

## COLLECTION AND TRANSPORT OF SPECIMENS

### I. PRINCIPLE:

- A. The type and quality of specimens submitted to the mycology laboratory are an initial factor in determining the success of isolating and identifying etiological agents of fungal infections. Each specimen received must be examined for quality. Proper specimen collection, container labeling, and culture requests are the responsibility of the ordering physician.
- B. The most important steps for the successful isolation of etiological agents of mycoses are
  - 1. proper collection of the specimens,
  - 2. rapid transport of the specimens to the laboratory,
  - 3. the prompt and correct processing of the specimens, and
  - 4. inoculation of specimens onto appropriate culture media and incubation at suitable temperatures.

### II. COLLECTION OF SPECIMENS:

- A. Specimens should be collected aseptically, placed in sterile containers, delivered to the laboratory within 2 hours, processed, and then inoculated to primary isolation media within a few hours of collection. Viability may decrease with prolonged specimen storage.
- B. Swabs are not encouraged; however, specimens from the environment or certain body sites such as the ear canal, nasopharynx, throat, vagina, and cervix are not readily collected by other means. Swabs for collection of material from open wounds or draining lesions are frequently contaminated with environmental microorganisms.

### III. TRANSPORT OF SPECIMENS:

- A. Specimens should be transported in a sterile, humidified, leak-proof container. Dermatologic specimens, however, should be transported in a dry container. Transport medium should not be used unless the specimen can be easily and completely retrieved from the medium. Although fungi can be recovered at times from specimens submitted in anaerobic transport media, such media should be avoided.
- B. Specimens should be processed and inoculated to primary isolation media as soon as possible after collection, ideally within a few hours. Limited studies have shown significantly decreased viability for *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Aspergillus fumigatus* when stored at room temperature or on dry ice. *Rhizopus arrhizus* is also known to be difficult to recover from delayed specimens. It should not be assumed that successful methods for storage of fungal cultures are suitable for temporary storage of clinical specimens that harbor relatively few fungal cells.
- C. The effect of refrigeration on fungal specimens has not been well-studied, but if processing is to be delayed for more than several hours, it is recommended that specimens be stored under refrigeration at 4°C with the following exceptions: blood and cerebrospinal fluid are stored at 30-37°C; dermatologic specimens are stored at 15-30°C.

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### IV. SPECIMENS:

**NOTE:** These directions should be shared with clinicians and made part of the instructions for collection and submission of specimens distributed to staff.

- A. Sputum (tracheal lavage, bronchial lavage, and aerosol collection)
  - 1. Sputum should be fresh and collected in the early morning.
  - 2. Have patient remove dentures and rinse mouth.
  - 3. Sputum should be the result of a deep cough (not saliva) or induced by a aqueous aerosol.
  - 4. Collect 5-10 ml in sterile container.
- B. Respiratory specimens other than sputum, such as tracheal aspirates, lung biopsy material, and bronchoscopy specimens are collected aseptically by physicians and sent to the laboratory immediately for examination and processing.
- C. Blood
  - 1. Blood is collected aseptically to avoid microbial contamination. The collection site is cleaned with a disinfectant at the time of collection.
  - 2. Use sodium polyanethol sulfonate (SPS, Liquoid) as an anticoagulant. Collect 8 ml blood in a yellow vacutainer tube (#4960, contains 1.7 ml of 0.35% SPS; final concentration with blood is 0.05%). This can be used to inoculate a vented biphasic blood culture bottle containing either trypticase soy or brain/heart infusion agar and broth. A ratio of 1 part blood to 10 parts broth is used.
  - 3. The Isolater Lysis Centrifugation System is ideal for fungal blood cultures. Ten ml of blood is directly collected in an Isolater tube.
- D. Pus, Exudate, and Drainage - Using a sterile needle and syringe, aspirate material from undrained abscesses. Place the material in a sterile container.
- E. Miliary Abscesses - Using a sterile, sharp-pointed scalpel, express pus and place it into a sterile container.
- F. Vaginal Material - Using several sterile swabs, collect material from the vagina. Insert swabs into a sterile tube.
- G. Tissue
  - 1. Tissue is aseptically collected from the center and edge of the lesion.
  - 2. Place between moist gauze squares, add a small amount of sterile water or 0.85% NaCl to keep tissue from drying out, and send immediately to the laboratory. Keep refrigerated not exceeding 8-10 hours at 4 ° until processed.
- H. Bone Marrow - Aspirate approximately 3-5 ml of bone marrow and place it in a sterile container. SPS or heparin can be added as an anticoagulant. The pediatric isolater blood culture system may be used.
- I. Cerebrospinal Fluid - As much spinal fluid as possible is collected and placed in a sterile container. Generally, a number 3 tube is used.

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### IV. SPECIMENS: (cont'd)

#### J. Urine

1. The urine specimen most suitable for making a diagnosis of mycoses of the urinary tract is a catheterized specimen. A clean catch, mid-stream should be collected when aspiration or cystoscopy cannot be done.
2. Early morning specimens are aseptically collected in sterile containers. Twenty-four hour collections have no value. Urine may be stored at 4°C for up to 12-14 hours.

#### K. Body Fluids (pleural, synovial, and peritoneal) - Specimens are collected aseptically and placed in sterile containers.

#### L. Hair

1. No cleaning of scalp is needed.
2. Select infected areas and with forceps, epilate at least 10 hairs.
3. For hairs broken off at the scalp level, use a scalpel or a blade knife.
4. Place hairs between two clean glass slides or in a clean envelope labeled with the patient's data.

#### M. Nail

1. Clean nail with 70% alcohol.
2. Dorsal plate - Scrape outer surface and discard; scrape the deeper portion.
3. Remove a portion of debris from under the nail with a scalpel.
4. Collect whole nail or nail clippings.
5. Place all material in a clean envelope labeled with the patient's data.

#### N. Skin and Interspaces

1. Wipe lesions and interspaces between the toes with alcohol sponge or sterile water.
2. Scrape the entire lesion(s) and both sides of interspaces with a sterile scalpel.
3. Place scrapings between two clean glass slides or place in a clean envelope labeled with the patient's data.

### V. VETERINARY SPECIMENS:

#### A. Respiratory

1. Upper: Collect specimens from the mouth on a swab with an aerobic transport medium. Specimens from the nose may include biopsy and nasal flush and should be transported in a sterile tube. Nasal swabs are usually not sufficient for diagnosis. Sterile saline without a preservative may be added to the biopsy to prevent drying. Specimens should be cultured promptly but if there is a delay, store overnight at 4°C.
2. Lower: Transport bronchial, transtracheal, or tracheal washes or aspirates in sterile tubes. These may be stored overnight at 4°C.

#### B. Blood - same as IV-C.

#### C. Pus, Exudate, and Drainage - same as IV-D.

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### V. VETERINARY SPECIMENS:

- D. Miliary Abscesses - same as IV-E.
- E. Vaginal and Uterine - Collect vaginal specimens on a swab with an aerobic transport medium and uterine specimens either on a swab or in a sterile tube. Either may be stored overnight at 4°C.
- F. Tissue
  - 1. Place the specimen between two pieces of moist sterile gauze or in a sterile container. Keep moist with sterile saline without preservatives.
  - 2. Storage at 4°C for up to 8-10 hours is acceptable except if a zygomycete or *Pythium* spp. is suspected. These organisms do not survive well when stored at 4°C.
- G. Bone Marrow - same as IV-H.
- H. Cerebrospinal Fluid - same as IV-I.
- I. Urine - same as IV-J.
- J. Body Fluids - same as IV-K.
- K. Hair, Nail, and Skin - same as IV-K, L, N.
- L. Eye - Corneal scrapings should not be transported, but be inoculated directly to media. It is necessary, therefore, to provide media to be taken to the location where the specimen will be collected. Intraocular fluid should be transported in a sterile tube (the collecting syringe is acceptable if handled properly and taken immediately to the laboratory) and should be left at 25°C if there is a delay in processing.

### VI. ENVIRONMENTAL SAMPLES:

- A. Swabs - Sterile swabs can be used to collect environmental specimens when an allergen or pathogen is suspected. Collect on an area of at least one square inch. Two swabs per site are preferred. One swab will be used for direct mount and the other for culture. Store the swabs at room temperature and deliver to the laboratory promptly.
- B. Soil and touch plates - Collect in a sterile container and deliver to the laboratory. Store at room temperature.
- C. Culture plates - Once collected, plates should be sealed with tape and delivered to the laboratory immediately.

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### VII. SEROLOGY:

Specimens requested for fungal serology will be transported to the proper laboratory for further testing, if the test is available. Currently the following serological tests are available at this institution:

- A. Cryptococcus antigen - serum or CSF
- B. Fungal immunodiffusion - serum

Other tests requested will be sent to a reference laboratory by the central processing laboratory.

### VIII. ADDITIONAL READING:

1. Abid, HN, Walter, PA, Litchfield, H: Chromomycosis in a horse. J. Am. Vet. Med. Assoc. 191:711-712, 1987.
2. Clinkenbeard, KD, Cowell, RL, Tyler, RD: Disseminated histoplasmosis in cats: 12 cases (1981-1986). J. Am. Vet. Med. Assoc. 190:1445-1448, 1987.
3. Corrier, DE, Wilson, SR, Scrutchfield, WL: Equine cryptococcal rhinitis. Compend. Cont. Ed. Pract. Vet. 6:S556-S558, 1984.
4. Haley, L., Calloway, C: Laboratory Methods in Medical Mycology. HEW Publication No. (CDC) 79-8361, 1978.
5. Hariri, AR, Hempel, HO, Kimberlin, CL, Goodman, NL: Effects of time lapse between sputum collection and culturing on isolation of clinically significant fungi. J. Clin. Microbiol. 15:425-428, 1982.
6. Rippon, JW: Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes, Philadelphia, W. B. Saunders Co., 1988.
7. Thompson, DW, Kaplan, W, Phillips, BJ: The effect of freezing and the influence of isolation medium on the recovery of pathogenic fungi from sputum. Mycopathologia 61:105-109, 1977.