

SHANDS Jacksonville
MICROBIOLOGY
SPECIMEN COLLECTION MANUAL

MICROBIOLOGY SPECIMEN COLLECTION MANUAL

TABLE OF CONTENTS

| | <i>Page</i> |
|--|-------------|
| Foreword..... | 4 |
| General Information..... | 5 |
| Specimen Selection and Collection..... | 5 |
| Specimen Transportation..... | 5 |
| Specimen Labeling..... | 5 |
| Unacceptable Specimens..... | 6 |
| Collection Materials..... | 8 |
| Collection and Transport Supplies..... | 8 |
| Swabs and Transport Media..... | 10 |
| Blood Cultures..... | 12 |
| Miscellaneous..... | 14 |
| Collection Instructions..... | 16 |
| AFB Culture..... | 16 |
| Blood Culture..... | 17 |
| Chlamydia Culture..... | 18 |
| Ear Culture..... | 19 |
| Eye Culture..... | 20 |
| Fluid Culture..... | 21 |
| Fungus Culture..... | 21 |
| Respiratory Specimens..... | 23 |
| Sputum Specimens..... | 23 |
| Nasal Specimens..... | 24 |
| Nasopharyngeal Specimens for <i>Bordetella pertussis</i> | 24 |
| Sinus Specimens..... | 25 |
| Throat Cultures for Group A Strep..... | 25 |
| Nares Specimens for MRSA Surveillance..... | 26 |
| Stool Specimens..... | 27 |
| Routine Stool Culture..... | 27 |
| Cryptosporidium/Giardia DFA..... | 28 |
| Clostridium Difficile Toxin Assay..... | 29 |
| Scotch Tape Collection for Pinworm..... | 29 |
| Tissue Culture..... | 30 |
| Urine Culture..... | 30 |
| Collection of a Midstream Clean Catch Specimen..... | 31 |
| Transferring Urine Specimens into a Urine Preservative Tube..... | 31 |
| Urogenital Specimens..... | 32 |
| Collection of Specimens for Genital Culture..... | 32 |
| Collection of Specimens Using the ProbeTec Collection and Transport System..... | 33 |
| Collection of Specimens for <i>N. gonorrhoeae</i> Culture Using the JEMBEC Transport System..... | 34 |
| Virology Specimens..... | 34 |
| Culture Collection Guidelines..... | 34 |
| Culture Collection Procedures..... | 35 |
| Rotavirus Antigen..... | 36 |
| RSV Antigen..... | 36 |
| Influenza Antigen..... | 36 |
| Wound Culture..... | 37 |
| Serology Specimens..... | 38 |
| Cryptococcal Antigen..... | 38 |

**MICROBIOLOGY SPECIMEN COLLECTION MANUAL
TABLE OF CONTENTS**

| | <i>Page</i> |
|--|-------------|
| CMV IgG Antibody..... | 38 |
| CMV IgM Antibody..... | 38 |
| <i>Helicobacter pylori</i> IgG Antibody..... | 38 |
| Acute Hepatitis Panel..... | 38 |
| Hepatitis A Total Antibody..... | 38 |
| Hepatitis A IgM Antibody..... | 38 |
| Hepatitis B Surface Antibody..... | 38 |
| Hepatitis B Surface Antigen..... | 38 |
| Hepatitis C Antibody..... | 38 |
| Hepatitis B Core Total Ab..... | 38 |
| Hepatitis B Core IgM Ab..... | 38 |
| Herpes simplex IgG Antibody..... | 38 |
| HIV Antibody..... | 38 |
| Legionella Urine Antigen..... | 38 |
| Measles/ Rubeola Immune Status..... | 38 |
| Monoscreen..... | 38 |
| Rubella Immune Status..... | 38 |
| <i>S. pneumoniae</i> Urine Antigen..... | 38 |
| Syphilis – RPR..... | 39 |
| Syphilis – VDRL..... | 39 |
| <i>Toxoplasma</i> IgG Antibody..... | 39 |
| <i>Toxoplasma</i> IgM Antibody..... | 39 |
| Varicella (VZV) Immune Status..... | 39 |
| Molecular Microbiology Tests..... | 39 |
| CMV Viral Load..... | 39 |
| Enterovirus PCR..... | 39 |
| Hepatitis C Viral Load..... | 39 |
| Hepatitis C Genotype..... | 39 |
| Herpes Simplex Virus PCR..... | 39 |
| HIV Viral Load..... | 39 |
| Unlisted Tests..... | 39 |

FOREWORD

When collecting any specimens, employees must follow the guidelines for Standard Blood/Body Fluid Precautions, Isolation Precautions and OSHA Bloodborne Pathogen Standards as outlined in the **Exposure Control Plan for Bloodborne Pathogens** (IC-01-005) and **Airborne Pathogens Exposure Control Plan** (IC-01-006).

GENERAL INFORMATION

Specimen Selection and Collection:

1. Specimens should always be collected before antimicrobial therapy is initiated. Collection during therapy may suppress the growth of pathogenic organisms resulting in a false negative culture.
2. Material should be collected from a site where the suspected organism(s) is most likely to be found, with little or no external contamination. The site should be cleaned with soap and then isopropyl alcohol, if appropriate. Collection of normally sterile body fluids by percutaneous needle aspiration should always be preceded by proper skin decontamination.
3. Collect adequate specimen volume. Insufficient material may yield false negative results.
4. Place the specimen in a container designed to promote the survival of the suspected agent(s) and to eliminate leakage.
5. A source/site must be specified for all specimens submitted. All test requests, including any special requests must be included on the requisition or in the comment area in the computer.

Specimen Transport:

1. All specimens must be promptly transported to the laboratory, preferably within 2 hours of collection.
2. Optimal transport times for clinical specimens depend on the volume of material collected. Small volumes should ideally be submitted within 15-30 minutes to minimize evaporation, drying and exposure to ambient conditions.
3. Specimens should be transported in a device designed to promote the survival of the suspected agent(s). See **Collection Materials**.

SPECIMEN LABELING

Every specimen must be clearly labeled with the **patient's name**, the **specimen type** and the **date and time of collection**. Unlabeled or mislabeled specimens cannot be processed. Unlabeled or mislabeled specimens that cannot be recollected (i.e. CSF) will not be processed without the approval of the Microbiology Director, relabeling of the specimen by the person who collected the specimen and completion of a RAQ report. A notation will be made in the patient's report indicating the labeling error.

Labeling information must be placed on the specimen container or transport swab. Never put the label on an outer wrapper that could be removed.

UNACCEPTABLE SPECIMENS

1. The following conditions will adversely affect the processing of Microbiology specimens:
 - a. Specimens received after prolonged delay
 - b. Specimen containers that have leaked
 - c. Unlabeled or mislabeled specimens
 - d. Specimens received with no time of collection
 - e. Specimens collected improperly
 - f. Specimen unsuitable for request
 - g. Insufficient amount of material
 - h. Incorrect container
 - i. Incorrect anticoagulation
 - j. Duplicate specimens on the same day for the same test request (except bloods and tissue)

2. The following specimens are unsatisfactory for culture/testing and cannot be accepted by the laboratory:
 - a. Foley catheter tips
 - b. Needled syringes
 - c. Voided urines
 - d. Vaginal lochia
 - e. Decubitus ulcers
 - f. Perirectal abscesses
 - g. Pilonidal abscesses
 - h. Bowel contents
 - i. Vomitus
 - j. Gastric contents
 - Gastric aspirates are acceptable for mycobacteriology (AFB) culture
 - k. Mouth/oropharynx
 - Mouth specimens are acceptable for fungus culture
 - l. Nasopharynx/nose/nasal
 - These specimens are acceptable for the isolation of *Staphylococcus aureus* and *Neisseria meningitidis* only
 - m. Stool specimens on patients who have been in-house for greater than 5 days
 - These specimens are acceptable for *C. difficile* toxin testing only

3. In the event that a specimen cannot be processed, specimens will be held for 7 days. Both a verbal and written report will be rendered suggesting the recollection of another specimen. Physicians may consult with the Microbiology Director (Dr. Yvette McCarter 244-6684) regarding processing of such specimens.

4. Rejection Criteria for Lower Respiratory and Wound Specimens
 - a. When lower respiratory tract secretions or wounds demonstrate more squamous cells than neutrophils on direct Gram stain examination, a report will be rendered suggesting that the specimen represents superficial material and suggesting repeat collection avoiding contaminating and colonizing bacteria.

- b. Culture results are correlated with the Gram stain results of the direct smear. If there is evidence of superficial contamination and multiple potential pathogens are isolated, these may be reported generically without antimicrobial susceptibility testing and with the indication that isolates may not be related to infection.
- c. Cultures are held for 7 days pending any consultation to perform additional work on the specimen.

5. Urine Culture Workup

- a. Urine cultures with more than three isolates and cultures containing small numbers of organisms will be reported generically without antimicrobial susceptibility testing and with the indication that isolates may not be related to infection.

COLLECTION MATERIALS

Appropriate collection devices, specimen containers and transport medium must be used to ensure optimal recovery of organisms. The majority of collection materials are provided by Central Supply. The remainder of supplies may be obtained from the Microbiology Laboratory, as indicated.

All collection materials except sterile specimen cups have an expiration date. Always check the date before using any collection equipment and discard any outdated material.

Collection equipment may be obtained by calling (X42231) or faxing (X43472) Central Supply. The remaining items may be obtained in the Microbiology Laboratory.

Collection and Transport Supplies

See pictures on following page

| Item | Used for | Special considerations | Obtain from |
|--|---|--|---|
| (a) Cup, specimen, sterile | Sputum, stool, urine catheter tips, tissue | | Central Supply |
| (b) Cup, sputum collector | Sputum for AFB smear and culture | | Central Supply |
| Tube, vacutainer, lavender, EDTA | Blood parasites (malaria, <i>Babesia</i>) | Always send as a STAT | Central Supply |
| (c) Tube, sterile (black top) | Body fluids for culture | Do not submit CSF in this tube | Central Supply |
| (d) CSF collection set | CSF collection | | Central Supply |
| (e) Para-Pak (EcoFix) vial | Parasitology (routine ova and parasite exam, <i>Cryptosporidium</i> / <i>Giardia</i> DFA, <i>Isospora</i> / <i>Cyclospora</i>) | | Microbiology Laboratory |
| (f) Outpatient Para-Pak stool collection kit | Routine stool culture, parasitology, <i>C. difficile</i> toxin assay | Kit contains EcoFix vial (Parasitology), C&S vial (routine culture) and clean vial (<i>C. difficile</i>) | University of Florida Outreach Laboratory Client Services |

(a)



(b)



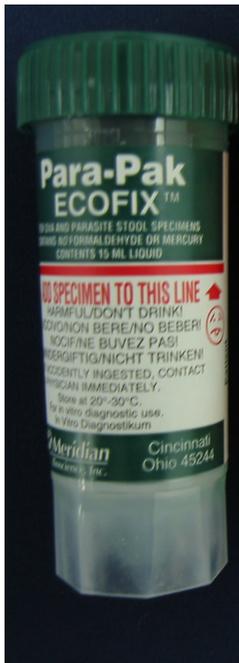
(c)



(d)



(e)



(f)



Swabs and Transport Media

See pictures on following page

Note: Swabs are not appropriate for the collection of mycobacteriology (AFB) or fungus cultures. Tissues, fluids or scrapings should be submitted.

| Item | Used for | Special considerations | Obtained from |
|---|--|---|-------------------------|
| (a) Swab and transport medium | Bacteriology cultures | | Central Supply |
| (b) Swab and transport medium (charcoal), mini-tip, wire | Bacteriology cultures (small orifices such as urethra and nasopharynx) | | Microbiology Laboratory |
| (c) Microtest medium Contains: – Multi-microbe transport medium – 2 Dacron swabs with plastic shaft Optional: – Rayon swab with aluminum shaft | Virus and <i>Chlamydia</i> cultures – Use swab with plastic shaft for large orifices – Use rayon swab with aluminum shaft for small orifices | Store and transport under refrigeration or on ice | Microbiology Laboratory |
| BD ProbeTec ET CT/NG swabs – Pink (female) – Blue (male) | Detection of <i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoeae</i> by nucleic acid amplification | Cannot be used for <i>Chlamydia</i> or <i>Neisseria gonorrhoeae</i> culture. Cannot be used for nasopharyngeal or conjunctival specimens | Central Supply |
| (e) Copan Transport Swab | Collection of nares specimens for MRSA surveillance | Cannot be used for routine bacteriology cultures | Central Supply |

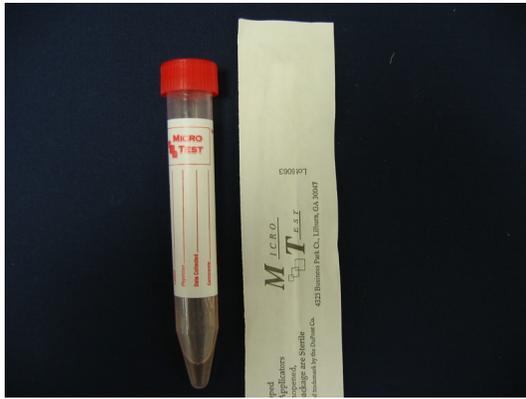
(a)



(b)



(c)



(d)



(e)



Blood Cultures

See pictures on following page

| Item | Used for | Special considerations | Obtained from |
|---|---|---|-------------------------|
| (a) Bottles, Blood Culture Procedure Tray (adult) | Bacteriology blood cultures in adults | Set includes an aerobic and anaerobic bottle and all necessary collection materials | Central Supply |
| (b) Bottle, Blood Culture Peds Plus (pediatric) | Bacteriology blood cultures in pediatric patients | | Central Supply |
| (c) Bottle, Myco F Lytic | AFB/Fungus blood cultures only | Not for routine bacteriology | Microbiology Laboratory |

(a)



(b)



(c)



Miscellaneous

See pictures on following page

| Item | Used for | Special Considerations | Obtained from |
|--|---|--|-------------------------|
| (a) <i>Bordetella pertussis</i> collection kit | Whooping cough | Kit includes: swab and transport medium | Microbiology Laboratory |
| (b) Corneal scraping collection kit | Submitting corneal scraping for culture | Kit includes: blood agar, chocolate agar, Sabouraud agar, thioglycolate and 2 glass slides | Microbiology Laboratory |
| (c) Acanthamoeba collection kit | Acanthamoeba culture of corneal scrapings | Kit contains small vial with sterile saline and collection instructions | Microbiology Laboratory |
| (d) <i>Neisseria gonorrhoeae</i> collection kit (Jembec plate) | | Kit includes: Jembec plate, CO ₂ tablet, Dacron swab, small plastic bag and collection instructions | Microbiology Laboratory |

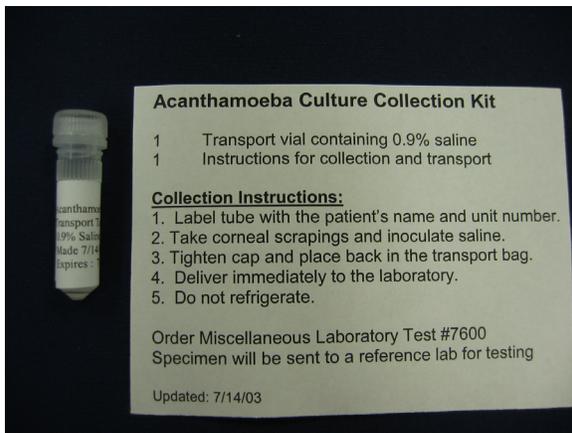
(a)



(b)



(c)



(d)



COLLECTION INSTRUCTIONS

AFB CULTURE

AFB Culture and Smear Test Code #4803
AFB Blood Culture Test Code #4804

Specimens received in the Microbiology Laboratory by **10:00 AM** will be processed the same day.

1. General Collection Guidelines
 - a. Collect specimens before chemotherapy is started, even a few days of drug therapy may kill or inhibit sufficient numbers of acid-fast bacilli to leave confirmation of disease in doubt.
 - b. Collect specimens in clean, sterile, leak proof, one-use, plastic, disposable containers. Sterile body fluids should be placed in sterile glass screw top tubes. Specimens should not be placed in anticoagulants such as EDTA, heparin, citrate or ACD.
 - c. Collect specimens aseptically minimizing contamination with indigenous flora
 - d. SPECIMENS SHOULD NOT BE COLLECTED ON SWABS
 - e. Collect sufficient material for the tests requested
 - f. DO NOT place specimens in preservative or fixative

2. Respiratory Specimens
 - a. Collect only the exudative material brought up from the lungs after a deep, productive cough.
 - b. At least 2 mL of sputum should be submitted for evaluation
 - c. First morning sputum specimens are the best.
 - d. Specimens should be refrigerated immediately after collection to minimize the growth of normal oral flora.
 - e. Collect a series of three early morning sputum specimens on successive days. The maximum number acceptable within a 7-day period is three specimens. A 24-hour sputum collection is not acceptable. The single random specimen is easier to handle in the laboratory and is less likely to be contaminated.

3. Extrapulmonary Specimens
 - a. Body fluids and tissues: collect aseptically by the physician using aspiration techniques or surgical procedure. Collect as much specimen as possible. Aseptically collected tissue specimens are placed in sterile containers without fixatives or preservatives.
 - b. Clean catch urine specimens: collect a single, early morning, midstream clean catch specimen or the total first morning specimen should be collected. 24 hr urine collections are not acceptable.
 - c. Blood cultures: collect aseptically in a Bactec Myco/F lytic bottle (see **Collection Materials and Blood Cultures**)
 - d. Swabs: Swabs are sub optimal for the recovery of mycobacteria and should not be collected. **Swabs received for AFB culture will not be processed.**

BLOOD CULTURE

| | |
|----------------------|-----------------|
| Culture Blood | Test Code #4812 |
| AFB Blood Culture | Test Code #4804 |
| Blood Culture Fungus | Test Code #4813 |

A total of three routine blood cultures collected during a 24-hour period is optimal for the detection of septicemia. Single blood culture sets are considered useless in the detection of septicemia and should be discouraged. Only one blood culture per 24-hour period is necessary for fungus and AFB blood cultures.

Blood cultures should always be taken before antimicrobial therapy is initiated. In case therapy has begun, please make a notation on the laboratory requisition and list the antibiotic(s). The effect of prior antibiotic therapy can be minimized by collection of blood for culture just prior to the next antibiotic dose (antibiotic trough).

Blood cultures should be collected directly into blood culture bottles and transported to the laboratory as soon as possible.

In adults the optimal volume of blood to collect per set (i.e. venipuncture) is 20 mL. A minimum of 5 mL and a maximum of 10 mL of blood should be placed in each routine blood culture bottle (aerobic and anaerobic). Culturing a suboptimal volume of blood may lead to false negative results.

Collection Procedure:

1. Always completely and accurately verify that the patient's identification exactly matches the name as it appears on the order.
2. Remove the tab from the top of each blood culture bottle. Swab the diaphragm tops with a fresh alcohol prep and allow to air dry.
3. Cleanse the venipuncture site
 - a. Using a fresh iodine pad, swab the skin by making concentric circles in an outward direction. Allow the iodine to dry for a minimum of 1 minute.
 - b. Using a fresh alcohol prep, swab the skin by making concentric circles in an outward direction.
 - c. Allow site to air dry.
 - d. Do not palpate vein after treating skin.
4. Special Venipuncture Notes
 - a. Use extra precaution in not allowing contact between the needle and any other objects (i.e. gauze) prior to inoculation.
5. Inoculate blood culture bottles by aseptically inoculating each bottle of the twin pack with 10mL of blood per bottle (optimal).
 - a. Place bottles on a secure and flat surface. Do not hold bottles during inoculation.

- b. If less than 20mL of specimen is obtained first inoculate the aerobic bottle with a full 10mL. Inoculate the second bottle with the remaining volume of specimen.
 - c. Mix both bottles by rotation (not inversion).
6. For AFB and/or Fungus blood cultures, aseptically inoculate each Myco F Lytic bottle.
 - a. Inoculate blood by aseptically inoculating with 10mL of blood per bottle (optimal).
 - b. A separate Myco F Lytic bottle must be submitted for AFB and Fungus requests.
 7. Label each bottle of each set before leaving the patient.
 - a. Record the date and the time of collection and your initials on the bottle(s).
 8. Collect subsequent set(s) of blood cultures following the above procedure.
 - a. Subsequent sets should be collected from a second venipuncture site.
 9. Procedure Notes
 - a. Consultation with the Microbiology Director (244-6684) is required if more than four cultures are requested in any 24 hour period.
 - b. If there is additional blood work ordered with the cultures, inoculate the vacutainer tubes after the culture bottles.
 - c. Always use extreme caution when inoculating blood culture bottles. Never hand-hold bottles during inoculation.

CHLAMYDIA CULTURE

Culture Chlamydia Test Code #4875

Specimen collection and transport are very important for the accuracy of *C. trachomatis* diagnostic testing. The sensitivity and specificity of *Chlamydia* culture are directly related to the adequacy of the specimen collected.

Appropriate specimens for *Chlamydia* culture include: ocular specimens; male and female genital tract specimens; bubo pus; respiratory specimens from infants < 1-month-old (specimens on older infants and adults should be cultured for *Chlamydia pneumoniae*); tissues and needle aspirates; and rectal swabs and biopsies of the gastrointestinal tract (stool is not acceptable).

Procedure for Collection of Specimens for *C. trachomatis* Culture:

1. Collection of male urethral specimens
 - a. Open Microtest Medium pack. Collect specimen using rayon swab with aluminum shaft.
 - b. Collect the urethral specimen at least one hour after urination.
 - c. Insert the rayon swab 2-4 cm into the urethra and rotate for 3-5 seconds. Note that epithelial cells must be collected, not pus.
 - d. Withdraw the swab and place the Microtest Medium tube.
 - e. Make sure the cap is tightly secured to the tube.

- f. Label the tube with patient information and date/time collected.
 - g. Transport the swab immediately on ice to the laboratory.
2. Collection of female endocervical specimens
 - a. Open Microtest Medium pack. Remove the Dacron swabs.
 - b. Remove excess mucus from the cervical os with one of the Dacron swabs and discard. Note that vaginal specimens are acceptable for *Chlamydia* isolation in pre-pubertal females only.
 - c. Insert the other Dacron swab into the cervical canal and rotate for 15-30 seconds. Note that epithelial cells must be collected, not pus or mucous.
 - d. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.
 - e. Immediately place the swab into the Microtest Medium tube. Make sure the cap is tightly secured to the tube.
 - f. Label the tube with patient information and date/time collected.
 - g. Transport the swab immediately on ice to the laboratory.
 3. Collection of conjunctival specimens
 - a. Open Microtest Medium pack. Collect specimen using rayon swab with aluminum shaft.
 - b. Gently remove pus or discharge and discard.
 - c. Swab inside of lower, then upper lid. Specimens should be collected in a vigorous manner in order to remove a representative sample of epithelial cells.
 - d. Immediately place the swab into the Microtest Medium tube. Make sure the cap is tightly secured to the tube.
 - e. Label the tube with patient information and date/time collected.
 - f. Transport the swab immediately on ice to the laboratory.
 4. Other Specimens
 - a. Respiratory specimens, aspirates and tissue should be collected and transported to the laboratory in a sterile container or tube.
 - b. Rectal swabs should be collected using Microtest Medium and a Dacron swab. Routine bacteriology transport swab devices are unacceptable.

EAR CULTURE

Culture Ear and Gram Stain Test Code #4827

External

Cultures of the **external ear** are of limited value since normal skin flora is found in this area. In order to obtain an adequate, significant culture, there should be a visible purulent drainage.

Middle

Cultures from the **middle ear** cannot be collected using a sterile transport swab. The physician must collect an aspirate from the middle ear using a syringe. The fluid should be inoculated into a sterile tube for transport to the laboratory.

Procedure for collection of swab specimens:

1. The transport swab consists of 2 Dacron swabs on a plastic shaft secured to a cap. The swabs contained within the cap are inserted into a plastic transport tube containing a semi solid gel to keep the specimen from drying during transport.
2. The external ear is thoroughly cleansed with a sterile swab and saline.
3. Open transport swab pack, and peel apart at the point labeled "TO OPEN" until the swab cap is visible.
4. Remove the sterile swabs, and collect new drainage as it appears.
5. Remove the transport tube of medium from the package.
6. Remove and discard the cap from the tube. Place the swabs into the medium, and push the swab cap firmly onto the tube.
7. Label and send to the laboratory immediately.
8. Specimens should be stored at room temperature prior to transportation to the laboratory.

EYE CULTURE

Culture Eye and Gram Stain Test Code #4830

"Eye" cultures are actually cultures of the conjunctiva. Cultures should be labeled as to which eye they were obtained from i.e. left or right. If both eyes are cultured, a separate specimen should be collected from each eye.

Corneal scrapings may have the culture media inoculated at the bedside. A corneal scraping collection kit is available from the Microbiology Laboratory (see **Collection Materials**)

Acanthamoeba cultures are sent to a Reference Laboratory. An Acanthamoeba collection kit is available from the Microbiology Laboratory (see **Collection Materials**)

Procedure for collecting conjunctival specimen:

1. The transport swab consists of 2 Dacron swabs on a plastic shaft secured to a cap. The swabs contained within the cap are inserted into a plastic transport tube containing a semi solid gel to keep the specimen from drying during transport.
2. Open transport swab pack, and peel apart at the point labeled "TO OPEN" until the swab cap is visible.
3. Remove the sterile swabs and collect conjunctival specimen.
4. Remove the transport tube of medium from the package.

5. Remove and discard the cap from the tube. Place the swabs into the medium, and push the swab cap firmly onto the tube.
6. Label and send to the laboratory immediately.
7. Specimens should be stored at room temperature prior to transportation to the laboratory.
8. Conjunctival specimens can also be collected using a Mini Tip Swab. This swab has small rayon swab and flexible aluminum wire shaft and is used as listed above.

Note: If *Chlamydia trachomatis* is suspected a conjunctival specimen should be collected for *Chlamydia* Culture using Microtest medium (see **Collection Materials** and **Chlamydia Culture**).

FLUID CULTURE

Culture Body Fluid and Gram Stain Test Code #4815

Collection of normally sterile body fluids by percutaneous needle aspiration should always be preceded by appropriate skin decontamination.

The physician should attempt to collect as much fluid as possible.

Fluids for culture SHOULD NOT be placed into vacutainer tubes containing EDTA, heparin or citrate since these compounds are inhibitory to bacteria.

Spinal fluid is transported to the laboratory in the original collection tube; all other fluids should be inoculated into a sterile tube. Specimens should be kept at room temperature and transported to the laboratory immediately.

Gram stains are performed on centrifuged sediments from all fluid specimens.

FUNGUS CULTURE

Culture Fungus and Smear Test Code #4833

Blood Culture Fungus Test Code #4813

1. General Specimen Collection Guidelines
 - a. Collect specimens aseptically in clean, sterile, one-use plastic disposable containers.
 - b. Fluids should not be collected in tubes with EDTA, heparin or citrate anticoagulant.
 - c. Swabs are not advisable for routine mycology culture except for ear canal, throat, vagina and cervical specimens and scalp and skin specimens for dermatophytes.
 - d. Transport specimens to the laboratory as soon as possible. Specimens should be received in the laboratory within 2 h of collection.

e. Label the specimen with patient name and date and time collected.

2. Specimen Collection Procedures

a. Sputum

- 1) Have the patient remove dentures, if present, and rinse mouth
- 2) Collect only the exudative material brought up from the lungs following a deep productive cough.

b. Bronchial washings, lung, biopsy, trans-tracheal aspiration, bronchial aspiration and bronchial lavage are collected aseptically and sent to the laboratory in a sterile container.

c. Pus, exudate, drainage and abscess material

- 1) Using a sterile needle and syringe, aspirate material from an undrained abscess. Place in a sterile container.
- 2) If it is not possible to use a syringe, use a sharp-pointed scalpel to express pus and place in a sterile container.

d. Tissue

- 1) Collect tissue aseptically from center and edge of lesion.
- 2) Place specimen in a sterile container, add a small amount of sterile water or non-bacteriostatic saline to keep tissue from drying out and send immediately to the laboratory.

e. Vaginal material

- 1) Using a sterile swab collect material from the vagina. Insert swab into transport media.

f. Bone Marrow

- 1) Aspirate approximately 3 to 5 mL of bone marrow and place in a sterile container. DO NOT place specimen in blood culture bottles.

g. Urine

- 1) Collect a clean catch midstream, catheterized specimen, or cystoscopy specimen
- 2) First morning specimens are preferred
- 3) 24 h urine specimens are not acceptable

h. Body fluids (CSF, pleural, synovial, and peritoneal)

- 1) Collect specimens aseptically and place in a sterile container. Collect as much specimen as possible.
- 2) Do not place fluids in tubes with EDTA, heparin or citrate anticoagulant.

i. Nail

- 1) Clean nail with 70% alcohol
- 2) For a specimen of the dorsal plate, scrape the outer surface and discard the scrapings. Then scrape the deeper portion for a specimen.
- 3) Remove a portion of debris from under the nail with a scalpel.
- 4) Collect the whole nail or nail clippings.
- 5) Place all material in a clean sterile container.

j. Skin and interspaces

- 1) Wipe lesions and interspaces between the toes/fingers with an alcohol sponge or sterile water.
- 2) Scrape the entire lesion(s) and both sides of the interspaces with a sterile scalpel.
- 3) Place scrapings in a clean sterile container.

k. Scalp or skin swabs for dermatophytes

- 1) Rub area vigorously to remove fungal elements.
- 2) Place swab in routine bacterial culture transport medium
- I. Hair
 - 1) Remove hairs from the scalp with sterile forceps. Try to obtain the root of the hair.
 - 2) Place hair in a clean sterile container.
- m. Blood: collect aseptically in a Bactec Myco/F lytic bottle (see **Collection Materials and Blood Culture**)
- n. Mouth/throat
 - 1) Remove oral secretions and debris from the surface of the lesion with a swab and discard.
 - 2) Using a second swab, vigorously sample the lesion, avoiding any areas of normal tissue
 - 3) Place swab in routine bacterial culture transport medium

RESPIRATORY SPECIMENS

| | |
|------------------------------------|-----------------|
| Quant BAL Culture | Test Code #4872 |
| Culture Respiratory and Gram Stain | Test Code #4845 |
| Legionella Culture and Smear | Test Code #4869 |
| Pneumocystis Smear | Test Code #4955 |

Sputum Specimens

Procedure for collection of expectorated sputum:

1. Before collecting a sputum specimen, the patient should rinse their mouth with water and remove dentures. Rinsing the mouth lessens the contamination of sputum specimens from oropharyngeal secretions and their associated normal oral flora.
2. Sputum specimens must contain lower respiratory tract secretions.
3. Patients should be instructed to cough as deeply as possible. Appropriately collected induced specimens or aspirations are recommended for adult patients who cannot produce acceptable sputum samples. Consultation with the respiratory therapy department may be required.
4. Collect the sputum specimen generated from a deep, productive cough in a clean, sterile specimen cup. The traps used with suction devices are also acceptable.
5. The specimen should be refrigerated and transported to the laboratory immediately.

First morning sputum specimens are the best especially if a mycobacteria (AFB) culture has been ordered.

One sputum specimen per 24-hour period is accepted.

Expectorated sputum specimens are unacceptable for Pneumocystis testing. An induced sputum or bronchoscopy specimen should be submitted.

Note: The laboratory performs a Gram stain on all sputum specimens for routine culture. Specimens containing a significant number of squamous epithelial cells indicate the presence of superficial (oropharyngeal) contamination.

In such cases the specimen will not be processed for routine culture. The laboratory will note the presence of oropharyngeal contamination and suggest the collection of another specimen.

Nasal Specimens

Culture Respiratory and Gram Stain Test Code #4845

Nasal /nose cultures are of limited value and should only be used for the detection of *Staphylococcus aureus* and *Neisseria meningitidis* colonization. Nasal cultures should not be used for the diagnosis of sinusitis or sinus tract pathogens. Gram stains will not be performed on nasal cultures.

Procedure:

1. The transport swab consists of 2 Dacron swabs on a plastic shaft secured to a cap. The swabs contained within the cap are inserted into a plastic transport tube containing a semi solid gel to keep the specimen from drying during transport.
2. Open transport swab pack, and peel apart at the point labeled "TO OPEN" until the swab cap is visible.
3. Remove the sterile swabs, and collect culture by inserting swab at least 1 to 1.5 inches into the nose.
4. Remove the transport tube of medium from the package.
5. Remove and discard the cap from the tube. Place the swabs into the medium, and push the swab cap firmly onto the tube.
6. Label and send to the laboratory immediately.
7. Specimens should be stored at room temperature prior to transportation to the laboratory.
8. Nasopharyngeal specimens can be collected using a Mini Tip Swab. This swab has small rayon swab and flexible aluminum wire shaft and is used as listed above.

Nasopharyngeal Specimens for *Bordetella Pertussis* PCR

Pertussis PCR Test Code #7600

Procedure:

1. Kit Contents

Transport system containing charcoal transport media and wire swab

2. Precautions
 - a. For optimal results, specimens should be obtained early in the course of disease, preferably during the first week and before the characteristic cough occurs.
 - b. DO NOT use the transport medium if it has expired.
 - c. For optimal results, each side of the nasopharynx should be separately sampled.

3. Instructions for Use
 - a. Immobilize the patient's head and gently pass the swab through one nostril until it reaches the posterior nares. Leave the swab in place for 15 to 20 seconds.
 - b. After removing the swab from the nares, insert it into the transport medium.
 - c. Specimens should be kept at room temperature.
 - d. Transport the specimen(s) to the laboratory immediately, preferably within 2 hours of specimen collection.

Sinus Specimens

Culture Respiratory and Gram Stain Test Code #4845

Procedure:

1. If a bacterial sinusitis is suspected, the physician should obtain a needle aspirate of sinus fluid. Nose and nasopharyngeal cultures are sub optimal and may produce misleading results.

2. This aspirated fluid should be inoculated into a sterile tube and transported to the Laboratory immediately.

Throat Culture for Group A Strep

Throat Culture Test Code #4854

Procedure:

1. The transport swab consists of 2 Dacron swabs on a plastic shaft secured to a cap. The swabs contained within the cap are inserted into a plastic transport tube containing a semi solid gel to keep the specimen from drying during transport.

2. Open transport swab pack, and peel apart at the point labeled "TO OPEN" until swab cap is visible.

3. Remove the sterile swabs, and collect specimen.
 - a. Only the back of the throat between and around the tonsillar area should be swabbed.
 - b. Avoid the tongue, teeth and cheeks.

- c. A tongue blade depressor should be used when swabbing the throat.
4. Remove the transport tube of medium from the package.
5. Remove and discard the cap from the tube. Place the swabs into the medium, and push the swab cap firmly onto the tube.
6. Label and send to the laboratory immediately.
7. Specimens should be stored at room temperature prior to transportation to the laboratory.

Nares Specimens for MRSA Surveillance

MRSA Surveillance Nares

Test Code #4371

Procedure:



1. A Copan (red capped) swab must be used to collect the nares specimen

2. Insert the dry swabs 1-2 cm into the nostril and rotate swabs against the inside of the nostril for 3 seconds while applying pressure with a finger on the outside of the nose



3. Repeat Step 2 in second nostril with the same swabs



4. Place the swabs back into the tube

5. Transport the specimen at room temperature as soon as possible to the Microbiology Laboratory

Specimens other than nares are not acceptable for testing. Specimens must be collected using a red-capped Copan swab. Other swab types are not acceptable for testing.

STOOL SPECIMENS

Routine Stool Culture

| | |
|-------------------------------|-----------------|
| Culture Stool | Test Code #4842 |
| Culture Stool <i>Yersinia</i> | Test Code #4811 |
| Culture Stool <i>Vibrio</i> | Test Code #4814 |

The ideal specimen for a stool culture is a non-formed, preferably diarrheal, sample. Bacterial enteric pathogens should not be ruled out based on a single negative specimen; therefore multiple specimens should be collected. Three separate specimens, collected on different days, should be obtained.

Routine stool culture will not be performed on patients hospitalized for more than 5 days. Diarrhea that develops after prolonged hospitalization may be due to *C. difficile* toxin but is rarely if ever due to a bacterial enteric pathogen. Consultation with the Microbiology Director is required in order for the specimen to be processed.

Procedure:

1. Collect the stool in sterile specimen cup.
2. The specimen should be transported to the laboratory immediately.
3. Specimens should be stored at room temperature prior to transportation to the laboratory.

Routine culture includes culture for *Salmonella*, *Shigella* and *Campylobacter*. If other bacterial enteric pathogens, mycobacteria, parasites or viruses (rotavirus) are suspected, tests for these organisms must be ordered separately.

Ova and Parasite Examination

Cryptosporidium/Giardia DFA Test Code #4943

Non-formed, preferably diarrheal, stool specimens are preferred. Rectal swabs are not acceptable.

Specimen Collection:

1. In-patients:
 - a. Fresh stool samples are collected in a sterile container and then placed in a Para-Pak EcoFix vial. Stool must be added to the "fill line" on the EcoFix vial. Preserved specimens should be transported to the laboratory as soon as possible at room temperature.
 - b. Alternatively, fresh stool samples are collected in a sterile container and transported to lab immediately. This method is sub optimal and may result in the loss of parasites.
 - c. *Cryptosporidium/Giardia* DFA will not be performed on patients hospitalized for more than 5 days. Diarrhea that develops after prolonged hospitalization may be due to *C. difficile* toxin and is rarely if ever due to parasites. Consultation with the Microbiology Director is required in order for the specimen to be processed.
2. Outpatients:
 - a. Fresh stool samples are collected in a sterile container and then placed in the Para-Pak stool system. This system contains 3 vials, one of which is a green EcoFix vial that is used as a parasite preservative. Stool must be added to the "fill line" on the EcoFix vial.

Procedure:

1. Collect the stool specimen into a clean, wide mouthed container.
2. Open the EcoFix vial.
3. Using the collection spoon built into the lid of the vial, place small spoonfuls of stool, especially portions that appear bloody, slimy, or watery, into the vial until the contents reach the red fill line. DO NOT OVERFILL OR UNDERFILL THE VIAL.
4. Transport vials to the laboratory as soon as possible.

Giardia lamblia and *Cryptosporidium parvum* are the most prevalent parasites detected in this area. Due to the low prevalence of parasites in this population overall routine ova and parasite examination is not routinely performed. Requests for Ova and Parasite examination will be converted to *Cryptosporidium/Giardia* DFA.

To request an Ova and Parasite examination on a *Cryptosporidium/Giardia* DFA negative specimen, contact the Microbiology Laboratory.

The presence of parasites cannot be ruled out based on one negative examination. Repeat examinations may be indicated when the first examination is negative. There are a number of substances that can lead to the masking of parasites, such as antacids, enemas, and antimicrobial or antiparasitic drugs. Specimens collected within 10 days of a barium enema are unacceptable.

Note: If pinworm (*Enterobius vermicularis*) is suspected a scotch tape preparation or pinworm paddle should be collected. See **Scotch Tape Collection** procedure.

Clostridium Difficile Toxin Assay

C. Difficile Toxin Test Code #4060

At least 5 grams of stool specimens must be collected.

Only non-formed, preferably diarrheal, stools should be submitted. The submission of formed stools may produce erroneous false positive results and will not be tested.

Only one request will be processed per 7-day period. Studies have shown that more frequent testing does not yield clinically useful results. Any additional testing requires consultation with the Microbiology Director.

Procedure:

1. Collect stool in a sterile specimen cup
2. The specimen should be refrigerated until transported to the laboratory. Do not freeze. Alternatively the specimen may be placed in a Para-Pak Clean vial.

SCOTCH TAPE COLLECTION FOR PINWORM

Pinworm Prep Test Code #4950

Scotch tape or pinworm paddle collections are performed exclusively for the detection of *Enterobius vermicularis* (pinworm).

Procedure:

1. The specimen must be collected first thing in the morning before the patient gets out of bed, bathes or goes to the bathroom.
2. Collect the specimen using a tongue depressor wrapped with clear (not "Magic") scotch tape, sticky side out or a pinworm paddle.
3. Press the sticky surface onto the right and left perianal folds (do not insert the blade into the rectum).

4. The tongue depressor with attached tape is sent to the laboratory after breaking the tongue depressor and putting it into a sterile specimen cup, or by placing the pinworm paddle into the plastic transport tube.
5. The specimen should be transported to the laboratory as soon as possible.

TISSUE CULTURE

Culture Tissue and Gram Stain
Culture Anaerobes

Test Code #4857
Test Code #4809

THE COLLECTION OF TISSUE IS PREFERABLE TO THE COLLECTION OF SPECIMENS ON SWABS.

Procedure for Collection of Tissue Specimens:

1. Tissue specimens are usually obtained during a surgical procedure. Therefore it is mandatory that sufficient specimen is collected for both microbiological and histopathologic examination.
2. Swabs provide a sub optimal specimen and their use should be avoided at all cost.
3. Tissue should be placed in a sterile container with a small amount of sterile, nonbacteriostatic saline. **DO NOT PLACE TISSUE SPECIMENS FOR MICROBIOLOGICAL EXAMINATION IN FORMALIN.** The use of gauze should be avoided since small pieces of tissue can become lost in gauze fibers.
4. The specimen should be transported to the laboratory as quickly as possible.

URINE CULTURE

Culture Urine Test Code #4860

All urine specimens should be identified by collection type i.e. clean catch, straight catheter, indwelling Foley catheter, etc.

A first morning urine sample is the best for recovering pathogenic organisms, but samples collected at other times are acceptable.

Urine specimens should always be collected into a sterile container (sterile specimen cup). Specimens collected in non-sterile systems such as bedpans, urinals, non-sterile catheter tips, etc. are **NOT** acceptable for urine culture.

Acceptable types of collection:

Urine, clean catch – The urine sample should be collected by a health care professional trained in this procedure.

Urine, straight catheterization – Catheterization of the patient should be performed by a health care professional according to established procedure.

When collecting urine from a straight catheter, the first urine emerging from the catheter should be discarded to minimize contamination by urethral flora.

“Mini-caths” are not acceptable devices for collecting a urine culture specimen since these catheters usually have urethral contaminants.

Urine, indwelling catheter – Culture results on urine specimens collected from indwelling catheters are often misleading due to colonization of the catheters.

Decontaminate the line or port and collect the specimen. Never collect urine from a Foley bag.

Procedure for Collecting a Midstream Clean Catch Specimen:

1. Unscrew the cap of the urine specimen cup. Place the cup on the counter. Place the cap on the counter, face up. Do not touch the inside of the cup or cap.
2. Thoroughly cleanse genitalia with towelettes as follows:
 - a. Male:
 - Wipe the head of the penis in a single motion with the first towelette. Repeat with two other towelettes. If not circumcised, hold the foreskin back before cleansing.
 - Urinate a small amount in the toilet.
 - Proceed to the next step.
 - b. Female:
 - Separate the labia. Wipe the inner labial folds front to back in a single motion with two towelettes. Wipe down through the center of the labial folds with a third towelette.
 - Keep the labia separated, and urinate a small amount into the toilet.
 - Proceed to the next step.
3. Place the cup under the stream of urine, and continue to urinate into the cup.
4. Finish voiding into the toilet.
5. Place the cap on the cup.
6. Label the specimen and transport to the laboratory immediately. If a delay in transport is expected the specimen should be refrigerated or placed into a urine preservative tube (see below).

Procedure for Transferring Urine Specimens into a Urine Preservative Tube:

1. This transport media for urine cultures prevents the rapid multiplication of bacteria in urine that cannot be immediately transported to the laboratory.
2. URINE TRANSFERRED TO A TRANSPORT PRESERVATIVE TUBE MAY **NOT** BE USED FOR ROUTINE URINALYSIS.

3. Instructions
 - a. Open pouch and remove transfer device and tube.
 - b. Submerge straw of transfer device below surface of urine to bottom of urine container. Tip the container if there is not a large volume of urine.
 - c. Place gray stoppered tube in holder position.
 - d. Push the tube down so the puncture point pierces the stopper.
 - e. Hold in position until urine stops flowing.
 - f. Remove the tube and shake to mix the urine and the material in the tube.
 - g. Label the tube with the patient's name and date and time of collection.
 - h. DO NOT send the holder to the lab. This holder should be treated as a sharp and discarded appropriately.
 - i. If a urinalysis is also required, send the remainder of the urine in the properly labeled, sterile specimen cup to the laboratory.

UROGENITAL SPECIMENS

| | |
|--------------------------------|-----------------|
| Culture GC | Test Code #4836 |
| Culture Group B Strep Screen | Test Code #4840 |
| Culture Genital and Gram Stain | Test Code #4839 |
| Chlamydia/GC Detect Amplified | Test Code #9534 |

Routine genital cultures of both males and females are of minimal value. Cultures should specify specific organisms such as group B strep or *Neisseria gonorrhoeae* or conditions such as bacterial vaginosis.

Discharge around Foley catheters is of no value for culture. Urine should be sent for urine culture if discharge is present (See **Urine Cultures**).

Procedure for Collecting Genital Specimens:

1. The transport swab consists of 2 Dacron swabs on a plastic shaft secured to a cap. The swabs contained within the cap are inserted into a plastic transport tube containing a semi solid gel to keep the specimen from drying during transport.
2. Open transport swab pack, and peel apart at the point labeled "TO OPEN" until the swab cap is visible.
3. Remove the sterile swabs and collect specimen.
 - a. Female
 - 1) If gonorrhea is suspected, cultures should be taken of the discharge from the cervix.
 - 2) Specimens for bacterial vaginosis should be collected from the vagina.
 - 3) A vaginal/rectal specimen should be collected from pregnant females to screen for group B strep colonization.
 - b. Male
 - 1) Specimens should be collected using a Mini-tip wire swab. Insert the swab 2 to 4 cm into the urethra. Rotate for approximately 5 sec and withdraw the swab.

- 2) If gonorrhoea is suspected, penile discharge may also be collected.
4. Remove the transport tube of medium from the package.
5. Remove and discard the cap from the tube. Place the swabs into the medium, and push the swab cap firmly onto the tube.
6. Label and send to the laboratory immediately.
7. Specimens should be stored at room temperature prior to transportation to the laboratory.
8. If a viral agent is suspected the specimen must be collected using the Microtest medium collection and transport system.

Procedure for Collection of Specimens for *N. gonorrhoeae* and/or *C. trachomatis* using the BD ProbeTec ET CT/NG Collection and Transport System:

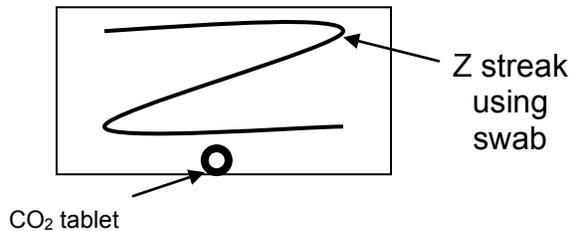
1. Collection of male urethral specimens
 - a. Open pack. Remove the transport swab.
 - b. Collect the urethral specimen at least one hour after urination.
 - c. Insert the Mini-Tip CULTURETTE Direct swab 2-4 cm into the urethra and rotate for 3-5 seconds.
 - d. Withdraw the swab and place the cap/swab into the transport tube.
 - e. Make sure the cap is tightly secured to the tube.
 - f. Label the tube with patient information and date/time collected.
 - g. Store swab at 2-30°C (refrigerated or room temperature) prior to transport to the laboratory.
2. Collection of female endocervical specimens
 - a. Open pack. Remove the transport swab.
 - b. Remove excess mucus from the cervical os with the large-tipped cleaning swab provided with the CULTURETTE Direct Collection and Transport System and discard.
 - c. Insert the CULTURETTE Direct swab into the cervical canal and rotate for 15-30 seconds.
 - d. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.
 - e. Immediately place the cap/swab into the transport tube. Make sure the cap is tightly secured to the tube.
 - f. Label the tube with patient information and date/time collected.
 - g. Store swab at 2-30°C (refrigerated or room temperature) prior to transport to the laboratory.

Note: This system should only be used to collect male urethral and female endocervical samples. This system cannot be used for the collection of other specimen types. This system cannot be used for collection of specimens for *Chlamydia* or *Neisseria gonorrhoeae* culture.

Procedure for Collection of Specimens for *N. gonorrhoeae* using the JEMBEC Transport System:

1. Remove the sterile swab and collect specimen.

- a. Female
 - 1) Use swab to collect discharge from the cervix.
 - b. Male
 - 1) Specimens should be collected using a Mini-tip wire swab. Insert the swab 2 to 4 cm into the urethra. Rotate for approximately 5 sec and withdraw the swab.
 - 2) Penile discharge may also be collected.
2. Using swab inoculate the JEMBEC plate by streaking a large "Z" across the surface of the agar.



3. Insert CO₂ tablet in the well and place one drop of water on it. Cover plate with lid.
4. Label plate with patient information and place immediately in the small rectangular bag provided.
5. Place into specimen bag and send to the laboratory immediately.
6. Specimens should be stored at room temperature prior to transportation to the laboratory. **DO NOT REFRIGERATE JEMBEC PLATES.**

VIROLOGY SPECIMENS

| | |
|--------------------------------------|-----------------|
| Culture CMV/Shell Vial | Test Code #4878 |
| HSV Culture/Shell Vial | Test Code #4881 |
| Respiratory Virus Culture/Shell Vial | Test Code #4884 |
| Rotavirus Antigen | Test Code #4130 |
| RSV Antigen | Test Code #4160 |
| Influenza Antigen | Test Code #4760 |

1. Culture Collection Guidelines
 - a. Many viruses are recovered from clinical specimens only during the first 4 days of illness. Therefore, specimens must be collected as soon as possible after the onset of illness, preferably within the first three days.
 - b. Viruses are very labile; therefore, IMMEDIATE DELIVERY to the laboratory is essential for viral culture. A new specimen will be requested if there is a delay of more than 2 hr of the time of collection to receipt in the laboratory. Labeled specimen containers must be placed in WET ICE immediately after collection to preserve viability. Exceptions include:
 1. When hand carried to laboratory immediately after collection.
 2. Outpatient or remote clients: specimen may be refrigerated prior to delivery to laboratory.
 - c. With the exception of stool and urine specimens, one properly collected specimen from the appropriate body site is sufficient.

- d. Microtest transport medium should be used for specimens that may dry during transport (swabs). Fluids, urine, respiratory secretions (sputum), and stool can be sent without Microtest transport medium.

2. Culture Collection Procedures

a. Respiratory

- 1) Sputum: Collect only the exudative material brought up from the lungs following a deep productive cough.
- 2) Throat
 - Swab: Using a swab, vigorously sample the lesion, avoiding any areas of normal tissue. Place swab in Microtest medium (see **Collection Materials**).
 - Gargle: Have patient gargle with non-bacteriostatic saline and expectorate the saline into a sterile container.
- 3) Nasopharyngeal swab
 - Immobilize the patient's head and gently pass a mini tip swab through one nostril until it reaches the posterior nares. Leave the swab in place for 15 to 20 seconds.
- 4) Bronchial washings, lung, biopsy, trans-tracheal aspiration, bronchial aspiration and bronchial lavage: Collected aseptically and sent to the laboratory in a sterile container.

b. Eye

- 1) Using a sterile swab collect conjunctival specimen.
- 2) Place swab in Microtest medium (see **Collection Materials**).

c. Tissue

- 1) Collect tissue aseptically from center and edge of lesion.
- 2) Place specimen in a sterile container, add a small amount of sterile water or non-bacteriostatic saline to keep tissue from drying out and send immediately to the laboratory.

d. Genital specimens

- 1) See **Urogenital Specimens**.
- 2) Place swab in Microtest medium (see **Collection Materials**).

e. Urine

- 1) Collect a clean catch midstream, catheterized specimen, or cystoscopy specimen and place in sterile specimen cup.
- 2) First morning specimens are preferred
- 3) 24 h urine specimens are not acceptable

f. Body fluids (CSF, pleural, synovial, and peritoneal)

- 1) Collect specimens aseptically and place in a sterile container. Collect as much specimen as possible.
- 2) Do not place fluids in tubes with EDTA, heparin or citrate anticoagulant.

g. Skin

- 1) Fresh dermal eruptions should be sampled for optimal virus detection.
- 2) Disrupt the surface of the lesion and collect the fluid on a swab.
- 3) Place swab in Microtest medium (see **Collection Materials**).

h. Stool

- 1) Collect 2-4 grams in a clean, dry, leak proof container

- 2) Rectal swabs are acceptable and should be collected by inserting a swab 4-6 cm into the rectum and rotating the swab against the mucosa. Place rectal swabs in Microtest medium (see **Collection Materials**).

Rotavirus Antigen

Test Code #4130

Stool specimens should be obtained during the acute phase of the illness when the greatest amount of virus shedding occurs. Samples collected 8 days or more after the onset of symptoms may not contain enough antigens to produce a positive reaction.

1. A minimum of 1mL of raw stool should be collected in a container free of media, preservatives, metal ions, detergents, and serum, all of which interfere with the assay.
2. Stool specimens collected on swabs are acceptable for testing, but not optimal.
3. It is not necessary to submit specimens on ice unless a viral culture is also requested.

Respiratory Syncytial Virus (RSV) Antigen

Test Code #4160

1. Nasopharyngeal washes are the only acceptable specimens for testing. Nasopharyngeal/nasal aspirates or swabs are not acceptable.
2. Wash volumes of 1mL are recommended. Excessive wash volumes can dilute the antigen and cause false negative results.
3. Specimens should be transported in a sterile container.
4. Specimens received in viral transport medium, semi-solid, charcoal based or liquid bacterial transport media are not acceptable for testing.
5. It is not necessary to submit specimens on ice unless a viral culture is also requested.

Influenza Antigen

Test Code #4760

1. The following specimen types are acceptable for testing:
 - a. Nasal Aspirate (Preferred Specimen)
Insert a depressed bulb syringe deeply into either nare and suction while withdrawing. Expel collected specimen into a sterile cup.
 - b. Sputum
Obtain sputum by deep cough either spontaneously or following mechanical induction using a throat swab. Collect specimen in a sterile cup.
 - c. Throat swab
Vigorously rub a rayon swab on both tonsillar surfaces and the posterior pharynx. Insert swab in 15 mL conical tube for transport.
 - d. Nasopharyngeal swab

Insert a Dacron wire swab beneath the inferior turbinate of either nare and vigorously rub against mucosal surface. Insert swab in 15 mL conical tube for transport.

2. Specimens received in viral transport medium, semi-solid, charcoal based or liquid bacterial transport media are not acceptable for testing.
3. It is not necessary to submit specimens on ice unless a viral culture is also requested.

WOUND CULTURES

Culture Wound/Abscess and Gram Stain Test Code #4863
Culture Anaerobes Test Code #4809

All wound cultures must be clearly labeled with specific designations as to the site and nature of the wound. Example: Abscess from right thumb or drainage from trach site. Simply labeling as "Wound Culture" is not acceptable.

Open draining lesions are difficult sites to culture since these lesions are often heavily contaminated with superficial skin flora.

THE COLLECTION OF FLUID OR TISSUE IS PREFERABLE TO THE COLLECTION OF SPECIMENS ON SWABS.

Procedure for Collection of Wound Specimens:

1. The transport swab consists of 2 Dacron swabs on a plastic shaft secured to a cap. The swabs contained within the cap are inserted into a plastic transport tube containing a semi solid gel to keep the specimen from drying during transport. The transport medium supports the recovery of both aerobic and anaerobic bacteria.
2. Open transport swab pack, and peel apart at the point labeled "TO OPEN" until the swab cap is visible.
3. Remove the sterile swabs and collect the specimen.
 - a. The collection of superficial cultures is discouraged.
 - b. Pass the swabs deep into the lesion to firmly sample the lesion's fresh border.
4. Remove the transport tube of medium from the package.
5. Remove and discard the cap from the tube. Place the swabs into the medium, and push the swab cap firmly onto the tube.
6. Label and send to the laboratory immediately.
7. Specimens should be stored at room temperature prior to transportation to the laboratory.
8. Anaerobic cultures are useful only for deep wounds. A foul odor and copious of pus are indications that an anaerobic culture should be requested.

9. The collection of decubitus ulcers and pilonidal cysts on swabs is strongly discouraged. Tissue or aspirates should be submitted.

SEROLOGY TESTS

Collection of sufficient specimen volume is required when multiple tests are ordered.

| Test | Test Code | Acceptable Specimens | Comments |
|---|------------------|---------------------------------|--|
| Cryptococcal Antigen | 4070 | Red top tube CSF | If positive, reflexes to titer |
| CMV IgG Antibody | 4312 | Red top tube | |
| CMV IgM Antibody | 4314 | Red top tube | |
| <i>Helicobacter pylori</i> IgG Antibody | 4085 | Red top tube | |
| Acute Hepatitis Panel | 4349 | Red top tube Purple top tube | Includes: HBsAg, HAV IgM Ab, HBc IgM Ab and HCV Ab |
| Hepatitis A total Ab | 7449 | Red top tube Purple top tube | Sendout test |
| Hepatitis A IgM Ab | 4028 | Red top tube Purple top tube | |
| Hepatitis B surface Ab | 4032 | Red top tube Purple top tube | |
| Hepatitis B surface Ag | 4030 | Red top tube Purple top tube | |
| Hepatitis C Ab | 4037 | Red top tube Purple top tube | Confirmatory testing performed on all initially positive specimens |
| Hepatitis B core total Ab | 4034 | Red top tube Purple top tube | |
| Hepatitis B core IgM Ab | 4029 | Red top tube Purple top tube | |
| Herpes simplex IgG antibody | 4348 | Red top tube | |
| HIV Antibody | 4100 | Red top tube Purple top tube | If positive, reflexes to Western blot |
| Legionella urine antigen | 4876 | Urine | |
| Measles/ Rubeola Immune Status | 4394 | Red top tube | |
| Monoscreen Monospot | 4090 | Red top tube Purple top tube | |
| Rubella Immune Status | 4170 | Red top tube | |
| <i>S. pneumoniae</i> urine antigen | 4950 | Urine | |

| Test | Test Code | Acceptable Specimens | Comments |
|--------------------------------|-----------|----------------------|--|
| Syphilis | | | |
| RPR | 4140 | Red top tube | If positive, reflexes to RPR titer and TP-PA |
| VDRL | 4200 | CSF | If positive, reflexes to VDRL titer |
| <i>Toxoplasma</i> IgG antibody | 4404 | Red top tube | |
| <i>Toxoplasma</i> IgM antibody | 4406 | Red top tube | |
| Varicella (VZV) Immune Status | 4190 | Red top tube | |

MOLECULAR MICROBIOLOGY TESTS

| Test | Test Code | Acceptable Specimens | Comments |
|--------------------------|-----------|--|--|
| CMV Viral Load | 4313 | Purple top tube | |
| Enterovirus PCR | 4317 | CSF | |
| Hepatitis C Viral Load | 4320 | Purple top tube or Red top tube | Transport to the laboratory as quickly as possible |
| Hepatitis C Genotype | 44307 | Purple top tube Yellow top (ACD) tube Red top tube | Transport to the laboratory as quickly as possible |
| Herpes Simplex Virus PCR | 44309 | CSF | |
| HIV Viral Load | 4319 | Purple top tube | Transport to the laboratory as quickly as possible |

UNLISTED TESTS

If there are any unusual specimens or test requests that are not covered in this manual, you may contact Dr. Yvette McCarter, Director of Microbiology (244-6684) or Noel Gomez, Microbiology Supervisor (244-6060) to discuss specimen collection or transport requirements as well as test appropriateness.