Proc.#

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| Procedure | Fungal Cultures |

**Principle**: Describes the proper procedure for the collection and processing of specimens for fungal culture.

**Specimen**:

1. ***Patient Preparation***: Not applicable
2. ***Type of Specimen***: Appropriately collected specimens for culture as described below in “Procedure” section.
3. ***Specimen Handling***: All specimens are transported to the lab in a timely manner with adherance to laboratory safety and infection control policies

**Equipment and Materials**:

1. ***Equipment***: 30°C incubator
2. ***Materials***: Sabouraud’s Dextrose or SABHI agar, BHI with 10% sheep blood and gentamicin & chloramphenicol, and, when indicated, mycosel agar for dermatophytes.
3. ***Materials*** ***Preparation***: Media plates are warmed enough to write on the backs with marker pen.
4. ***Performance Parameters***: Not applicable
5. ***Storage Requirements***: Media is stored at 2-8°C in the orange Jewett refrigerator.

**Calibration**: Not Applicable

**Quality Control**: Media is quality controlled according to NCCLS guidelines

**Procedure**:

1. ***Collection***: Most collection is performed by nursing service or the physician. If lab personnel is involved in collection, the following guidelines may be used.
	1. *Skin &/or Nails*
		1. Wash infected area with 70% isopropanol.
		2. After drying, scrape the lesion with a sterile scalpel blade and place the material in a sterile petri dish, a slide, or directly onto the media.
	2. *Hair*
		1. A sample of the infected area should be clipped or plucked.
		2. Place in a sterile petri dish or other container and transport to lab.
	3. *Subcutaneous mycoses* specimens (includes pus, exudate, and tissue samples) are usually collected by physician or nursing staff and transported to lab in sterile containers or culturettes.
	4. *Systemic mycoses* specimens (includes blood, csf, sputum, bone marrow & tissue) are collected by physician or nursing staff with the exception of the blood cultures, and transported to the lab in sterile containers.
2. ***Processing***
	1. All specimens for fungal culture are to be logged in the laboratory information computer system and assigned an accession number. Once the specimen is in microbiology, it is given a mycology number which is an “M” placed in front of the accession number. (i.e. M123456) Also place a mycology 2” x 4” label on the requisition (see example at end of procedure) to aid in reading cultures.
	2. If a routine culture is also ordered, use the mycology number and the regular accession number for the routine culture.
	3. Centrifuge all nonviscous fluid specimens when possible and culture the sediment. Use the centrifuge in Specimen Reception and centrifuge for 10 minutes.
	4. If clot or membranous material is present in body fluids, mince it using sterile scalpels and combine with the centrifuged sediment. If necessary, fluids that are too dense for adequate centrifugation may be thinned with sterile distilled water prior to centrifugation.
	5. Blood cultures are collected and processed as noted in the blood culture procedure.
	6. Fungal cultures are examined every Monday, Wednesday, and Friday. Whenever cultures are examined, date and initial the log sheet. Issue reports at two weeks and 4 weeks.
3. ***Direct Smears***
	1. Direct smears should be performed on al specimens except blood and bone marrow if at all possible.
	2. Examination may be completed by any of the following methods: Unstained wet mound with or without KOH, or India Ink (on CSF specimens). Gram stains may also be used, but they are the least desirable type of stain for fungal elements. The Wright stain may be used when examining specimens for *Histoplasma* spp.
4. ***Inoculation and Incubation***
	1. As noted above, centrifuge fluids when possible.
	2. Inoculate the following media: Sabourad’s Dextrose or SABHI agar and BHI with 10% sheep blood and antibiotics. Also inoculate mycosel agar if skin, hair or nails are submitted or dematophytes are suspected.
	3. Since fungal elements are generally in smaller numbers than bacterial elements in an infection, it is important to culture a sufficient amount of fluid. Generally speaking, 1-2 ml of fluid should be divided up on the plates.
	4. Seal the plates with shrink seals that are in the large bacti refrigerator. Place the seals around the plates and then place the plates in the 35°C incubator until the seals have shrunk. Alternatively, masking tape may be used. If flasks or tubes are used, be sure and leave the screw caps slightly loose. Do not close tight!
	5. Incubate approximately 30 days (12 readings) at 30°C.
5. ***Examination***
	1. Examine the plates every Monday, Wednesday & Friday.
	2. If there is mold present, examine a specimen stained with lactophenol cotton blue. The fungi may be identified with the aide of reference materials. If it is not an *Aspergillus* sp. or *Penicillium* sp. or there is doubt as to the identification, refer the specimen to a reference laboratory. All isolates of *Aspergillus* or *Penicillium* should be referred to the section tech for final identification approval.
	3. Yeast like colonies need to be confirmed as yeast.
		1. If yeast, run a *C. albicans* disk screen (1008.118) or germ tube test on isolate (1008.122).
		2. If above tests are positive, report as “*Candida albicans*.” If negative, report as “Yeast–not *C. albicans*.”
		3. If further identification is needed, the isolate should be set up using the API 20C strip by techs approved for the procedure. If not available, refer the isolate to a reference lab.
		4. Yeasts that generally need full identification beyond *C. albicans* are those isolates origination from blood & CSF specimens or when there is a physician request. Check with section technologist or pathologist when in question as to extent of workup.

**Calculations**: Not applicable

**Reporting Results**: Results are reported using the microbiology computer system

**Medical Alert Values**: Isolates from blood and CSF are considered “Panic Values” and need to be referred to the physician.

**Notes**:

1. ***Reference Ranges***: Not applicable
2. ***Abnormal Results***: Not applicable
3. ***Reporting Format***: Results are reported using the microbiology computer system.

**Limitations**:

When *Malasezia furfur* is suspected, three drops of preservative-free olive oil are to be added to a duplicate set of inoculated media.

Any questions concerning setup & reading of fungal cultures should be referred to the microbiology section technologist.

**References**:

1. Baron, EJ, LR Peterson, & SM Finegold, *Diagnostic Microbiology*, 9th Ed., CV Mosby Co., St. Louis 1994.
2. *Manual of Clinical Microbiology*, 5th Ed., ASM, Washington, DC 1991
3. Koneman, EW et el, *Practical Laboratory Mycology*, 2nd Ed., Williams & Wilkins, 1979.
4. Larone, Davise H., *Medically Important Fungi: A Guide to Identification*, 2nd Ed., Elsevier Science Publishing Co., New York, NY 1987.

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