# Organizing a Reference Laboratory for Medical Mycology

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## Objectives

The objective is to provide the conceptual basis as well as some specifics needed for establishing a laboratory for mycology. A functional self-contained mycology laboratory, include three definitive areas, a clerical office-library-sample area, the laboratory and an isolation room. All such lab would need media preparation, autoclaving, and glassware cleaning and disposable of materials. A total space requirement of 2500 square feet which include laboratory specialized for isolation, identification and susceptibility of fungal isolates, stock culture collection and maintenance including preservation of reference culture. And it should also provide comprehensive laboratory services for investigating fungal infections with the help of specialist services. Regular training programs should be conducted for the technologists regarding all necessary technical and intellectual aspects of medical mycology with latest update to provide best services.

## Importance

It is now a well-established fact that the number of reported deaths worldwide due to scarlet fever, typhoid, whooping cough, diphtheria, dysentery, malaria and other infectious disease are keep on decreasing with the development of medical science whereas there is no much change in the frequency of deaths reported due to mycoses.(2) The significance of reference mycology laboratory increases due to increase in mobility and travel of modern man to a geographical area where a fungus exists as part of the commensal flora of the local population, or is endemic to the area. Demographics changes and Microbial adaptation are among few major factors which also promote the emergences of these fungal infections. All Conditions which are responsible for lowering immunity increases the number of susceptible host. Patient with advanced HIV infection, bone marrow transplantation, cancer, and severe malnutrition are few factors among many which lowers the immune response. Other factor such as Environmental air and water pollution; over processed & "fast" foods; fad diets, ageing population, and Immunology of the Mycoses add to the situation which indirectly increases the significance of a reference laboratory for the diagnosis and prevention.

## Requirements

**Facility environmental component:** Environmental condition in the facility such as lighting, temperature and humidity must be under control. The main lab must be under negative air pressure. Such condition will prevent movement of airborne particles from the laboratory areas. Air flow is always into the laboratory and out the exhaust. A single pass ventilation system moves from moves air from outside the building, filters, heats, and humidifies as necessary. Usually 50% relative humidity is considered ideal.

**Isolation room component**: Require 300 square feet. It is in this area that the culture will be prepared. This room should have a laminar flow hood with lights, a HEPA filter, an UV light source and appropriate utilities. A safety centrifuge operating at 2000g will be needed. The room will need a sink, storage cabinets and incubators.

**Safety cabinet**: A separate certified biological safety cabinet with proper filters air flow must be available for mycological work.

**Incubator**: BOD incubator to meet with the environmental conditions for the isolation of fungi.

**Media**: Media should be sterile and meet the entire quality standard so that it supports growth of desired organism. It must also be controlled for quality using the stock of reference organisms.

**Reagents/ Kits/ Antibiotic discs**: Storage should be proper and should not be used beyond their expiry date. The antibiotic susceptibility testing should be done as per CLSI recommendation and the hospital antibiotic policy.

## Specimen Collection & Transport

**Blood and bone marrow:** Sterile technique should be used for taking the sample and for injecting in blood culture bottles used for the isolation of desired organism.

**CSF:** Aseptic technique should be used for collecting the sample, transport in sterile container, sediments after centrifugation should be used for make slides and for processing even direct CSF sample may be used.

**Hair:** Wood's lamp may be used to see infected areas. Use sterile tweezers to pull out hair from roots. Transport in sterile container.

**Nails:** Clean with 70% alcohol. Scrape away outer layers of nail and nail beds material should be collected in a sterile container.

**Skin:** Clean with normal saline or 70% alcohol. Scrape the actively growing edge and collect on a slide for staining or in a sterile container for processing.

**Respiratory (sputum, throat swab, bronchial washings and Transtracheal aspirates):** Early morning specimen should be collected and all the precaution must be taken to avoid throat flora and bacterial outgrowth.

**Tissue biopsies:** Should be kept moist with sterile saline in a sterile container until processed and should include normal tissue and tissue from the center and edge of lesions**.** Granules, pus and exudates must be looked.

**Urine:** Early morning clean catch or catheterized specimen is best. Bacterial overgrowth should be avoided.

**Vaginal, uterine cervix, prostatic secretion:** sterile technique for collection and transport.

**Wounds, subcutaneous lesions, and mucocutaneous lesions, exudates:** sterile technique should be used for collection and transport. Material should be aspirated if possible from cysts and abscesses and processing of granules if present.

**Specimen Collection Issues:** sterile technique should always be used, avoid contamination with hands other means**.** Samples should be adequate, must be properly labeled and should not be any processing delay. Prevent overgrowth of bacteria and ubiquitous molds as most Pathogenic molds grows slow.

**Diagnostic Test**

For identification of all suspected group of fungi, direct examination, fungal culture and None culture methods which include, Serological methods (antigen detection, antibody detection Test), for detection of metabolites and Tests for detection of CMI (cell mediated immunity) should be done. Other important method includes Molecular technique and Radiology.

**Staining techniques**

1. KOH mount
2. LCB staining
3. Gram staining
4. India ink stain
5. Nigrosin stain
6. Calcofluor white stain
7. PHOL stain
8. Neutral Red stain
9. Modified acid fast staining
10. H & E staining
11. PAS staining
12. Grigley's fungal staining
13. Grocott-Gomori's Methenamine Silver stain
14. Mayer's Mucicarmine stain
15. Masson-Fontana Silver staining
16. Toluidine Blue Ostain for P. jirovecii
17. Acridine Orange Stain
18. Fluorescent-Antibody Staining

**Conventional mycological technique**

1. Germ tube test
2. Hair perforation test
3. Hair bait technique
4. Adhesive tape technique
5. Fungal slide culture
6. Exo-antigen test

## Antifungal Drug Susceptibility

**Testing of yeasts using:** ketoconazole, micafungin, miconazole, nystatin, posaconazole, voriconazole. anidulafungin, amphotericin, caspofungin, clotrimazole, econazole, fluconazole, flucytosine, Itraconazole.

**Testing of moulds using**: clotrimazole, griseofulvin, itraconazole Amphotericin, caspofungin, natamycin, posaconazole, terbinafine and voriconazole. Other drugs may be included on request.

**Method for susceptibility testing:** (1) Broth macrodilution method. (2) Broth microdilution method. (3) SPOTi test for sensitivity

**Serological tests**

1. Serology of non-indigenous mycoses**:** histoplasmosis, coccidioidomycosis, blastomycosis, paracoccidioidomycosis
2. tests for antibodies to Aspergillus, Candida, Thermophilic actinomycetes (farmer's lung) and avian allergens
3. tests for Aspergillus (galactomannan), Candida (mannan) and Cryptococcus antigens
4. Beta 1-3 Glucan antigen detection for invasive fungal infections (this test has a very high negative predictive value)

**Histological examination (Staining technique)**

1. Hematoxylin and eosin (H&E) – On staining Color of fungi will be pink to pinkish blue. Allows determination of innate pigmentation by invading fungus, Stains most nuclei of yeast-like fungi.
2. Gomori's methenamine silver (GMS) often referred to as 'silver stain'. It gives black brown color to fungi with a light green background and can stain filamentous bacteria.
3. Periodic acid-Shiff (PAS) – Color of fungi will be red pink on a green background and does not stain filamentous bacteria.(4)
4. Gridley fungus (GF) – Color of fungi will be purplish red on a yellow background, Non-viable cells do not stain and does not stain filamentous bacteria.
5. Mucin (mucicarmine) stains – Mayer's or Southgate's preparations – Application: stains of mucopolysaccharide capsular material of fungi, e.g., Cryptococcus – Limitation: Not specific for Cryptococcus
6. Modified Gram's stains – Brown-Hopps' and MacCallum-Goodpasture preparations – stains

Gram-positive filamentous bacteria but does not selectively stain.

7. Modified Fontana-Masson – Stains cell walls of Cryptococcus and other melanin producing fungi and accentuates weakly pigmented agents of phaeohyphomycosis.
8. Whitening agents – Calcofluor White, Uvitex, and others – stains cell walls of fungi and seen under fluorescent microscope.

**Test for Detection of Metabolites**

This is to demonstrate a distinctive fungal metabolic byproduct in the body fluids of patients. Metabolites like D-Arabinitol are produced in large amount by all strains of C. albicans, C. tro picalis, C. parapsilosis, whereas no strain of C. glabrata, C. krusei, or C. neoformans produce D-arabinitol. It can be used as serum marker for diagnosis of candida spp. and also as prognostic indicator. Production of D-Arabinitol is detected by Gas chromatography(GC) in serum and/or urine or by quantifying D-arabinitol enzymatically and detected spectrophotometrically.(5)

**Molecular Technique**

**Diagnostic polymerase chain reaction (PCR) on blood, fluids and tissue:** PCR specific for Aspergillus, Candida and other should be done. Typing of common fungal pathogens should also be available and genomic DNA is being extracted for all of the fungal cultures in the collection.

**Proficiency testing for the lab:** The laboratory shall participate in External Quality Assessment Scheme (EQAS)/ Inter laboratory comparison as defined in NABL. The laboratory shall document any corrective actions taken based on the EQAS evaluation report. For those analytes where a formal EQAS is not available the laboratory shall exchange samples with other NABL accredited laboratories.(3) The laboratory shall adopt alternate methods to validate performance for certain tests for which inter-laboratory comparisons are not possible.

**Safety Issue:** The presence of containers, safety shower in the laboratory or nearby is essential. Other laboratory safety equipment should include appropriate fire extinguisher, an eye wash station near a sink, first aid kits, spill retention supplies, a fire blanket, safety glasses, laboratory coat, paper face masks, rubber or latex gloves, disposable containers, safety labels, and a respirator with an emergency air supply depending upon the chemicals and the organism used in the laboratory. Work place information and the hazard communication standard or worker right to know booklet with material safety data sheets, training records, the chemical inventory, safety procedures, and related required information should be at hand.

## Conclusion

This review article provides an overview of the major components needing consideration in the development of a research mycological laboratory. It is assumed that such an endeavor is initiated from an initial and beginning point in time. Planning for such an undertaking is noted as being of ultimate importance. The prime component discussed herein include the desired environmental condition needed in such a facility, the clerical office-library-sample receiving area, the mycology laboratory itself including the isolation room and the material & methods required for the diagnosis of fungal infection.

**Conflict of Interest: None**

**Source of Support: Nil**

## Reference

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