. 2015; 8 (2): 1105 – 1110.
- Ahmed Hassan Fahal. Aggressive actinomycetoma. *Khartoum Med J*. 2015; 8 (2): 1116.
- Emmanuel E. Siddig, Aghapy E. Siddig, Ali Edreis, Ahmed Omer Almo-barak, Ahmed H Fahal. The Utility of Fine Needle Aspiration for cytology for the Diagnosis of Breast Lesions in low-resource Areas. *Khartoum Med J*. 2015; 8(3):1153-1157.
- Emmanuel Edward Siddig, Najwa A. Mhmoud, Ahmed Hassan Fahal. Identification of *M. mycetomatis* fungus in pleural fluid and sputum of a patient with aggressive gluteal eumycetoma with pulmonary spread. *Khartoum Med J* 2015; 8(3): 1164-1167.
- EE Zijlstra, WWJ van de Sande, AH Fahal. Mycetoma, Mycetoma: A Long Journey from Neglect. Editorial, *PLoS NTD*, DOI:10.1371/journal.pntd.0004244, January 21, 2016.
- Zijlstra EE, van de Sande WW, Welsh O, Mahgoub ES, Goodfellow M, Fahal AH. Mycetoma. *Lancet Infect Dis*. 2016; 16(1):100-112. doi: 10.1016/S1473-3099(15)00359-X.
- Scolding PS, Abbas MAQ, Omer RF, Fahal AH. *Madurella mycetomatis*-Induced Massive Shoulder Joint Destruction: A Management Challenge. *PLoS Negl Trop Dis* 2016; 10(8): e0004849. doi:10.1371/journal.pntd.0004849
- Omer RF, Seif EL Din N, Abdel Rahim FA, Fahal AH. Hand Mycetoma:

The Mycetoma Research Centre Experience and Literature Review. PLoS Negl Trop Dis 2016;10(8): e0004886. doi:10.1371/journal.pntd.0004886

- Mohamed EISW, Seif El Din N, Fahal AH. Multiple Mycetoma Lung Secondaries from Knee Eumycetoma: An Unusual Complication. PLoS Negl Trop Dis. 2016 Jul 21; 10(7):e0004735. doi: 10.1371/journal.pntd.0004735. eCollection 2016.
- Fahal AH, Finkelman MA, Zhang Y, Van de Sande WW. Detection of (1→3)-β-D-glucan in eumycetoma patients. J Clin Microbiol. 2016;54(10):2614-7. doi: 10.1128/JCM.01478-16
- Nasr A, Abushouk A, Hamza A, Siddig E, Fahal AH. Th-1, Th-2 Cytokines Profile among *Madurella mycetomatis* Eumycetoma Patients. PLoS Negl Trop Dis. 2016 Jul 19; 10(7):e0004862. doi: 10.1371/journal.pntd.0004862. eCollection 2016.
- Suleiman SH, Wadaella el S, Fahal AH. The Surgical Treatment of Mycetoma. PLoS Negl Trop Dis. 2016 Jun 23; 10(6):e0004690. doi: 10.1371/journal.pntd.0004690. eCollection 2016
- Smit S, Derks MF, Bervoets S, Fahal A, van Leeuwen W, van Belkum A, van de Sande WW. Genome Sequence of *Madurella mycetomatis* mm55, Isolated from a Human Mycetoma Case in Sudan. Genome Announc. 2016 May 26;4(3). pii: e00418-16. doi: 10.1128/genomeA.00418-16
- ELbadawi HS, Mahgoub E, Mahmoud N, Fahal AH. Use of immunoblotting in testing *Madurella mycetomatis* specific antigen. Trans R Soc Trop Med Hyg. 2016 May;110(5):312-6. doi: 10.1093/trstmh/trw023.
- Emmanuel E. Siddig, Badr EL Din M. Yousif, Ali M. Edris, Ahmed H. Fahal. The Touch Imprint Cytology: A simple method for the diagnosis of mycetoma causative agents. Khartoum Med J. 2016; 9(1):1212-1216
- Wadal A, Elhassan TA, Zein HA, Abdel-Rahman ME, Fahal AH. Predictors of Post-operative Mycetoma Recurrence Using Machine-Learning Algorithms: The Mycetoma Research Center Experience. PLoS Negl

Trop Dis. 2016; 10(10): e0005007. doi:10.1371/journal.pntd.0005007

- Mohamed NA, Fahal AH. Mycetoma Pulmonary Secondaries from a Gluteal Eumycetoma: An Unusual Presentation. PLoS Negl Trop Dis. 2016; 10(10): e0004945. doi:10.1371/journal.pntd.0004945.
- Mitjà O, Marks M, Bertran L, Kollie K, Argaw D, Fahal AH, Fitzpatrick C, Claire Fuller L, Izquierdo BG, Hay R, Ishii N, Johnson C, Lazarus JV, Meka A, Murdoch M, Ohene SA, Smal PI, Andrew Steer A, Tabah EN, Tiendrebeogo A, Lance Waller L, Yotsu R, Walker SL, Asiedu K. Integrated Control and Management of Neglected Tropical Skin Diseases. PLoS Negl Trop Dis 2017, 11(1): e0005136. Doi
- Fahal AH. Mycetoma: A global medical and socio-economic dilemma. PLoS Negl Trop Dis 2017; 11(4): e0005509. <https://doi.org/10.1371/journal.pntd.0005509>
- Eadie K, Parel F, Helvert-van Poppel M, Fahal A, van de Sande W. Combining two antifungal agents does not enhance survival of *Galleria mellonella* larvae infected with *Madurella mycetomatis*. Trop Med Int Health. 2017 Mar 25. doi: 10.1111/tmi.12871.
- Ahmed AA, van de Sande W, Fahal AH. Mycetoma laboratory diagnosis: Review article. PLoS Negl Trop Dis. 2017; 11(8): e0005638. <https://doi.org/10.1371/journal.pntd.0005638>
- Siddig EE, Fahal AH. Histopathological Approach for the Diagnosis of Mycetoma Causative Agents: A Mini Review. J Cytol Histol. 2017; 8: 466. doi: 10.4172/2157-7099.1000466.
- Saad ESA, Fahal AH. Broncho-pleuro-cutaneous fistula and pneumothorax: Rare challenging complications of chest wall eumycetoma. PLoS Negl Trop Dis 2017; 11(9): e0005737. <https://doi.org/10.1371/journal.pntd.0005737>
- Ahmed HM Ibrahim, Ali Mohamed Osman, Ahmed H Fahal, Alnada A M Ibrahim. The use of a documentary drama film to improve the knowledge, attitude and practice of an endemic village population towards mycetoma at Sennar State, Sudan. Khartoum Med J. 2017;

10(2):1385 – 1391

- Queiroz-Telles F, Fahal AH, Falci DR, Caceres DH, Chiller T, Pasqua-lotto AC. Neglected endemic mycoses. *Lancet Infect Dis.* 2017; 17(11):e367-e377. doi: 10.1016/S1473-3099(17)30306-7. Epub 2017 Jul 31.
- Kloezen W, Parel F, Brüggemann R, Asouit K, Helvert-van Poppel M, Fahal A, Mouton J, van de Sande W. Amphotericin B and terbinafine but not the azoles prolong survival in *Galleria mellonella* larvae infected with *Madurella mycetomatis*. *Med Mycol.* 2018; 1;56(4):469-478. doi: 10.1093/mmy/myx064.
- van de Sande W, Fahal A, Ahmed SA, Serrano JA, Bonifaz A, Zijlstra E. Closing the mycetoma knowledge gap. *Med Mycol.* 2018 Apr 1; 56(suppl\_1):153-164. doi: 10.1093/mmy/myx061.PMID:28992217
- Bakhiet SM, Fahal AH, Musa AM, Mohamed ELSW, Omer RF, Ahmed ES, El Nour M, Mustafa ERM, Sheikh A Rahman ME, Suliman SH, El Mamoun MAG, El Amin HM. A holistic approach to the mycetoma management. *PLoS Negl Trop Dis.* 2018; 12(5):e0006391. <https://doi.org/10.1371/journal.pntd.0006391>
- Abdallah MOE, Algizouli UK, Suliman MA, Abdulrahman RA, Koko M, Fessahaye G, Shakir JH, Fahal AH, Elhassan AM, Ibrahim ME Mohamed. HSEBV-Associated Breast Cancer Whole Methylome Analysis Reveals Viral and Developmental Enriched Pathways. *Front. Oncol.* 2018; 8:316. doi: 10.3389/fonc.2018.00316
- Lim W, Eadie K, Horst-Kreft D, Ahmed SA, Fahal AH, van de Sande WWJ. VNTR confirms *Madurella mycetomatis* heterogeneity and is a promising typing tool for *Madurella mycetomatis*. *Med Mycol.* 2018 Jul 31. doi: 10.1093/mmy/myy055.
- Bakhiet SM, Edwar Siddig EE, Abdallah OB, Mohamed SW, Fahal AH. Fine Needle Aspiration Cytology Utility in the Identification of Mycetoma Causative Agents: The Mycetoma Research Center Experience. *Khartoum Med J.* 2018; 11(0): 1499 – 1506.

- Nyuykonge B, Lim W, Fahal AH, van de Sande WWJ. Mycetoma. Dutch Journal of Infectious Diseases. 2018; 13(6): 143-150.
- Fahal AH, Suliman SH, Hay R. Mycetoma – The Spectrum of Clinical Presentation. Trop. Med. Infect. Dis. 2018, 3, 97; doi:10.3390/tropicalmed 3030097
- Abbas M, Scolding PS, Yosif AA, EL Rahman RF, EL-Amin MO, Elbashir MK, Groce N, Fahal AH. The disabling consequences of Mycetoma. PLoS Negl Trop Dis. 2018; 12(12): e0007019. <https://doi.org/10.1371/journal.pntd.0007019>
- Bakhiet SM, Mohamed SW, Najwa A Mhmoud, Siddig EE, Ibrahim DM, Ahmed ES, Fahal AH. Madurella mycetomatis causing Multiple mycetoma lesions: A rare presentation. Khartoum Med J. 2018: 11(0): 1499 – 1506.
- Suleiman SH, Yosif AA, Bakhiet SM, Fahal AH. Massive progressive scroto-perineal eumycetoma and its therapeutic challenges. Khartoum Med J. 2019; 12(2): 1622 - 1627
- Mhmoud NA, Santona A, Fiamma M, Siddig EE, Deligios M, Bakhiet SM, et al. Chaetomium atrobrunneum causing human eumycetoma: The first report. PLoS Negl Trop Dis. 2019; 13(5): e0007276. <https://doi.org/10.1371/journal.pntd.0007276>
- Siddig EE, Mohammed Edris AM, Bakhiet SM, van de Sande WWJ, Fahal AH. Interleukin-17 and matrix metalloprotease-9 expression in the mycetoma granuloma. PLoS Negl Trop Dis. 2019; 13(7): e0007351. <https://doi.org/10.1371/journal.pntd.0007351>
- Abushouk A, Nasr A, Masuadi E, Allam G, Siddig EE, Fahal AH. The Role of Interleukin-1 cytokine family (IL-1 $\beta$ , IL-37) and interleukin-12 cytokine family (IL-12, IL-35) in eumycetoma infection pathogenesis. PLoS Negl Trop Dis. 2019; 13(4): e0007098. <https://doi.org/10.1371/journal.pntd.0007098>
- Siddig EE, Mhmoud NA, Bakhiet SM, Abdallah OB, Mekki SO, El Dawi NI, Van de Sande W, Fahal AH. The Accuracy of Histopathological and

Cytopathological Techniques in the Identification of the Mycetoma Causative Agents. *PLoS Negl Trop Dis*. 2019 Aug 29;13(8): e0007056. doi: 10.1371/journal.pntd.0007056. [Epub ahead of print]

- Nyuykonge B, Croughs P, Fahal AH, Verbon A, van de Sande WWJ. Pyomelanin Secretion in *Madurella mycetomatis* Interferes with Spectrophotometrically End-point Reading using the Sensititre® YeastOne Alamar Blue Assay but not with Visual End-point Reading. *Antimicrob Agents Chemother*. 2019 Oct 14. pii: AAC.01532-19. doi: 10.1128/AAC.01532-19.
- Hay R, Denning DW, Bonifaz A, Queiroz-Telles F, Beer K, Bustamante B, Chakrabarti A, Chavez-Lopez MG, Chiller T, Cornet M, Estrada R, Estrada-Chavez G, Fahal A, Gomez BL, Li R, Mahabeer Y, Mosam A, Soavina Ramarozatovo L, Rakoto Andrianarivelo M, Rapelanoro Rabenja F, van de Sande W, Zijlstra EE. The Diagnosis of Fungal Neglected Tropical Diseases (Fungal NTDs) and the Role of Investigation and Laboratory Tests: An Expert Consensus Report. *Trop Med Infect Dis*. 2019 Sep 24;4(4). pii: E122. doi: 10.3390/tropicalmed4040122.
- Amir Arastehfar, Wilson Lim, Farnaz Daneshnia, Wendy WJ van de Sande, Ahmed H Fahal, Marie Desnos-Ollivier, Gerrit S de Hoog, Teun Boekhout, Sarah A Ahmed. *Madurella* Real-Time PCR, a Novel Approach for *Eumycetoma* Diagnosis *PLoS Negl Trop Dis*. 2020 Jan 15[Online ahead of print] PMID: 31940343 DOI: 10.1371/journal.pntd.0007845
- Rita O Oladele, Iorhen E Akase, Ahmed H Fahal, Nelesh P Govender, Martin Hoenigl, Jean Pierre Gangneux, Tom M Chiller, David W Denning, Oliver A Cornely, Arunaloche Chakrabarti. Bridging the Knowledge Gap on Mycoses in Africa: Setting Up a Pan-African Mycology Working Group. *Mycoses*. 2020 Mar;63(3):244-249. doi: 10.1111/myc.13044. Epub 2019 Dec 30.
- Hussein HS, Bakhiet SM, Fahal AH. The late presentation of mycetoma patients: the challenges. *Khartoum Med J*. 2018; 13(01): 1681 – 1687.
- Wilson Lim, Kimberly Eadie, Mickey Konings, Bart Rijnders, Ahmed

H. Fahal, Jason D. Oliver, Mike Birch, Annelies Verbon, Wendy van de Sande. The eumycetoma causative agent *Madurella mycetomatis* is highly susceptible towards olorofim. *J Antimicrob Chemother* 2020; 75: 936–941 doi:10.1093/jac/dkz529.

- Khidir ES, Ahmed A, Fahal AH, Ibrahim AA. Draft Genome Sequences of Three Clinical Isolates of *Madurella mycetomatis*, the Major Cause of Black-Grain Mycetoma. *Microbiol Resour Announc*. 2020 Apr 16;9(16). pii: e01533-19. doi: 10.1128/MRA.01533-19.
- Sheehan G, Konings M, Lim W, Fahal A, Kavanagh K, van de Sande WWJ. Proteomic analysis of the processes leading to *Madurella mycetomatis* grain formation in *Galleria mellonella* larvae. *PLoS Negl Trop Dis*. 2020 Apr 8;14(4):e0008190. doi: 10.1371/journal.pntd.0008190. eCollection 2020 Apr.
- Ali RS, Newport MJ, Bakhiet SM, Ibrahim ME, Fahal AH. Host genetic susceptibility to mycetoma. *PLoS Negl Trop Dis*. 2020; 14(4): e0008053. <https://doi.org/10.1371/journal.pntd.0008053>.
- Kwizera R, Bongomin F, Meya DB, Denning DW, Fahal AH, Lukande R. Mycetoma in Uganda: A neglected tropical disease. *PLoS Negl Trop Dis*. 2020; 14(4): e0008240. <https://doi.org/10.1371/journal.pntd.0008240>
- Elkheir LYM, Haroun R, Mohamed MA, Fahal AH. *Madurella mycetomatis* causing eumycetoma medical treatment: The challenges and prospects. *PLoS Negl Trop Dis*. 2020; 14(8): e0008307. <https://doi.org/10.1371/journal.pntd.0008307>
- Kunna E, Yamamoto T, Fahal A. The use of traditional medicines among mycetoma patients. *Trans R Soc Trop Med Hyg*. 2021 Apr 14;115(4):297-306.
- Zaman S, Nahara P, MacGregor H, Barker T, Bayiseng J, Calloway C, Fairhead J, Fahal AH, Hounsoumea N, Roemer-Mahler A, Mugumec P, Tadelef G, Davey G. Severely stigmatised skin neglected tropical diseases: a protocol for social science engagement. *Trans R Soc Trop Med*

Hyg 2020; 114: 1013–1020 doi:10.1093/trstmh/traa141

- Fahal AH. The Khartoum call for action, Khartoum, Sudan – 2019. Trans R Soc Trop Med Hyg. 2021;115:295–296. doi:10.1093/trstmh/trab043. Advance Access publication 5 March 2021.
- Bertrand Nyuykonge, Kimberly Eadie, Willemien H A Zandijk, Sarah A Ahmed, Marie Desnos-Ollivier, Ahmed H Fahal, Sybren de Hoog, Annelies Verbon, Wendy W J van de Sande, Corné H W Klaassen. A Short-Tandem-Repeat Assay (Mmy STR) for Studying Genetic Variation in *Madurella mycetomatis*. J Clin Microbiol 2021 Feb 18;59(3): e02331-20. doi: 10.1128/JCM.02331-20. Print 2021 Feb 18.
- Konings M, Kimberly E, Lim W, Fahal A, Mouton J, Tesse N, van de Sande W. The synthetic synergistic cinnamon oil CIN-102 is active against *Madurella mycetomatis*, the most common causative agent of mycetoma. PLoS Negl Trop Dis. 2021 Jun 9;15(6):e0009488. doi: 10.1371/journal.pntd.0009488. eCollection 2021 Jun.
- Lim W, Siddig E, Eadie K, Nyuykonge B, Ahmed S, Fahal AH, Verbon A, Smit S, van de Sande WJJ. The development of a novel diagnostic PCR for *Madurella mycetomatis* using a comparative genome approach. PLoS Negl Trop Dis. 2020 Dec 16;14(12):e0008897. doi: 10.1371/journal.pntd.0008897. eCollection 2020 Dec.
- Siddig EE, Ahmed A, Ali Y, Bakhiet SM, Mohamed NS, Ahmed ES, Fahal AH. Eumycetoma Medical Treatment: Past, Current Practice, Latest Advances and Perspectives. Microbiol. Res. 2021, 12, 899–906. <https://doi.org/10.3390/microbiolres12040066>
- Nyuykonge B, van Amelsvoort L, Eadie K, Fahal AH, Verbon A, van de Sande W. Comparison of Disc Diffusion, Etest, and a Modified CLSI Broth Microdilution Method for In Vitro Susceptibility Testing of Itraconazole, Posaconazole, and Voriconazole against *Madurella mycetomatis*. Antimicrob Agents Chemother. 2021 Aug 17;65(9):e0043321.
- Hassan R, Simpson H, Cano J, Bakhiet S, Ganawa E, Argaw D, Newport MJ, Deribe K, Fahal AH. Modelling the spatial distribution of mycetoma

in Sudan. *Trans R Soc Trop Med Hyg.* 2021 Oct 1;115(10):1144-1152.

- Oladele RO, Ly F, Sow D, Akinkugbe AO, Ocansey BK, Fahal AH, van de Sande WWJ. Mycetoma in West Africa. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):328-336.
- Azrag RS, Bakhiet SM, Mhmoud NA, Almalik AM, Mohamed AH, Fahal AH. A possible role for ticks in the transmission of *Madurella mycetomatis* in a mycetoma-endemic village in Sudan. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):364-374.
- Yosif AA, Bakhiet SM, Abdalla TH, Mhmoud NA, Siddig EE, Fahal AH. Invasive, aggressive mastoid bone eumycetoma: a treatment challenge. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):431-435.
- Ganawa ETS, Bushara MA, Musa AEA, Bakhiet SM, Fahal AH. Mycetoma spatial geographical distribution in the Eastern Sennar locality, Sennar State, Sudan. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):375-382.
- Kébé M, Ba O, Mohamed Abderahmane MA, Mohamed Baba ND, Ball M, Fahal A. A study of 87 mycetoma patients seen at three health facilities in Nouakchott, Mauritania. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):315-319.
- Fahal AH. Mycetoma: the journey from neglect to recognition as a neglected tropical disease. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):292-294.
- Bahar ME, Bakheet OELH, Fahal AH. Mycetoma imaging: the best practice. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):387-396.
- Mohamed ESW, Bakhiet SM, El Nour M, Suliman SH, El Amin HM, Fahal AH. Surgery in mycetoma-endemic villages: a unique experience. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):320-323.
- Mhmoud NA, Siddig EE, Nyuykonge B, Bakhiet SM, van de Sande WWJ, Fahal AH. Mycetoma caused by *Microascus gracilis*: a novel agent of human eumycetoma in Sudan. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):426-430.

- Omer RF, Ahmed ES, Ali BM, Alhaj HE, Bakhiet SM, Mohamed ESW, Strub-Wourgaft N, Fahal AH. The challenges of recruitment in clinical trials in developing countries: the Mycetoma Research Centre experience. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):397-405.
- Ezaldeen EA, Ahmed ES, Fahal AH. Massive complicated secondary inguinal mycetoma: a case series. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):420-425.
- Siddig EE, van de Sande WWJ, Fahal AH. Actinomycetoma laboratory-based diagnosis: a mini-review. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):355-363.
- Santana A, Mhmoud NA, Siddig EE, Deligios M, Fiamma M, Bakhiet SM, Barac A, Paglietti B, Rubino S, Fahal AH. Metagenomics of black grains: new highlights in the understanding of eumycetoma. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):307-314.
- Zaid DM, Bakheet OE, Ahmed ES, Abdalati F, Mhmoud NA, Mohamed ESW, Bakhiet SM, Siddig EE, Fahal AH. Multiple extensive *Madurella mycetomatis* eumycetoma lesions: a case report and review of the literature. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):411-414.
- Bakheet OE, Hassan MA, Fahal AH. Extensive perineal *Actinomadurea pelletieri* actinomycetoma-induced urethral stricture: a rare complication. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):415-419.
- Siddig EE, Nyuykonge B, Ahmed MT, Hassan R, Saad ESA, Mhmoud NA, Bakhiet SM, van de Sande WWJ, Fahal AH. Human actinomycetoma caused by *Actinomadurea mexicana* in Sudan: the first report. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):406-410.
- Traxler RM, Beer KD, Blaney DD, van de Sande WWJ, Fahal AH, Asiedu KB, Bower WA, Chiller T. Development of the Global Mycetoma Working Group. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):437-440.
- Hay RJ, Asiedu KB, Fahal AH. Mycetoma - a long journey out of the shadows. *Trans R Soc Trop Med Hyg.* 2021 Apr 14;115(4):281-282.

- Siddig EE, Verbon A, Bakhiet S, Fahal AH, van de Sande WWJ. The developed molecular biological identification tools for mycetoma causative agents: An update. *Acta Trop.* 2022 Jan; 225:106205. doi: 10.1016/j.actatropica.2021.106205. Epub 2021 Oct 21. PMID: 34687643
- Fahal LA, Ahmed ES, Bakhiet SM, Siddig EE, Fahal AH. The Mycetoma Research Centre experience during the COVID-19 pandemic: obstacles and beyond. *Trans R Soc Trop Med Hyg.* 2022 Jan 19;116(1):1-3. doi: 10.1093/trstmh/tra111. PMID: 34313305
- Hashizume H, Taga S, Sakata MK, Taha MHM, Siddig EE, Minamoto T, Fahal AH, Kaneko S. Detection of multiple mycetoma pathogens using fungal metabarcoding analysis of soil DNA in an endemic area of Sudan. *PLoS Negl Trop Dis.* 2022 Mar 11; 16(3):e0010274. doi: 10.1371/journal.pntd.0010274. eCollection 2022 Mar. PMID: 35275915
- Nyuykonge B, Lim W, van Amelsvoort L, Bonifaz A, Fahal A, Badali H, Abbastabar M, Verbon A, van de Sande W. Eumycetoma Causative Agents are Inhibited in vitro by Luliconazole, Lanoconazole and Ravuconazole. *Mycoses.* 2022 Apr 10. doi: 10.1111/myc.13442. Online ahead of print. PMID: 35398930
- Lim W, Konings M, Parel F, Eadie K, Strepis N, Fahal A, Verbon A, van de Sande WWJ. Inhibiting DHN- and DOPA-melanin biosynthesis pathway increased the therapeutic value of itraconazole in *Madurella mycetomatis* infected *Galleria mellonella*. *Med Mycol.* 2022 Feb 2;60(2):myac003. doi: 10.1093/mmy/myac003. PMID: 35064672
- Lim W, Nyuykonge B, Eadie K, Konings M, Smeets J, Fahal A, Bonifaz A, Todd M, Perry B, Samby K, Burrows J, Verbon A, van de Sande W. Screening the pandemic response box identified benzimidazole carbamates, Olorofim and ravuconazole as promising drug candidates for the treatment of eumycetoma. *PLoS Negl Trop Dis.* 2022 Feb 4;16(2):e0010159. doi: 10.1371/journal.pntd.0010159. eCollection 2022 Feb. PMID: 35120131.
- Fahal AH, Otieno LA, Ahmed ES, Kashif T, Khalid M, Mahmoud AH, Ododo L, Kyalo T, Bakhiet SM. Visual ethnographic documentation: a

novel tool for mycetoma awareness and advocacy. *Trans R Soc Trop Med Hyg.* 2022 May 30: trac048. doi: 10.1093/trstmh/trac048. Online ahead of print. PMID: 35640654

- Rawaa Adlan Elhady Ahmed, Alkhair Abd Almahmoud Idris, Nadia Ibrahim Eldawi, Ahmed Hassan Fahal. Role of special stains in the identification of fungi in eumycetoma among Sudanese patients in Soba University Hospital. *Microbes Infectious Dis.* 2022; 3(1): 185-191. DOI: 10.21608/mid. 2021.96355.1193
- Siddig EE, El Had Bakhait O, El Nour Hussein Bahar M, Siddig Ahmed E, Bakhiet SM, Motasim Ali M, Babekir Abdallah O, Ahmed Hassan R, Verbon A, van de Sande WWJ, Fahal AH. Ultrasound-guided fine-needle aspiration cytology significantly improved mycetoma diagnosis. *J Eur Acad Dermatol Venereol.* 2022 Jun 24. doi: 10.1111/jdv.18363. Online ahead of print. PMID: 35748131
- Musa EA, Abdoon IH, Bakhiet SM, Osman B, Abdalla SA, Fahal AH. Mycetoma management and clinical outcomes: the Mycetoma Research Center experience. *Trans R Soc Trop Med Hyg.* 2022 Jul 28: trac069. doi: 10.1093/trstmh/trac069. Online ahead of print. PMID: 35903002
- Watson AK, Kepplinger B, Bakhiet SM, Mhmoud NA, Chapman J, Allenby NE, Mickiewicz K, Goodfellow M, Fahal AH, Errington J. Systematic whole-genome sequencing reveals an unexpected diversity among actinomycetoma pathogens and provides insights into their antibacterial susceptibilities. *PLoS Negl Trop Dis.* 2022 Jul 25;16(7):e0010128. doi: 10.1371/journal.pntd.0010128. Online ahead of print. PMID: 35877680
- Hassan R, Deribe K, Fahal AH, Newport M, Bakhiet S. Clinical epidemiological characteristics of mycetoma in Eastern Sennar locality, Sennar State, Sudan. *PLoS Negl Trop Dis.* 2021 Dec 13;15(12):e0009847. doi: 10.1371/journal.pntd.0009847. eCollection 2021 Dec. PMID: 34898611
- Emanwell Siddig, Antonella Santona, Najwa A Mhmoud , Massimo Deligios Maura Fiamma, Bianca Paglietti, Sahar Mubarak Bakhiet, Salvatore Rubino, Ahmed Hassan Fahal. Metagenomic detection of eumy-

cetoma causative agents from households of patients residing in two Sudanese endemic villages in White Nile State. PLoS NTD Accepted.

- Nyuykonge B, Siddig EE, Konings M, Bakhiet S, Verbon A, Klaassen CHW, Fahal AH, van de Sande WWJ. *Madurella mycetomatis* grains within a eumycetoma lesion are clonal. *Med Mycol.* 2022 Jul 29;60(7):myac051. doi: 10.1093/mmy/myac051. PMID: 35833294
- Hassan R, Deribe K, Simpson H, Bremner S, Elhadi O, Alnour M, Fahal AH, Newport M, Bakhiet S. Individual Risk Factors of Mycetoma Occurrence in Eastern Sennar Locality, Sennar State, Sudan: A Case-Control Study. *Trop Med Infect Dis.* 2022 Aug 10;7(8):174. doi: 10.3390/tropicalmed7080174. PMID: 36006266
- Nyuykonge B, Siddig EE, Mhmoud NA, Nyaoke BA, Zijlstra EE, Verbon A, Bakhiet S, Fahal AH, van de Sande WWJ. Epidemiological cut-off values for itraconazole and ravuconazole for *Madurella mycetomatis*, the most common causative agent of mycetoma. *Mycoses.* 2022 Jul 30. doi: 10.1111/myc.13509. Online ahead of print. PMID: 36005544
- Siddig EE, Ahmed A, Hassan OB, Bakhiet SM, Verbon A, Fahal AH, van de Sande WWJ. Using a *Madurella mycetomatis*-specific PCR on grains obtained via non-invasive fine-needle aspirated material is more accurate than cytology. *Mycoses.* 2023 Jun;66(6):477-482. doi: 10.1111/myc.13572. Epub 2023 Feb 19. PMID: 36740735.
- Siddig EE, Nyuykonge B, Mhmoud NA, Abdallah OB, Bahar MEN, Ahmed ES, Nyaoke B, Zijlstra EE, Verbon A, Bakhiet SM, Fahal AH, van de Sande WWJ. Comparing the performance of the common used eumycetoma diagnostic tests. *Mycoses.* 2023 May;66(5):420-429. doi: 10.1111/myc.13561. Epub 2023 Jan 17. PMID: 36583225
- Fahal AH, Ahmed KO, Saeed AA, Elkhawad AO, Bakhiet SM. Why are mycetoma patients are still neglected. *PLoS Negl Trop Dis.* 2022 Dec 29;16(12):e0010945. doi: 10.1371/journal.pntd.0010945. eCollection 2022 Dec. PMID: 36580447
- Adam SAY, Ahmed ES, Mohammed Adam SI, Abdallah OB, Siddig EE,

Fahal AH. Eumycetoma with pulmonary dissemination: an unusual complication: Case series and literature review. *PLoS Negl Trop Dis*. 2022 Nov 10;16(11):e0010867. doi: 10.1371/journal.pntd.0010867. eCollection 2022 Nov. PMID: 36355778

- Fongwen N, Asiedu KB, Bakhiet S, Bonifaz A, Cruz I, Argaw D, Estrada-Chavez G, Fahal AH, Litvintseva A, Marks M, Salinas-Carmona MC, Sow D, van de Sande WWJ. Diagnostics to support mycetoma management-Development of two target product profiles. *Trop Med Int Health*. 2022 Dec;27(12):1059-1064. doi: 10.1111/tmi.13828. Epub 2022 Nov 14. PMID: 3632962
- Litvintseva AP, Bakhiet S, Gade L, Wagner DD, Bagal UR, Batra D, Norris E, Rishishwar L, Beer KD, Siddig EE, Mhmoud NA, Chow NA, Fahal A. Genomics and metagenomics of *Madurella mycetomatis*, a causative agent of black grain mycetoma in Sudan. *PLoS Negl Trop Dis*. 2022 Nov 2;16(11):e0010787. doi: 10.1371/journal.pntd.0010787. eCollection 2022 Nov. PMID: 36322569
- Hounsborne N, Hassan R, Bakhiet SM, Deribe K, Bremner S, Fahal AH, Newport MJ. Role of socioeconomic factors in developing mycetoma: Results from a household survey in Sennar State, Sudan. *PLoS Negl Trop Dis*. 2022 Oct 17;16(10):e0010817. doi: 10.1371/journal.pntd.0010817. eCollection 2022 Oct. PMID: 36251732
- Hassan R, Cano J, Fronterre C, Bakhiet S, Fahal A, Deribe K, Newport M. Estimating the burden of mycetoma in Sudan for the period 1991-2018 using a model-based geostatistical approach. *PLoS Negl Trop Dis*. 2022 Oct 14;16(10):e0010795. doi: 10.1371/journal.pntd.0010795. eCollection 2022 Oct. PMID: 36240229
- Nyuykong B, Siddig E, Mhmoud NA, Bakhiet S, Zijlstra E, Verbon A, Fahal AH, van de Sande WWJ. Wako  $\beta$ -D-glucan assay can be used to measure serum  $\beta$ -D-glucan in Sudanese patients to aid with the diagnosis of eumycetoma caused by *Madurella mycetomatis*. *J Eur Acad Dermatol Venereol*. 2023 Apr;37(4):783-786. doi: 10.1111/jdv.18642. Epub 2022 Oct 17. PMID: 36201367

- Siddig EE, El Had Bakhait O, El Nour Bahar M, Siddig Ahmed E, Bakhiet SM, Motasim Ali M, Babekir Abdallah O, Ahmed Hassan R, Verbon A, van de Sande WWJ, Fahal AH. Ultrasound-guided Fine Needle Aspiration Cytology significantly improved mycetoma diagnosis. *J Eur Acad Dermatol Venereol*. 2022 Jun 24.
- Siddig EE, Nyuykonge B, Bakheit OEH, Hassan OB, Ahmed ES, Osman AA, Bakhiet SM, van de Sande WW, Fahal AH. *Staphylococcus aureus* causing primary foot botryomycosis mimicking actinomycetoma: a case report from Sudan. *Int J Infect Dis*. 2022 Nov;124:224-226. doi: 10.1016/j.ijid.2022.10.010. Epub 2022 Oct 12. PMID: 36241164.
- Fahal AH. The International Research Collaboration: A room for improvement. *Khartoum Med J*. 2022; 15(3): 1987.
- Ali HO, Fahal AH. Why is mycetoma still a public health dilemma and a unique neglected tropical disease? *Khartoum Med J*. 2022; 15(3): 1990–1999.
- Ahmed KO, Osman IA, Abdalnabi AM, Bakhiet SM, Elkhawad A, Fahal AH. Are pharmacists neglecting the neglected mycetoma in the most endemic area in Sudan? An opportunity for improvement. *Khartoum Med J*. 2022; 15(3): 2000-2008.
- Siddig EE, Ahmed A, Hassan OB, Bakhiet SM, Verbon A, Fahal AH, van de Sande WWJ. Using a *Madurella mycetomatis* specific PCR on grains obtained via non-invasive Fine Needle Aspirated material is more accurate than cytology. *Mycoses*. 2023 Feb 5. doi: 10.1111/myc.13572. Epub ahead of print. PMID: 36740735.
- Siddig EE, Ahmed A, Eltigani HF, Bakhiet SM, van de Sande WWJ, Fahal AH. The First Case of *Fusarium falciforme* Eumycetoma in Sudan and an Extensive Literature Review about Treatment Worldwide. *J Fungi (Basel)*. 2023 Jul 6;9(7):730. doi: 10.3390/jof9070730. PMID: 37504719
- Eltigani HF, Elsheikh SM, Ahmed KO, Bakhiet SM, Fahal AH. Mycetoma National Campaign Report. *Khartoum Med J*. 2022; 15; (3); 2018 - 202
- Yoshioka I, Mori Y, Fahal AH, Siddig EE, Kaneko S, Yaguchi T. Specific

and sensitive loop-mediated isothermal amplification (LAMP) method for *Madurella* strains, eumycetoma filamentous fungi causative agent. *PLoS Negl Trop Dis*. 2023 Sep 18;17(9):e0011644. doi: 10.1371/journal.pntd.0011644. PMID: 37721946; PMCID: PMC10538720.

- Nyuykonge B, Siddig EE, Nyaoke BA, Zijlstra EE, Verbon A, Bakhiet SM, Fahal AH, van de Sande WWJ. Using (1,3)- $\beta$ -D-glucan concentrations in serum to monitor the response of azole therapy in patients with eumycetoma caused by *Madurella mycetomatis*. *Mycoses*. 2023 Oct 23. doi: 10.1111/myc.13664. Epub ahead of print. PMID: 37872649.
- Konings M, Eadie K, Strepis N, Nyuykonge B, Fahal AH, Verbon A, van de Sande WWJ. The combination of manogepix and itraconazole is synergistic and inhibits the growth of *Madurella mycetomatis* in vitro but not in vivo. *Med Mycol*. 2023 Nov 13: myad118. doi: 10.1093/mmy/ myad118. Epub ahead of print. PMID: 37960934.
- Fahal AH, Bakhiet SM. Mycetoma and the environment. *PLoS Negl Trop Dis*. 2023, 17(11): e0011736. <https://doi.org/10.1371/journal.pntd.0011736>.
- Badri R, Fahal AH (2023) The impact of the Sudan armed conflict on Mycetoma control. *PLoS Negl Trop Dis* 17(12): e0011783. <https://doi.org/10.1371/journal.pntd.0011783>
- Hashizume H, Taga S, Sakata MK, Hussein M, Siddig EE, Minamoto T, Fahal AH, Kaneko S. Environmental detection of eumycetoma pathogens using multiplex real-time PCR for soil DNA in Sennar State, Sudan. *Trop Med Health*. 2023 Dec 19;51(1):71. doi: 10.1186/s41182-023-00563-3. PMID: 38115141.
- Konings M, Gerrits van den Ende B, Raats MWJ, Fahal AH, van de Sande WWJ, Hagen F. Complete Genome Sequence of the Itraconazole Decreased Susceptible *Madurella fahalii* Type-Strain CBS 129176. *Mycopathologia*. 2024 Jan 17;189(1):6. doi: 10.1007/s11046-023-00807-0. PMID: 38231295; PMCID: PMC10794591.
- Gabani MH, Ahmed AA, Hassan AA, Abdalla MA, Mustafa SA, Alobaid

TA, Khatir AA, Mohammed RM, Awad NI, Abdellateef TA, Hassan A, Ahmed ES, Ali MZ, Fahal AH. The nutritional status of mycetoma-affected patients seen at the Mycetoma Research Center, Sudan. *PLoS Negl Trop Dis*. 2024 Jan 2;18(1):e0011726. doi: 10.1371/journal.pntd.0011726. Epub ahead of print. PMID: 38166142.

- Ali HO, Elkheir LYM, Fahal AH. The use of artificial intelligence to improve mycetoma management. *PLoS Negl Trop Dis*. 2024 Feb 8;18(2):e0011914. doi: 10.1371/journal.pntd.0011914. PMID: 38329930; PMCID: PMC10852264.
- Fahal A, Smith DJ, Nyaoke B, Asiedu K, Falves F, Warusavithanas S, Argaw D, Hay R. Towards enhanced control of mycetoma: a roadmap to achieve the UN's sustainable development goals by 2030. *Trans R Soc Trop Med Hyg*. 2024 Mar 26;trae016. doi: 10.1093/trstmh/trae016. Epub ahead of print. PMID: 38530874.
- Saeed AA, Mahdi MA, Elalawy IAMA, Alawad SS, Gibree OA, Fahal AH. Integrating Artificial Intelligence in Snakebite Management: An Innovative Approach. *Migrat. Lett. [Internet]*. 2024Mar.6 [cited 2024Mar.30];21(5):893-901. Available from: <https://migrationletters.com/index.php/ml/article/view/9285>
- Fahal AH, Abdelmunim AMS. El Mardi TES. Reviewing the Health Ramifications and Recovery Avenues for Sudan's April 2023 Armed Conflict. *SJMS*. 2023; 19(1): DOI 10.18502/13
- van de Sande WWJ, Fahal AH. An updated list of eumycetoma causative agents and their differences in grain formation and treatment response. *Clin Microbiol Rev*. 2024 May 1: e0003423. doi: 10.1128/cmr.00034-23. Epub ahead of print. PMID: 38690871.
- Alhaj AAM, Ahmed ES, Hassan A, Fahal AH. Epidemiological observations and management challenges in extrapedal mycetoma: A three-decade review of 420 cases. *PLoS Negl Trop Dis*. 2024 May 10;18(5):e0011841. doi: 10.1371/journal.pntd.0011841. Epub ahead of print. PMID: 38728359.

- Omar Ali H, Abraham R, Desoubieux G, Fahal AH, Tauber C. Evaluation of a computational model for mycetoma-causative agents identification. *Trans R Soc Trop Med Hyg.* 2024 Apr 6;118(4):253-263. doi: 10.1093/trstmh/trad057. PMID: 38088215.
- Ma J, Eadie K, Schippers M, Fahal A, Laleu B, Verbon A, van de Sande WWJ. Novel Compound MMV1804559 from the Global Health Priority Box Exhibits In Vitro and In Vivo Activity against *Madurella mycetomatis*. *Int. J. Mol. Sci.* 2024, 25, 6227. <https://doi.org/10.3390/ijms25116227>
- Ma J, Eadie K, Fahal A, Verbon A, van de Sande WWJ. The performance and costs of XTT, resazurin, MTS and luciferin as viability dyes in in vitro susceptibility testing of *Madurella mycetomatis*. *Trans R Soc Trop Med Hyg.* 2024 May 9:trae030. doi: 10.1093/trstmh/trae030. Epub ahead of print. PMID: 38721683.
- du Pré S, Konings M, Schoorl DJA, Fahal AH, Arentshorst M, Ram AFJ, van de Sande WWJ. Protoplast-mediated transformation of *Madurella mycetomatis* using hygromycin resistance as a selection marker. *PLoS Negl Trop Dis.* 2024 Apr 5;18(4):e0012092. doi: 10.1371/journal.pntd.0012092. PMID: 38578808; PMCID: PMC11108199.
- Fahal AH, Ahmed ES, Bakhiet SM, Bakhiet OE, Fahal LA, Mohamed AA, Mohamedelamin ESW, Bahar MEN, Attalla HY, Siddig EE, Mhmoud NA, Musa AM, van de Sande WWJ, Scherrer B, Oyieko P, Egondi TW, Onyango KO, Hata K, Chu WY, Dorlo TPC, Brüggemann RJ, Nyaoke BA, Strub-Wourgaft N, Zijlstra EE. Two dose levels of once-weekly fosravuconazole versus daily itraconazole in combination with surgery in patients with eumycetoma in Sudan: a randomised, double-blind, phase 2, proof-of-concept superiority trial. *Lancet Infect Dis.* 2024 Nov;24(11):1254-1265. doi: 10.1016/S1473-3099(24)00404-3. Epub 2024 Aug 1. PMID: 39098321.
- Elkheir LYM, Delaye PO, Penichon M, Eadie K, Mohamed MA, Besson P, Chesnay AG, Desoubieux G, Roger S, van de Sande WWJ, Fahal AH, Enguehard Gueiffier C. Emerging Therapeutics: The imidazo[1,2-b]pyridazine scaffold as a novel drug candidate for eumycetoma, a



neglected tropical disease. *Eur J Med Chem.* <https://doi.org/10.1016/j.ejmech.2024.116720>.

- Fahal AH, Ahmed IS, Saaed AA, Smith DJ, Alves F, Nyaoke B, Asiedu K, Hay R. Hope amidst neglect: Mycetoma Research Center, University of Khartoum. A holistic management approach to achieve the United Nations' Sustainable Development Goals. *PLoS Negl Trop Dis.* 2024 Sep 5;18(9):e0012420. doi: 10.1371/journal.pntd.0012420. PMID: 39235990; PMCID: PMC11376558.
- Fahal AH, Ahmed ES, Mahmoud AH, Saaed AA. The Mycetoma Research Center, University of Khartoum, Sudan's experience in community engagement initiatives spans 3 decades. *PLoS Negl Trop Dis.* 2024 Aug 22;18(8):e0012304. doi: 10.1371/journal.pntd.0012304. PMID: 39172748; PMCID: PMC11340884.
- El-Amin SO, El-Amin RO, El-Sadig SM, Fahal AH, Musa A. Painful mycetoma: a study to understand the risk factors in patients visiting the Mycetoma Research Centre (MRC) in Khartoum, Sudan. *Trans R Soc Trop Med Hyg.* 2024 Nov 29:trae093. doi: 10.1093/trstmh/trae093. Epub ahead of print. PMID: 39611590.
- Mohamed AF, Saeed AA, Mohamoud MA, Jama AA, Fahal AH. Mycetoma in Somalia. The Mycetoma in Somalia: An updated literature Review. *Ann Med Surg. Annals of Medicine and Surgery* ();10.1097/MS9.0000000000002851, December 19, 2024. | DOI: 10.1097/MS9.0000000000002851
- Chandler DJ, Davey G, Hay RJ, Fahal A; Mycetoma Activity and Severity clinical Scale (MASS) expert panel. Development of the Mycetoma Activity and Severity clinical Scale (MASS): an international Delphi study. *Lancet Infect Dis.* 2025 Feb 26:S1473-3099(24)00808-9. doi: 10.1016/S1473-3099(24)00808-9. Epub ahead of print. PMID: 40023182.
- Fahal AH, Smith DJ, Saeed AA, Nyaoke B, Alves F, Asiedu K, Hay R. Global health initiatives and mycetoma management: the unmet promise. *Trans R Soc Trop Med Hyg.* 2025 Feb 4:traf001. doi: 10.1093/trstmh/traf001. Epub ahead of print. PMID: 39901853.

- Hussein SME, Saeed AA, Fahal AH. Mycetoma management: Therapeutic challenges and the role of pharmacovigilance. *PLoS Negl Trop Dis*. 2025 Feb 20;19(2):e0012827. doi: 10.1371/journal.pntd.0012827. PMID: 39977423.
- Yoshioka I, Fahal AH, Kaneko S, Cao W, Yaguchi T. Itraconazole resistance in *Madurella fahalii* linked to a distinct homolog of the gene encoding cytochrome P450 14- $\alpha$  sterol demethylase (CYP51). *PLoS Negl Trop Dis*. 2025 Mar 27;19(3):e0012623. doi: 10.1371/journal.pntd.0012623. PMID: 40146733; PMCID: PMC11964275.
- Ouangré A, Yerbanga IW, Savadogo I, Ouédraogo H, Bado ND, Nagalo A, Traoré F, Dem AK, Bouda SAK, Koulybari DFD, Sawadogo A, Nakana-bo Diallo S, Gangneux JP, Fahal AH, Bamba S. Burden of mycetoma in Burkina Faso: case series and systematic review. *Trans R Soc Trop Med Hyg*. 2025 Feb 28: traf015. doi: 10.1093/trstmh/traf015. Epub ahead of print. PMID: 40036719.
- Fahal AH, Ahmed ES, Saeed AA, Fahal LA, Hussein SME, Nakano K, Hata K, Alves F, Nyaoke BA. A balancing act: Navigating the advantages and challenges of pioneering mycetoma treatment in Sudan- A landmark trial by the Mycetoma Research Center. *PLoS Negl Trop Dis*. 2025 Apr 21;19(4):e0013000. doi: 10.1371/journal.pntd.0013000. PMID: 40257994; PMCID: PMC12011228.
- Konings M, Siddig E, Eadie K, Minlekib CP, Faye M, Sow D, Fahal A, Verbon A, van de Sande W. The development of a multiplex recombinase polymerase amplification reaction to detect the most common causative agents of eumycetoma. *Eur J Clin Microbiol Infect Dis*. 2025 Apr 30. doi: 10.1007/s10096-025-05134-4. Epub ahead of print. PMID: 40304898.
- Ma J, Eadie K, Konings M, Fahal A, Verbon A, van de Sande WWJ. Ibrexafungerp prolongs survival and reduces the eumycetoma grain size in *Galleria mellonella* infection models of two different causative agents of eumycetoma. *J Antimicrob Chemother*. 2025 Apr 30:dkaf133. doi: 10.1093/jac/dkaf133. Epub ahead of print. PMID: 40304084.

- Abdulazeem Abbass O, Hassan AAA, Hamed RHA, Saeed AA, Fahal AH. Orbital actinomadura madurae actinomycetoma: Case report and literature review. *PLoS Negl Trop Dis*. 2025 Apr 29;19(4):e0013035. doi: 10.1371/journal.pntd.0013035. PMID: 40299927; PMCID: PMC12040158.
- Alyson M. Cavanaugh, Amanda Ribeiro dos Santos, Dayvison Francis Saraiva Freitas, James Venturini, Ahmed Fahal, Conceição de Maria Pedrozo e Silva de Azevedo, Jeremy A.W. Gold. Quality of Life, Disability, and Fungal Neglected Tropical Diseases. *Current Fungal Infection Reports* (2025) 19:4. <https://doi.org/10.1007/s12281-025-00503-0> REVIEW.
- Abugessaisa I, Konings M, Manabe RI, Murphy CM, Kawashima T, Hasegawa A, Takahashi C, Tagami M, Okazaki Y, Eadie K, Lim W, Doyle S, Verbon A, Fahal AH, Kasukawa T, van de Sande WWJ. Iron regulatory pathways differentially expressed during *Madurella mycetomatis* grain development in *Galleria mellonella*. *Nat Commun*. 2025 Jun 25;16(1):5324. doi: 10.1038/s41467-025-60875-2. PMID: 40562743; PMCID: PMC12198395.
- Chu WY, Fahal AH, Ahmed ES, Bakhiet SM, Bakhiet OE, Fahal LA, Mohamed AA, Mohamedelamin ESW, Bahar MEN, Attalla HY, Siddig EE, Mhmoud NA, Musa AM, Oyieko P, Egondi T, Brüggemann RJ, Hata K, Strub-Wourgaft N, Alves F, Nyaoke BA, Zijlstra EE, Dorlo TPC. Pharmacokinetics and Pharmacodynamics of Fosravuconazole, Itraconazole and Hydroxyitraconazole in Sudanese Patients With Eumycetoma. *J Infect Dis*. 2025 May 28;jiaf279. doi: 10.1093/infdis/jiaf279. Online ahead of print. PMID: 40433693
- Salah ME, Fearon Scales ML, Habib K, Alhamwi F, Abdelwahab S, Ahmed Y, Khalid MM, Smith DJ, Fahal A. Global sociodemographic, clinical, and epidemiological profiling of patients with mycetoma: A systematic review. *PLoS Negl Trop Dis*. 2025 Aug 14;19(8):e0013217. doi: 10.1371/journal.pntd.0013217. Epub ahead of print. PMID: 40811735.
- Mohammed Hussein, Elshimaa Ali, Yassin Kamal, Ali Elhouni, Yousif El-

tayeb, Ali Awadella Saaed, Ahmed Hassan Fahal. The Utility of Artificial Intelligence in the Management of Dengue Fever- Trans R S Trop Med Hyg- traf103, <https://doi.org/10.1093/trstmh/traf103>

- Hassan Fahal A, Ahmed AO, El Hassan L, Saeed AA. The contribution of mycetoma grains to suboptimal disease management. *Nat Commun.* 2025 Nov 7;16(1):9855. doi: 10.1038/s41467-025-64908-8. PMID: 41203632; PMCID: PMC12594801.
- Erber, Astrid and Dahal, Prabin and Bonifaz, Alexandro and Brack, Matthew and Brodersen, Joanna and Harriss, Eli and Jeleff, Maren and Maguire, Brittany J. and Nyaoke, Borna and Regensburger, Sandra and Srambickal, Amala and Strub Wourgaft, Nathalie and Tirado-Sanchez, Andrés and Weichselberger, Tobias and Fahal, Ahmed H. and Zijlstra, Eduard E. and Guérin, Philippe J., A Systematic Review of the Clinical Study Landscape for Eumycetoma, 1990-2024. Available at SSRN: <https://ssrn.com/abstract=5368722> or <http://dx.doi.org/10.2139/ssrn.5368722>
- Smith DJ, E Silva de Azevedo CMP, Fahal A, Lipner SR, Grijzen ML, Hay R. Approach to Diagnosing and Managing Implantation Mycoses. *Cutis.* 2025 Sep;116(3):88-104. doi: 10.12788/cutis.1266. PMID: 41183475.
- Saeed AA, Hussein SME, Al Zamel AM, Eisa LB, Daud AOA, Seedahmed HSM, Ahmed FOI, Al-Jabali TAKT, Kheir E, Fahal AH. Medical and health students' insights into mycetoma: A survey-based study on knowledge and clinical practices. *PLoS Negl Trop Dis.* 2025 Oct 9;19(10):e0013583. doi: 10.1371/journal.pntd.0013583. PMID: 41066411; PMCID: PMC12510600.
- Yoshioka I, Fahal AH, Sow D, Kaneko S, Mori Y, Ban S, Yaguchi T. Simultaneous Detection of Four *Madurella* Species Using Loop-Mediated Isothermal Amplification (LAMP) for Eumycetoma Diagnosis. *Myopathologia.* 2025 Oct 29;190(6):105. doi: 10.1007/s11046-025-01019-4. PMID: 41152542; PMCID: PMC12568910.
- Saeed AA, Osman MSS, Fahal AH. Molecular docking approaches in mycetoma: Toward improved patient management. *PLoS Negl Trop*

Dis. 2026 Feb 11;20(2):e0013963. doi: 10.1371/journal.pntd.0013963.  
PMID: 41671243

- Mosi L, Acharya B, Asiedu K, Bamisaiye A, Benbow ME, De Souza D, Elson L, Fahal A, Hay R, Nyangacha R, Quaye C, Shitu Sa'id A, Smith DJ, Jordan H. Integrated action for skin NTDs: Deconstructing transmission, addressing knowledge gaps, and championing one health strategies. *Nat Commun*. 2026 Feb 3. doi: 10.1038/s41467-026-69065-0. Epub ahead of print. PMID: 41629311.
- Ahmed R, Saeed AA, Fahal AH, Dube A. Advances in the treatment of mycetoma: the emerging role of nanomedicine. *Nanomedicine*. 2026;1-11. DOI: 10.1080/17435889.2026.2645392. <https://doi.org/10.1080/17435889.2026.2645392>.
- Badri R, Fahal AH. Left Gluteal and Perianal Eumycetoma Lesion Extending Into the Left Greater Sciatic Foramen, Pelvic Cavity to the External Anal Sphincter," *Clinical Case Reports*. 2026; 14(3): e72316, <https://doi.org/10.1002/ccr3.72316>.
- Dallas J. Smith, Doudou Sow, Wendemagegn Embiale, María Francisca Colom, John Lochuke Ekai, Bornha Nyaoke, Ahmed Fahal. The Neglected Burden of Eumycetoma in Africa. *The Lancet Regional Health - Africa*. 2026, Pages 100032.
- Yousif MH, Alsammani A, Abdalla MH, Bashier EBM, Mohammed MAY, Saeed AA, Fahal AH. Interpretable machine-learning prognosis of mycetoma from routine clinical data. *Trans R Soc Trop Med Hyg*. 2026 May 28;trag061. doi: 10.1093/trstmh/trag061. Epub ahead of print. PMID: 42206479.
- Fahal AH, Mycetoma in Mora-Montes HM, Leila M. Lopes-Bezerra L (Editors) *Current Progress in Medical Mycology*, Springer Nature 2017, pp 355-380. DOI 10.1007/978-3-319-64113-3
- Ottawa 2010, Assessment & Technology, Consensus Statement. Zubar Amin, David A. Cook, Rachel Ellaway, Ahmad Fahal, Roger Kneebone, Moira Maley, Doris Ostergaard, Gominda Ponamperuma, Andy Wearn,

Jack Boulet, Amitai Ziv, 2011

- van de Sande WWJ, Fahal AH, de Hoog GS, Van Belkum A, Madurella. In: Liu D, editor. *Molecular detection of human fungal pathogens*. Boca Raton: CRC Press, Taylor & Francis Group. 2011, pp. 117–128.
- Fahal, AH. Mycetoma in Richard, Guerrant, Walker, Peter, *Tropical Infectious Diseases: Principles, Pathogens and Practice*. Third edition, Elsevier Publisher, 2011, chapter 83, pp. 565-568
- Fahal AH. Mycetoma in Williams, Bulstrode, O'Connell, Bailey and Love's *Short Practice of Surgery* 26E: 26th Edition, Oxford University Press, 2013, pp 64-68.
- van Belkum A, Fahal, AH, van de Sande WW. Mycetoma by madurella mycetomatis: A completely neglected medico-social dilemma. In *Hot Topics in Infection and Immunity in Children IX*. Springer Science & Business Media New York 2012
- Fahal AH, *Mycetoma: Clinico-pathological Monograph*, University of Khartoum Press. 2006.
- Fahal AH, *Actinomycetoma in Africa*: in Serrano JA, Sandoval AH, Beaman BL. *Actinomicetoma*, Merida, Venezuela, 2006.
- Hassan MA, Fahal AH. *Mycetoma: in Tropical Surgery*, Kamil R, Lumbly J, Westminster Publication LTD, 2004.
- Fahal AH, *Evidence-Based Guidelines for the Management of Mycetoma Patients*, 2001.
- Scolding P, Fahal A, Yotsu RR. Drug therapy for Mycetoma. *Cochrane Database Syst Rev* 2018: <https://doi.org/10.1002/14651858.CD013082>



[www.mycetoma.edu.sd](http://www.mycetoma.edu.sd)