I. **GENERAL PRINCIPLE**

The most important steps for the isolation of fungus are:

- proper collection of specimens.
- rapid transport to the lab.
- prompt and correct processing of the specimen.
- inoculation onto appropriate media.
- incubation at suitable temperatures.

Each specimen submitted must be examined for quality and the importance of proper collection cannot be overemphasized. Fungi normally live a saprophytic existence in nature, capable of a life cycle without a human or animal host. Humans become an accidental host by inhalation of spores or by introduction of the fungus into tissue through trauma. Generally, humans are relatively resistant to these infections, but due to long-term catheterization, GI surgical procedures, chemotherapy and alterations of the immune system, exposure of fungi normally considered to be nonpathogenic can lead to infection. The laboratory is obligated to identify and report all isolates recovered from specimens submitted for fungal culture.

II. **SPECIMEN COLLECTION**

A. **General considerations**

1. All specimens should be collected aseptically and placed in sterile containers. A urine culture cup works well, as it is wide-mouthed, plastic with a screw-on lid, and leakproof.

2. Swabs are not encouraged, but specimens from the ear canal, nasopharynx, throat, vagina and cervix are not easily collected by any other means. It should be noted that the fungi commonly isolated from these sources can be and are recovered on routine aerobic culture of those sites and cultures for fungus only are not necessary. Swabs from open wounds or draining lesions are often contaminated with environmental fungi.

3. Many fungal infections are clinically similar to mycobacterial infections and often the same specimen is cultured for both fungi and AFB.

4. If a specific fungal agent is suspected, note on the request. Include travel and/or exposure history. Occupation is often helpful as well, since some types of work predispose a patient to certain specific fungal infections.

5. Fungus stains are determined by the type of specimen.

   - India ink - CSF
   - KOH wet prep - Hair, nails, and skin scrapings
   - Gram stain - All other specimens, but a fungus stain performed by the histology section of the laboratory yields better results, particularly for tissue specimens.
B.  **Abscess, pus, exudate, drainage**
   1. Using needle and syringe, aspirate specimen and place in sterile container.
   2. Small volume specimens may be submitted in the syringe, without the needle. Recap the syringe with the syringe cap.

C.  **Blood and bone marrow**
   1. Follow the routine procedure for collection.
   2. Place specimen in Isolator blood culture collection tube and mix vigorously.

D.  **Body fluids (pleural, synovial, peritoneal)**
   1. Cleanse proposed puncture site with alcohol, followed by iodine solution.
   2. Using needle and syringe, aspirate aseptically and place in sterile container.

E.  **CSF**
   1. Collect as much spinal fluid as possible and place in sterile tube.
   2. Tube 2 or 3 is preferred for culture.

F.  **Eye**
   1. Notify the lab before beginning procedure. Media will be provided by laboratory to be inoculated at the bedside.
   2. For suspected mycotic keratitis, an ophthalmologist will scrape the surface of the cornea, usually in a surgical procedure.

G.  **External ear**
   1. Use swab to collect specimen from ear canal or to sample drainage.
   2. Squeeze sponge to moisten swab in culturette sleeve and submit to lab.

H.  **Hair**
   1. No cleaning of scalp is necessary.
   2. From the infected area, use forceps to select at least 10 hairs.
   3. For hair broken off at the scalp, use a scalp or knife blade.
   4. Place hair in a clean, dry urine culture cup.
I. Nail
   1. Clean nail with alcohol.
   2. Use a scalpel to scrape the surface of the nail and discard the scraping. Then scrape the deeper portion for a specimen. Also, remove some of the debris under the nail with the scalpel. Place all the scrapings into a sterile urine culture cup.
   3. Alternatively, the whole nail or nail clippings may be submitted.

J. Skin and interspaces
   1. Wipe lesions and interspaces between toes with an alcohol sponge.
   2. Scrape the entire lesion(s) and both sides of the interspaces with a sterile scalpel blade.
   3. Place all scrapings into a sterile urine culture cup.

K. Sputum
   1. Collect the first morning specimen. Do not pool specimens.
   2. Have patient rinse mouth and collect sputum resulting from a deep cough, or if necessary by induction.
   3. Have patient expectorate immediately into a sputum collection container. **Do not let patient hold sputum in the mouth.**
   4. Disassemble collection container, place cap on conical centrifuge tube, tighten, label, and transport to the laboratory. Discard remaining pieces of the collection device at the patient's bedside.

L. Respiratory specimens other than sputum
   1. These include bronchial washings, lung biopsies, and tracheal aspirates.
   2. These specimens are collected aseptically by the physician, placed in the appropriate container and immediately transported to the lab.

M. Tissue
   1. Biopsy aseptically from both the center and edge of the lesion.
   2. Place tissue in a sterile container and if transport is delayed, it may be covered with a minimum of sterile saline to prevent drying.
N. Urine

1. A catheterized specimen is the most suitable for making a diagnosis of fungal infection of the urinary tract. A clean catch midstream urine is acceptable when aspiration or cystoscopy can not be performed.

2. Collect the first morning specimen aseptically as for any urine culture. A 24-hour collection is unacceptable.

O. Vaginal

1. Using several sterile swabs, collect discharge from the vagina and place swabs in a sterile tube that can be capped. This method of collection provides a specimen that is also suitable for performance of a wet prep.

2. Alternatively, vaginal material may be collected and submitted on a culturette.

III. SPECIMEN TRANSPORT:

A. Deliver to the lab within 2 hours of collection. Since overgrowth of the slower growing pathogenic fungi by contaminating bacteria and/or fungi is common, it is important to transport specimens to the lab as soon as possible.

B. If transport is delayed, store specimens under refrigeration at 4°C with the following exceptions:
   ♦ Hold blood, bone marrow and CSF at body temperature at 30-37 ° C.
   ♦ Hold dermatological specimens at room temperature.
   ♦ Be aware that viability may decrease with prolonged storage.

C. As always, transport specimen to the lab in a biohazard bag.

References:

Clinical Microbiology Procedures Handbook, 1992, Isenberg, American Society for Microbiology.
