LESSON ASSIGNMENT

LESSON 2 Collection and Processing Procedures for Mycological Studies.

TEXT ASSIGNMENT Paragraphs 2-1 through 2-15

TASK OBJECTIVES After completing this lesson, you should be able to:

2-1. Select the statement that correctly describes procedures for collecting a specific type of specimen for mycologic study.

2-2. Select the statement that correctly describes procedures for processing a specific type of specimen for mycologic study.

2-3. Select the statement that correctly describes procedures for examining a specific type of specimen for mycologic study.

SUGGESTION After completing the assignment, complete the exercises at the end of this lesson. These exercises will help you to achieve the lesson objectives.
LESSON 2
COLLECTION AND PROCESSING PROCEDURES
FOR MYCOLOGICAL STUDIES

2-1. INTRODUCTION

The principal goals of a clinical mycology laboratory are to isolate and accurately identify pathogenic fungi. Proper collection, transport, processing, and culturing of clinical mycology specimens is essential to achieve these goals. Laboratory personnel must be knowledgeable about specimen requirements if optimal results are to be obtained from the study of clinical specimens. This lesson covers information and procedures necessary for processing specimens for mycologic studies.

2-2. ABSCESS SPECIMENS

a. Collection. These specimens are collected from non-draining abscesses by aseptically aspirating the material with a sterile needle and syringe. Pus from miliary abscesses (abscesses resembling millet seeds) is collected by opening the abscess with a sterile scalpel and expressing the material into sterile tubes. Swabs should not be used to collect specimens for culture of fungi.

b. Processing. Process abscess material within 2 hours of collection. Refrigerate specimens prior to processing if delay is unavoidable.

   (1) Direct examination. Prepare specimen for direct examination using 10 percent KOH and/or periodic acid-Schiff stain (PAS). Fungal morphology can be observed using either a bright-field or phase-contrast microscope.

   (2) Inoculation. Inoculate material directly onto isolation media. If a large volume of specimen is available, centrifuge for 15 minutes at 2500 RPM and process the sediment. Media to be used for inoculation are Sabouraud dextrose agar (SDA), Mycosel™ or Mycobiotic™ agar (containing cycloheximide and chloramphenicol), and brain heart infusion agar (BHIA).

   (3) Incubation. Incubate inoculated media at 30ºC and examine for growth every 2-3 days. Identify all recovered fungi. Discard cultures as negative for fungi after four weeks of incubation.

2-3. BIOPSY MATERIAL

a. General. With the exception of a liver biopsy, tissue containing a portion of the wall, base, and center of the lesion should be obtained for mycotic studies. The specimen should be placed between sterile moist gauze squares, placed in a sterile container or petri dish, and sent to the laboratory immediately. Liver biopsies usually yield a small core of tissue that must be shared by the microbiology and pathology
laboratories. It is recommended that the portion to be subjected to microbiologic studies be placed directly into a tube of brain heart infusion broth or Sabhi broth and sent to the laboratory. A sterile glass rod should be used to crush the soft tissue in the broth. Microscopic studies will be made by the pathologist. If lymph nodes are biopsied, a portion of several nodes should be sent to the microbiology section.

b. Processing of Tissue for Mold or Yeast Isolation.

(1) Examine for presence of purulent, caseous, or necrotic areas. Such material should be inoculated directly to media and at least 5 thin smears made on glass slides not exceeding 1.0 mm in thickness. These smears may be subjected to various stains including the modified PAS stain. If indicated, one or more of these smears may be used for fluorescent antibody studies. A KOH preparation should also be examined. If indicated, an India Ink preparation may also be examined.

(2) Cut tissue into small fragments with sterile scissors and forceps.

(3) Place tissue fragments into a 15-mL homogenizer, or into a mortar, and thoroughly homogenize with pestle. Care should be taken to avoid any aerosol resulting from grinding the specimen.

(4) Two to 3 mL of Sabouraud or BHI broth may be added to the tissue as homogenization progresses.

(5) Pipette 0.5 mL of the homogenate to each plate of medium to be used. The remaining homogenate should be inoculated to BHB.

c. Processing Tissue for Mycetomas

(1) A mycetoma is a tissue tumor composed of granules. There are two basic types of mycetomas, actinomycotic and eumolytic. The actinomycotic mycetomas are caused by Nocardia species, Actinomyces species, or Streptomyces species of bacteria. The crushed granules of an actinomycotic mycetoma will show microscopic coccoid and bacillary forms. These organisms can also produce filaments that are easily confused with hyphae or mycelia. The crushed granules of eumycotic mycetomas microscopically will show hyphae and/or mycelia because the organisms causing the mycetomas are molds.

NOTE: Other bacteria such as, Escherichia coli, staphylococcus aureus, Proteus spp, and Pseudomonas aeruginosa may cause a similar disease called botryomycosis, which also gives rise to granules. Therefore, presence of a "granule" doesn't confirm that an actinomycotic mycetoma is present.

(2) In some instances, the organisms within a granule may be dead or dying and will not grow when inoculated to culture media. For this reason careful microscopic
studies must be made of one or more granules to determine whether an actinomycete, some other bacterium, or a mold, is the causative agent. The agent present determines therapy and prognosis.

(3) Examination of tissue for presence of granules.

(a) Place tissue in the bottom of a sterile petri dish.

(b) If granules are not visible through a hand lens, place the petri dish under a dissecting scope. With sterile teasing needles, gently but quickly dissect the tissue while looking through the dissecting scope.

(c) If granules are absent, streak any purulent and/or necrotic tissue directly to SDA, Mycosel™, and BHIA.

(4) Microscopic examination of a granule.

(a) Place the granule in a drop of water (do not use KOH since it may be necessary to do a Gram stain or a PAS stain on the material).

(b) Gently tease the granule apart.

(c) Place a coverslip over the preparation and examine microscopically. An actinomycotic granule will usually reveal beaded branching filaments that are approximately 1.5 to 2.0 microns in diameter. It is not possible to differentiate an aerobic actinomycete from an anaerobic strain by microscopic study of the granule. Cultures are necessary to confirm the identity of the organism. A eumycotic granule will contain hyphae that measure 4 to 7 microns in diameter. Swollen cells, suggestive of chlamydospores, may also be observed. Chlamydospores are thick-walled cells formed by the rounding-up of a cell. They may or may not germinate to form additional hyphae.

NOTE: Granules seen in botryomycosis may reveal filamentous, nonbranching bacilli, often in association with cocci.

(5) Preparation of granules for inoculation of media.

(a) Tease several granules free from the specimen and place them in a small test tube containing 2 to 3 mL of sterile, distilled water. Gently shake the tube and then transfer the washed granules with a pipette to a second tube of sterile, distilled water. Shake the second tube and content.

(b) Transfer the washed granules to a small centrifuge tube containing 0.5 to 1.0 mL of sterile, distilled water. Crush the granules with a glass rod. Inoculate the suspension using a sterile pipette. Streak agar plates with a loop. Inoculate broth media with several drops of the suspension.
2-4. BLOOD CULTURES.

a. The compromised host is very susceptible to mycotic infections. Candidiasis is the most common of the deep-seated fungal infections in these patients. Even when systemic candidiasis is suspected, only a small percentage of the cases are diagnosed before death. Thus, the physician and the laboratorian are faced with two major tasks, the first of which is to employ the most reliable method for isolation of yeast from blood as quickly as possible and, second, to obtain data that will assist in differentiating catheter associated fungemia from other forms of fungemia.

b. When attempting to differentiate catheter-associated fungemia from other septic episodes, the following specimens are to be collected within the same time period: (1) a blood culture collected from the arm not containing the catheter, (2) a blood culture drawn through the catheter, (3) culture of the catheter tip, and (4) additional blood cultures over a 24-to 48-hour period, all collected from the arm that did not have the catheter. Smears of the blood and material from the catheter tip should be stained immediately with either the PAS or Wright's stain. If any exudate is present at the insertion site of the catheter tip, this should also be stained and examined. Regardless of the method used for culturing blood, sodium polyanethol sulfonate (SPS, liquoid) should be used as an anticoagulant. Sodium polyanethol sulfonate is the least toxic of the anticoagulants and inactivates complement and leukocytes. The ratio of blood to broth in a culture should be 1:10 to 1:20. A higher concentration of blood will markedly reduce the chance of isolating an organism.

c. Prepare the skin for venipuncture.

(1) Clean the skin thoroughly with 70 percent ethyl or isopropyl alcohol at the site where venipuncture is to be made.

(2) If the patient is not allergic to iodine, swab the site concentrically with 2 percent tincture of iodine. Remove the iodine with an alcohol swab when venipuncture is completed.

d. Use the standard technique for blood culture.

(1) As outlined above, collect the blood in a Vacutainer tube or inoculate it directly to a biphasic blood culture bottle containing SPS. Biphasic blood culture bottles contain an agar slant to help isolate the disease-causing organism. Remember to maintain a ratio of 1:10 to 1:20 of blood to broth.

(2) Immediately and gently mix the contents of the bottle to prevent clotting and to wash the surface of the agar slant. If a biphasic blood culture bottle is not used, the blood-broth mixture must still be mixed.

(3) If blood was collected in a Vacutainer tube, it may be transferred to one or more blood culture bottles. Use of a transfer set greatly reduces the chance of contamination.
(4) Vent all blood culture bottles. Fungi will not survive under anaerobic conditions. Incubate at room temperature.

(5) If biphasic bottles are used, examine the agar slant daily for presence of fungal colonies. If no colonies are seen and broth is clear, gently "wash" the agar surface with the blood-broth mixture and reincubate. Culture media should be held at least 4 weeks before reporting as negative for fungi.

(6) If bottles containing a broth only are used, examine daily for gross evidence of growth. Subculture the broth every 48 hours. Blood cultures should be held for 4 weeks. *Candida guilliermondii* and *Torulopsis glabrata* may require 28 to 30 days to grow in routine blood culture bottles. Discard bottles as negative after four weeks.

2-5. BONE MARROW

a. This specimen is useful in the diagnosis of histoplasmosis, disseminated candidiasis, and cryptococciosis.

b. Procedure.

(1) Approximately 0.25 to 0.3 mL of bone marrow is collected in a heparinized syringe.

(2) A sterile cap is placed over the syringe and is sent immediately to the laboratory.

(3) The specimen is inoculated to several plates of HIA or Sabhi agar.

(4) Several fragments of marrow are used to prepare smears for PAS or Giemsa stains.

(5) Following inoculation of agar plates with the marrow specimen the syringe is ringed aseptically with sterile BHIB and the rinse is then incubated.

(6) All culture media are incubated at room temperature and are held at least 4 weeks before being discarded. If *Histoplasma capsulatum* is suspected, the cultures are incubated for 10 to 12 weeks before discarding as negative.

(7) If the specimen contains a large quantity of blood, it is centrifuged and the supernatant is removed with a sterile pipet and placed in a blood culture medium. The sediment is then inoculated to plates as outlined above.
2-6. **GENERAL SPINAL FLUID**

   a. At least 3 mL are needed for adequate mycologic studies. Use of the membrane filter technique will permit culturing for *Cryptococcus neoformans*, *Candida albicans*, and *Mycobacterium tuberculosis* simultaneously. If this technique is not used, it will not be possible to setup satisfactory cultures for fungi and *Mycobacterium tuberculosis* on the same spinal fluid sample.

   b. Membrane filter technique.

      (1) Centrifuge specimen at 2000 rpm for 15 minutes.

      (2) Do not decant supernatant. Using a capillary pipette, remove a small portion of the sediment and make an India Ink preparation and at least two thin smears that may be used for PAS, Acid Fast, or Gram stains.

      (3) Thoroughly mix the specimen on a mechanical mixer and pass the entire sample through a 0.45 micron membrane filter.

      (4) Place the membrane (inoculated surface up) on BHIA or Sabhi agar plates.

      (5) Seal each plate with parafilm or place in a plastic bag and incubate at 35°C for two weeks before discarding as negative.

      (6) All membrane filter cultures should be examined every 24 to 48 hours under a dissecting scope (leave top on Petri dish). Growth of yeast may be first observed as a "film" on the membrane surface, developing into actual colonies in another 24 to 48 hours. When a "film" is evident, sweep the membrane surface with a sterile loop and wash the loop in a drop of water onto a clean glass slide. Cover the drop with a coverslip and examine the preparation for presence of yeasts or bacteria. If desire, the coverslip may be removed and the slide allowed to air dry for Acid Fast or Gram stains to be done. Subculture any organism preset to SDA, Mycosel™, and BHIA.

2-7. **HAIR, SKIN, AND NAILS (DERMATOPHYTES)**

   a. **Hair**.

      (1) The patient's scalp should be examined with Wood's lamp for the presence of fluorescing hairs. No cleaning of the scalp is needed.

      (2) Using forceps, epilate (luck) at least 10 to 20 fluorescent or broken hairs and place them between clean glass slides or in a clean pill envelope sealed with cellophane tape. Wrap slides in a piece of paper. On this paper or envelope, write the patient's data including notation of therapy received before epilation of hair.
NOTE: Hairs that break off at the scalp level when using forceps may have been invaded by *T. tonsurans* and are best epilated with a knife blade. Scraping the scalp rarely yields hairs that have been invaded.

(3) Processing.

(a) Using KOH or PAS, mount the specimen for direct microscopic examination. Observe specimen for fungal elements and for ectothrix or endothrix hair invasion.

(b) Place 3 or 4 hairs each on SDA and Mycosel™ media.

(c) Incubate at 30°C for 4 weeks before discarding as negative.

(d) Examine every 2 days for growth. Identify all fungi.

b. Skin and Nails.

(1) Glabrous skin.

(a) Wipe lesion(s) well with an alcohol gauze sponge (cotton balls leave too many fibers on the skin that may interfere with interpretation of the direct examination). Instead of alcohol, use sterile water or broth if the area is irritated or the skin is broken.

(b) Scrape the entire periphery of a lesion(s) with a sterile scalpel (if more than one lesion is present, several should be scraped). Place scrapings in an envelope or between clean glass slides as discussed in para 2-7(a)(2).

NOTE: Lesions should be untreated with topical antifungal agents for at least one week if isolation of fungi is to be successful.

(2) Interspaces (between toes)

(a) Clean interspaces with a gauze square moistened with 70 percent alcohol or sterile water. Remove all dried exudate.

(b) With scalp, gently scrape both sides and base of each interspace. If fissuring is present, scrape only the sides.

NOTE: The fourth interspace of each foot should always be included when collecting specimens for a laboratory diagnosis of tinea pedis. A dermatophyte may be present in this area in patients with skin reactions or with asymptomatic infections.
(3) **Nails.**

(a) Clean nails with a gauze square.

(b) Remove a portion of debris from under the nail with a scalpel and place between clean glass slides.

(c) If the dorsal plate appears diseased, scrape the outer surface. It is recommended that the first four or five scrapings be discarded. This will help eliminate contaminating spores and bacteria. Collect scrapings through the diseased portion.

(d) An evulsed nail should be placed in petri dish or envelope.

(e) Nail clippings for isolation of dermatophytes require micronizing the nails before inoculation to media.

(4) **Skin.**

(a) Lesions due to *Candida* species--obtain scrapings from any portion of the lesion (center or edge) and place on a clean glass slide. If scrapings are moist, place a "match stick" or a small piece of folded paper between the bottom slide (containing scrapings) and the slide used to cover the specimen. This will prevent the two slides from "freezing" together when the scrapings dry.

(b) Lesions due to *Malassezia furfur*--collect scrapings from more than one lesion. The entire lesion may be scraped.

(c) Skin biopsy specimen--place the specimen between two moistened gauze squares. Place these in a petri dish. This permits microscopic examination of the specimen for white blood cells (pus). If the specimen is collected by punch biopsy, place it in a small tube of sterile water or sterile saline. Process the specimen in the laboratory immediately.

(5) **Processing.**

(a) Place the specimen fragments into a drop of ten percent KOH and then cover with a cover glass. Pass the slide several times through a flame; DO NOT OVERHEAT OR BOIL! Examine preparation by bright-field or phase-contrast microscopy. The more sensitive PAS stain may be used if fungi are suspected but not observed in the KOH preparation.

(b) Inoculate portions of the specimen on SDA and Mycosel™ media.

(c) Incubate at 30°C and examine every 2-3 days. Discard negative cultures after 4 weeks.

(d) Identify all fungi.
2-8. DRAINING FISTULA, SINUS TRACT

a. Swabs used to collect material from these lesions rarely yield fungi. The specimen should be obtained by curettage from deep in the lesion and should include part of the wall. Such specimens are more likely to contain granules or fungal elements.

b. The specimen should be placed in a sterile tube containing 2 to 3 mL of sterile water or saline.

c. Occasionally, if the lesion is due to an actinomycete, granules may be sloughed out into the tract. Flushing the tract with sterile saline may yield these granules. These washings should be collected in a sterile test tube. Covering the tract opening with a gauze pad for 12 to 24 hours may permit entrapment of granules in the pad; bacterial contamination of a specimen collected in this manner may suppress the growth of actinomycetes or molds. However, the morphology of organisms in these granules can usually be determined.

d. Both the washings and gauze pad should be examined with a hand lens for presence of granules. Granules should be removed with either a sterile capillary pipet or forceps and processed as outlined under "Biopsic Material."

2-9. JOINT FLUID

This specimen should be collected in a sterile tube containing either heparin or SPS to prevent clotting.

a. Centrifuge the specimen at 2500 rpm for approximately 30 minutes.

b. With sterile pipet, place the supernatant into a tube of BHI broth.

c. Inoculate 0.1 mL of the sediment to two plates each of SDA, Mycosel\textsuperscript{tm}, and BHIA.

d. Incubate one set of plates at room temperature and the second set at 35°C. Hold all cultures at least 4 weeks before discarding as negative.

2-10. NASOPHARYNGEAL

Nasopharyngeal (NP) specimens are not routinely cultured for fungi and are only one of several specimen types that may be collected when a diagnosis of systemic or disseminated aspergillosis or candidiasis is suspected.

a. Place the NP swab in a sterile dry tube or a tube containing 2 to 3 mL of Sabouraud broth. Do not use transport media. Specimens should reach the laboratory within 30 minutes.
b. Streak the swab over a SDA plate and a Mycosel™ plate.

c. If the swab is received in broth, incubate the broth with culture plates at room temperature.

2-11. PLEURAL FLUID

   a. The specimen should be collected with heparin to prevent clotting.

   b. If the specimen is not purulent, centrifuge at 2500 rpm 15 minutes.

   c. Decant and inoculate 0.1 mL of the sediment to SDA, Mycosel™, and BHIA.

   d. Make a KOH preparation and several thin smears of the sediment.

   e. Incubate the plates and remainder of specimen at room temperature for at least 4 weeks.

   **NOTE:** If the specimen is purulent, it is processed and inoculated to media in the same manner as outlined for sputum and bronchial washings. If culturing for Nocardia is requested, inoculate media to isolate these organisms before adding chloramphenicol to the sediment.

2-12. SPUTUM. BRONCHIAL WASHINGS

   a. One of the greatest challenges to the clinician and the microbiologist is diagnosis of pulmonary disease seen in the compromised host. The need for teamwork between the clinician and microbiologist is crucial. A sputum specimen must represent material from the lungs. Saliva and nasal secretions are unsatisfactory. An early morning specimen that is likely to contain secretions from the tracheobronchial tree is desirable. Collect one specimen per day for three days.

   b. Sputum should be collected and then placed in a sterile screw cap container; bronchial washings are usually placed in a sterile tube. The specimen should be examined carefully for flecks containing pus, and caseous or bloody material. Such flecks are most likely to contain fungal elements. They should be plated and smears made from them. If careful examination of the specimen does not reveal such flecks, it should be concentrated, since there may be only a few fungal cells in sputum or bronchial washings.

   c. Processing. Sputum specimens should be processed within 2-4 hours. If processing must be delayed, refrigerate the specimen.

      (1) Using ten percent KOH and/or PAS, mount the specimen for microscopic examination. Observe the specimen for fungal elements using bright-field or phase-contrast microscopy.
The sediment of concentrated specimens should be inoculated to plates (or bottles) of media rather than to agar slants. Surface area is of great importance in attempting to isolate fungi from contaminated material.

(a) Concentration of specimen for isolation of yeasts and molds.

1. Place equal volumes of digestant (N-acetyl-l-cysteine or Dithiothreital without NaOH) and the specimen in a 50-mL graduated centrifuge tube, preferably with a screw cap.

2. Mix on a Vortex mixer for 5 to 10 seconds; mixing time may be extended if sputum is tenacious.

3. Add enough M/15 phosphate buffer solution (pH 6.8-7.1) to bring the volume up to 500 mL.

4. Centrifuge at 2500 rpm for 15 minutes.

5. Decant supernatant.

6. Add enough chloramphenicol to the sediment to give final concentration of 0.05-0.1 mg/mL. If *Histoplasma capsulatum* is suspected, substitute penicillin (20 units/mL) or streptomycin (40 mg/mL) because the yeast phase of *H. capsulatum* is susceptible to chloramphenicol.

7. Mix thoroughly and place 0.1 mL of the sediment on each plate (or in each bottle) of medium.

8. Make a KOH preparation of a drop of the sediment; make three or four thin smears, and allow to air dry. These may be used for PAS, Giemsa, Acid Fast, or Gram stains.

(b) Concentration of sputum for isolation of *Nocardia* species.

1. Concentrate as outlined above.

2. Do not add chloramphenicol to sediment.

3. Inoculate plates as for mold isolation; thin smears should also be made. Cultures of molds, yeasts, and *Nocardia* species can be made using one concentrated specimen, if a portion of the sediment is inoculated to 7H10 medium before chloramphenicol is added.

(3) Inoculate portions of the specimens on SOA, Mycosel™ yeast extract phosphate plate.
(4) Incubate at 30°C and examine daily. Discard negative cultures after 4 weeks. If *Histoplasma* is suspected, discard negative cultures after 12 weeks.

(5) Identify all fungi.

2-13. THRUSH LESIONS OF ORAL CAVITY

a. Split a tongue depressor in half along its long axis.

b. Use one half the tongue depressor to gently scrape the lesion.

c. Insert the depressor into a sterile test tube and send to the laboratory immediately.

d. Tease a portion of the material on the stick, into a drop of KOH on a clean glass slide.

e. Mount with a cover slip and examine for presence of pseudohyphae and blastoconidia.

f. Scrape the remainder of the specimen onto the agar surface of a plate of Sabouraud dextrose agar containing only chloramphenicol. With a sterile loop, spread the inoculum over the medium surface.

g. Incubate the plate at 30°C for one week before discarding.

**NOTE:** Material from thrush lesions should not be collected with swabs. The fungus is usually firmly attached to the mucous membranes and only blastoconidia may be obtained using the swab. The laboratory diagnosis of thrush requires the demonstration of pseudohyphae as well as blastoconidia.

2-14. URINE SPECIMEN

a. This specimen may yield *c. neoformans* in a patient with cryptococcosis before the organism is revealed in sputum or spinal fluid. It also may be a valuable specimen when testing a patient for disseminated candidiasis or candidiasis of the urinary tract. Infections of the upper bladder or upper urinary tract may sometimes require urine specimens collected from each kidney by means of a cystoscope. Before collecting specimens, the bladder should be flushed to reduce chances of contaminating specimens obtained from the kidneys with yeasts that may be in the bladder. The three specimens (one from each kidney and one from the bladder) should be clearly labeled and the laboratorian must be careful not to mix them during processing.

b. A urine specimen collected by percutaneous needle biopsy reduces the problem of contamination with organisms present in the lower urethra or external genitalia. A clean catch midstream specimen should be collected when percutaneous bladder aspiration and cystoscopy are thought to be unnecessary by the physician.
c. Process urine within 2 to 4 hours of collection. Refrigerate specimens if a delay in processing is unavoidable.

(1) Centrifuge urine at 2000 rpm for 10 to 15 minutes. Decant the supernatant.

(2) Direct examination. Prepare the specimen for direct examination by mixing a drop of sediment with one percent KOH and/or PAS on a clean glass slide, then coverslip. Observe for fungal elements.

(3) Place 0.05 to 0.1 mL of the sediment on each plate of SDA, Mycosel\textsuperscript{tm}, and BHIA.

(4) Incubate at 30\degree C for 2 weeks before discarding as negative.

(5) Examine every 2 to 3 days for growth. Identify all fungi.

2-15. VAGINAL

a. Several swabs containing material from the vagina should be inserted into tubes of Sabouraud broth and sent to the laboratory immediately. A transport medium is not recommended.

**NOTE:** Although *Candida albicans* will survive in most transport media, insufficient data is available concerning the survival of other fungi in these media. When transport media are used, an adequate specimen adhering to the swab is usually not available for KOH preparations.

b. Tease material from a swab directly into a drop of KOH on a clean glass slide. Mount with a coverslip and examine the preparation carefully for any fungal elements.

c. Use a second swab to streak a plate of Sabouraud agar containing chloramphenicol.

d. Incubate plates at 25\degree C for 4 to 5 days before discarding as negative.

Continue with Exercises
EXERCISES, LESSON 2

INSTRUCTIONS: Answer the following exercises by marking the lettered response that best answers the exercise, by completing the incomplete statement, or by writing the answer in the space provided at the end of the exercise.

After you have completed all of these exercises, turn to "Solutions to Exercises" at the end of the lesson and check your answers. For each exercise answered incorrectly, reread the material referenced with the solution.

1. Direct examination of smears for fungal morphology is done using:
   a. A monocular microscope.
   b. Phase-contrast microscopy.
   c. Smears made from culture growth.
   d. Gram stain.

2. Media used to isolate fungi from abscess specimens include:
   a. Mycosel™.
   b. Sabouraud dextrose agar.
   c. Brain heart infusion agar.
   d. All of the above.

3. Mycology specimens obtained from a liver biopsy usually include:
   a. Portions of several nodes.
   b. Portions of the wall, base, and center of the lesion.
   c. A single small tissue core.
   d. Purulent material.
4. The presence of "granules" in biopsy tissue confirms the presence of a *Nocardia* species infection.
   a. True.
   b. False.

5. Microscopic examination of an actinomycotic granule shows:
   a. Hyphae 4-7 mcm in diameter.
   b. Beaded branching filaments.
   c. Filamentous nonbranching bacilli.
   d. Differences between aerobic and anaerobic actinomycetes.

6. Collection of blood for the detection of fungemia requires the use of ______________________ as an anticoagulant.
   a. EDTA.
   b. Sodium citrate.
   c. Citrate-phosphate-dextrose.
   d. Sodium polyanethol sulfonate (SPS).

7. The most commonly found fungal infection is:
   a. Candidiasis.
   b. Histoplasmosis.
   c. Tuberculosis.
   d. Botryomycosis.
8. Blood culture bottles for fungal growth must be:
   a. Incubated at 30°C.
   b. Incubated under anaerobic conditions.
   c. Examined every 2-3 days for growth.
   d. Vented to create aerobic conditions.

9. The syringe used to collect bone marrow for fungal study should be:
   a. Coated with heparin before specimen collection.
   b. Rinsed with BHIB and the rinse fluid incubated.
   c. Capped for transportation.
   d. All of the above.

10. Use of the membrane filter technique for cerebral spinal fluid permits the:
    a. Use of the Sabhi agar culture medium.
    b. Simultaneous culture of fungi and *H. tuberculosis*.
    c. Identification of *c. neoformans*.
    d. Subculturing of early growth.

11. Specimens of hair, for isolation of fungi, should be cultured on:
    a. BHIB medium.
    b. Mycosel™ medium.
    c. KOH medium.
    d. Sabhi broth.
12. Collection of a specimen from glabrous skin is done by:
   a. Flushing the infected area with sterile water.
   b. Covering an open lesion with a gauze pad for 12 to 24 hours.
   c. Scraping the periphery of a lesion with a sterile scalpel.
   d. Removing loose cells with sterile forceps.

13. The best method for collecting a specimen for fungal studies from a draining fistula is the use of a swab.
   a. True.
   b. False.

14. Joint fluid specimens are inoculated on/into:
   a. Sabouraud broth.
   b. BHI broth.
   c. A membrane filter.
   d. Blood culture medium.

15. Flecks which may be seen in sputum specimens are likely to:
   a. Contain fungal elements.
   b. Indicate bacterial infection.
   c. Interfere with mycology studies.
   d. Indicate specimen contamination.
16. How long must culture plates be retained if *Histoplasma* is suspected?
   a. 2 weeks.
   b. 4 weeks.
   c. 8 weeks.
   d. 12 weeks.

17. Laboratory diagnosis of thrush:
   a. Is usually made from the culture of a sputum specimen.
   b. Depends on the demonstration of blastoconidia.
   c. Depends on the demonstration of pseudohyphae.
   d. Requires the demonstration of blastoconidia and pseudohyphae.

18. Culture of a urine specimen:
   a. Is helpful in looking for candidiasis of the urinary tract.
   b. May yield *c. neoformans*.
   c. Is helpful in looking for disseminated candidiasis.
   d. All of the above.

Check Your Answers on Next Page
1. b (para 2-2b(1))
2. d (para 2-2b(2))
3. c (para 2-3a)
4. b (para 2-3c)
5. b (para 2-3c(4)(c))
6. d (SPS) (para 2-4b)
7. a (para 2-4a)
8. d (para 2-4d(4))
9. d (para 2-5b)
10. b (para 2-6a)
11. b (para 2-7a(3)(b))
12. c (para 2-7b(1))
13. b (para 2-8(a))
14. b (para 2-9)
15. a (para 2-12b)
16. d (para 2-12c(4))
17. d (para 2-13)
18. d (para 2-14a)

End of Lesson 2