

PROCESSING AND CULTURING

I. PRINCIPLE:

Clinical specimens must be processed as soon and as carefully as possible to provide fungi present with the opportunity to grow on the appropriate isolation media and temperature. Recovery of pathogens is necessary for their identification and evaluation against antifungal agents. The method of processing and inoculation will vary depending upon the specimen (Table 1).

NOTE: Routine processing and culturing of specimens from within UTMB will be carried out by the Clinical Microbiology Laboratory staff.

II. DIRECT INOCULATION:

Many specimens can be directly inoculated to media. Three to 5 drops of fluid should be inoculated to each tube of media. For media in plates, inoculate with 2 or 3 drops and then streak over the medium surface. Up to 0.5 ml of certain sterile fluids, such as CSF, may be inoculated to tube media. When *Nocardia* is specifically suspected, the specimen will be cultured by the AFB laboratory and the Mycology laboratory to optimize recovery.

- A. Abscess aspirates
- B. Bone marrow aspirates
- C. Cerebrospinal fluid volume < 2.0 ml. Use multiple tubes to culture the entire volume.
- D. Vaginal swabs. Vortex swab in 0.5 ml of sterile water or directly swab the medium surface.
- E. Body fluids volume < 2.0 ml. If the fluid has a clot or membranous material, mince with scalpels and inoculate to media. Use multiple tubes to culture the entire specimen.
- F. Hairs
- G. Skin scrapings and nail
- H. Bronchial brushings. Vortex brush in sterile water. Directly inoculate media. Place brush on IMA agar.
- I. Bronchial washings volume < 1.0 ml, fluid
- J. Procedure for CSF specimens (> 2.0 ml volume necessary):
 1. Centrifuge the CF specimen for 5 minutes at 2000 xg.
 2. Without disturbing the sediment, aseptically remove the supernatant with a sterile pipette.
 3. Resuspend the sediment.
 4. Use the sediment to inoculate the culture media.

III. CONCENTRATION:

Large volumes of fluids should be concentrated by centrifugation (1500-2000 xg for 5 minutes) before inoculation to isolation media as a means to enhance the detection and recovery of fungi and aerobic actinomycetes.

PROCESSING AND CULTURING

III. CONCENTRATION: (cont'd)

- A. Body fluids volume > 2.0 ml for mycology. If clot or membranous material is present, inoculate it to the isolation media.
- B. Urines > 2.0 ml (do not process more than 50.0 ml).
- C. Cerebrospinal fluids volume > 2.0 ml for mycology.

IV. MINCED OR HOMOGENIZATION:

- A. Biopsies, tissues, and nails must be processed to increase the surface area of the specimen and expose the microorganisms to the isolation media. Mince specimens other than nails with sterile scalpels in a Petri dish with a few drops of sterile water. Nails can be ground in a nail pulverizing mill. Inoculate small pieces of the tissue or nail to the isolation media. It may be necessary to homogenize a specimen with a tissue grinder. The specimen should be minced first and then a portion placed in a sterile tissue grinder with a small volume of sterile water. Gently grind the tissue. Excessive force in grinding a tissue can result in the destruction of viable hyphae (especially zygomycetes). Inoculate the homogenate, as well as pieces of the minced tissue, to the appropriate media.
- B. Horses may present with lesions on the extremities caused by either a zygomycete or *Pythium* spp. Excised tissue from the infected area may have obvious "kunkers" which appear as cream-colored, irregular, branching structures containing actively growing hyphae. These specimens are often contaminated because of their location on the animal. To decontaminate the specimen, remove the branching structures using forceps and soak them in a 1:500 dilution of roccal for 5 to 15 minutes. Remove the specimen from the roccal and rinse 3 to 4 times in sterile water. Blot the material in sterile paper towel and cut it into small pieces.

V. INCUBATION:

Routine fungal cultures must be incubated at 30°C for 3-4 weeks before discarding. Cultures should not be discarded when a fungus is first isolated. They should be held for the entire incubation period to ensure that slow growing fungi are not overlooked.

- A. Vaginal cultures. Routine vaginal cultures are screened for yeasts and accordingly, are incubated at 30°C for 7 days only.
- B. Blood cultures. Blood cultures are incubated at 35°C for three weeks. Refer to the Isolator Blood Culture Procedure for details.
- C. Tissue cultures. Tissue cultures are incubated for 3 weeks at 30°C.
- D. All other routine specimens are incubated at 30°C for 3 weeks.
- E. Extended incubation. If *Histoplasma capsulatum* is suspected, tissue and respiratory specimens are incubated for twelve weeks. This is important for patients without AIDS.

PROCESSING AND CULTURING

VI. PLATE READING:

- A. A common view of clinical mycology, rooted in its past, holds that fungi grow slowly and one must be patient for results. In the last 15 years, there has been a considerable increase in the number and incidence of opportunistic pathogens. Many of these emerging pathogens, such as *Fusarium*, *Bipolaris*, and *Curvularia* spp. are very rapidly growing fungi. Newer media are providing faster growth and greater sensitivity than traditional media, and optical brighteners such as calcofluor white allow far more sensitive microscopic examination than the traditional KOH procedures. It is no longer adequate to read cultures as little as once a week. If possible, cultures should be read daily for the first 5 to 7 days. Most cases of serious mycotic infection in many parts of the continent can be detected in 24-72 hours under optimal laboratory conditions. Daily culture reading for significant positive KOH specimens will speed the identification of the organism so that proper therapy can be initiated.
- B. Reading Schedule. Based upon use of inhibitory mould agar incubated at 30°C. Refer to the Isolator Blood Culture Procedure for details about the schedule or reading.
1. Read all cultures Tuesdays and Fridays. Read cultures and account for all culture plates. Review culture requisitions for any overdue cultures.
 2. Discard three-week-old cultures on Tuesdays; finalize cultures before the week ends.
 3. Pull out positive growths and divide into stacks according to type: molds, yeasts, or aerobic actinomycetes.
 4. Log new findings in log book with date, type of fungus, and type of medium when the fungus was isolated.
 5. Preliminary results should be put in as soon as possible with proper charges.
 6. Worklists should be made for the new findings using the LIS; use WGT for new yeast and WLACTO for new molds.
 7. Use the proper identification scheme depending on the colony morphology of the organisms.

VII. ADDITIONAL READING:

1. McGinnis, MR: Laboratory Handbook of Medical Mycology, New York, Academic Press, 1980.
2. Roberts, GC, Goodman, NL, Land, GA, Larsh, HW, McGinnis, MR: Manual of Clinical Microbiology, 4th Edition, Lennette, EH, Balows, A, Hausler, WJ, Shadomy, HJ (eds), Washington, DC, AMS, pp. 500-513, 1985.

PROCESSING AND CULTURING

TABLE 1. Clinical Specimens and Isolation Media

Specimen	Collection	Undesirable Specimens	Processing	Media*
Abscess	Asceptically with needle and syringe from undrained abscesses; pus expressed from miliary abscesses opened with a scalpel	Swabs	< 3 cc media > 3 cc conc.	BHI+GC+10% b1 BHI
Biopsy	See Tissue			
Blood	See section on Isolator Blood Culture System			
Bone Marrow	0.2 - 0.3 ml in sterile citrated tube or Isostat tube	Clotted specimen	Direct; store at 37°C prior to processing	BHI BHI+GC+10% b1
Bone Marrow Biopsy	Sterile container with 1.0 ml sterile physiological saline			BHI BHI+GC+10% b1
Bronchial Brush	Place in sterile vial or tube with sterile water	Brush in carbon transport medium	Vortex brush small volume (8-10 drops) of sterile water	BHI IMA
Bronchial Washing	Leukens tube		See "Sputum"	
Cerebrospinal Fluid	3.0 ml in sterile screw cap tube	< 0.5 ml inferior quantity	< 2.0 cc direct > 2.0 conc.	IMA BHI
Cornea	Corneal tissue in 1.0 ml sterile distilled water	Dried specimen	Direct	Emmons SAB Sheep blood Agar plate or SAB DeX tube
Ear	Swab in sterile container or culturette	Dried specimen	Direct	IMA BHI
Eye Fluid	Filter paper and filtered fluid	Swabs	Concentrate fluids; divide filter	Emmons SAB IMA Sheep Blood Agar plate or BHI
Gastric Washing or Gastric Aspirate	Central Processing area will neutralize with equal volume sodium carbonate		Handle as Per "Sputum"	BHI+GC+10% b1 IMA
Hair	Hair and base of shaft in sterile petri dish or in paper envelope	Clippings	Direct inoculation	Mycosel IMA BHI
Joint Fluid, Pleural Fluid, Peritoneal Fluid	Sterile tube or syringe	Swabs	< 3 cc direct > 3 cc conc	IMA BHI

PROCESSING AND CULTURING

Specimen	Collection	Undesirable Specimens	Processing	Media*
Liver	Sterile container with 1.0 ml sterile physiological saline	Swabs	Mince; direct	IMA BHI
Lung	Sterile container with 1.0 sterile physiological saline	Swabs	Mince 1/2	IMA BHI
Lymph Node	Sterile container with 1.0 sterile physiological saline	Swabs	Mince 1/2; direct inoculation	IMA BHI
Nails	Clean site with 70% ETOH; collect shavings and material under the nail plate; discard first scrapings; sterile container	Swabs, random clippings	Pulverize nails, direct inoculation	BHI IMA Mycosel
Pleural	Sterile container with 1.0 sterile physiological saline	Swabs	Mince; direct	IMA BHI
Rhinocranial	Sterile container with 1.0 sterile physiological saline		Mince only; direct	IMA BHI Sterile bread or Malt agar
Skin Scrapings	Clean site with 70% ETOH; center and edge of any lesion any exudate; sterile container	Swabs	Direct inoculation	IMA Mycosel BHI
Sputum	5.0 - 1.0 ml early morning prior to eating; sterile sputum cup	Saliva, nasal secretions, throat swabs, 25 hour collections	Direct inoculation	IMA BHI+GC+10% b1
Stool	Will not be processed for fungus culture			
Subcutaneous or Cutaneous	Sterile container with 1.0 sterile physiological saline	Swabs	Mince 1/2; direct inoculation	IMA BHI
Urine	25-50 ml clean catch specimen; first morning, in sterile container. Catheterized and suprapubic aspirates are also acceptable	24 hour collection small volumes	Concentrate	BHI+GC+10% b1 IMA
Vaginal	Discharge material from vagina; swabs kept moist in sterile tube	Swabs in transport medium or dry swabs	Direct	BHI IMA or a Biggy slant will be accepted from the clinics.

NOTES: * 3. All pediatric or nursery specimens for culture will be set up routinely, but a BHI+GC+10% SB with olive oil will be

PROCESSING AND CULTURING

added for the isolation of *Malassezia furfur*. Any culture suspected of *Nocardia* or aerobic actinomycete will be set up also on a Mitchinson 7H11 plate.

4. Any respiratory specimen that has not been set up for AFB (Mycobacteriology Laboratory) will be set up in the Mycology Laboratory by using a BHI plate instead of the IMA plate.