General Guidelines for Microbiology Specimen Collection

1. Collect material for cultures before antibiotic therapy.

2. Avoid contamination of specimen with normal flora. Properly cleanse skin sites, and use proper technique when obtaining throat cultures and sputum cultures.

3. Use appropriate collection containers. All containers must be sterile. Obtain anaerobic culturettes and special sputum containers for Acid Fast Bacilli (AFB) from central supply. Containers must seal tightly to prevent leakage.

4. Properly label containers with patient identification information to include: Patient’s name, medical record number, date and time collected.

5. Accurately define specimen source and patient diagnosis when ordering the culture. This information is VITAL for proper handling of the culture by the microbiologists. Please be as descriptive as necessary.

6. Indicate on request form if the presence of a particular organism is suspected, since special handling or additional media may be required.

Anaerobic Culture Collection

A. The most desirable method of collecting material for anaerobic culture is by aspirating with a syringe and needle. Once collected, express the air from the syringe, remove needle, cap and send to the lab.

B. If specimens cannot be collected by aspiration. The anaerobic culturette is available upon request from Microbiology, or Distribution.

C. Specimens acceptable for anaerobic culture:
   - Excised tissue
   - Pus from foul smelling discharge
   - Post operative wounds
   - Fractures
   - Deep ulcers
   - Empyema fluid
   - Gangrenous tissue
   - Products from septic abortions
   - Body cavity fluids

D. Specimens NOT acceptable for anaerobic cultures:
   - Sputum
   - Urine
   - Genital specimens
   - Stools
   - Decubitus ulcer material
   - Throat
   - Ileostomy or colostomy material
   - Gastric washings
General Culture Collection

A. Most specimens may be collected using swabs, ideally, the Culturette II. Once the material has been collected, return the swabs to the container and crush the ampule in the bottom of the tube.

B. If the specimen is liquid, please use a sterile container with a screw cap lid.

Miscellaneous

A. All specimens must be properly labeled. Unlabeled or mislabeled specimens will be rejected.

B. All specimens must be accompanied by a Microbiology requisition that has been completed by Nursing Service or medical office personnel. All outpatient requests must be completed properly including the five elements required for a valid order.

C. Be very specific as to the site or source of the specimen. For example, do not mark “Wound” without an explanation of the site of the wound.

D. Number of orders needed: One order is needed for each general examination requested. One order would be needed for each of the general examinations of AFB, Fungal, and Bacterial. For example, orders for AFB, Fungal, and Bacterial would require three orders. However, orders for aerobic and anaerobic cultures on the same specimen would require only one order.

E. Specimens must be delivered to the Laboratory Accessioning area as soon as possible. Spinal fluid specimens, STAT Gram stains, KOH preps, and wet preps must be delivered IMMEDIATELY.

F. If a delay in the transport of a urine specimen for routine culture is anticipated, the urine should be refrigerated at 2-8°C for up to 24 hours or inoculated into a urine culture transport tube containing preservative if the specimen is from an outlying office.

G. Stools for culture on inpatients should be collected during the first three days of admission with no more that a total of two stools submitted during the admission.
Microbiology Collection Devices

I. Double-walled Sputum Collection Containers (Obtained from Distribution)
   A. Instruct patient to rinse mouth out with water.
   B. Patient should cough deeply to bring up any material from lungs.
   C. Lift the top of the container and expel sputum into the container.
   D. Do not submit saliva as a sputum specimen.
   E. Recap the container.
   F. Do not disassemble the container; transport intact to the Laboratory ASAP.

II. Urine Preservative Tubes

   Urine preservative tubes are used by Out-patients, Family Care Centers and certain Doctor's offices where the transport time may be prolonged. In-house patients should have urines collected appropriately and transported to the Laboratory within two hours.

   A. Place transfer device (white plastic tube) into the urine container.
   B. Place glass tube into transfer device, rubber stopper first.
   C. Push glass tube firmly into transfer device to puncture rubber stopper with needle.
   D. After flow of urine has stopped and glass tube is about half full, remove the glass tube from the transfer device.

III. N/P Culturette (mini-tip culturette collection and transport system) for collection of specimens from small or hard-to-reach areas such as eye, ear, nose or urogenital tract.

   A. Peel open one-third length. Remove culturette from package.
   B. Remove cap/swab from tube, or if longer swab is desired (with cap in place), compress tube to hold wire and pull cap to desired length.
   C. Take sample and return cap/swab to tube.
   D. Return cap/swab tube to package.
   E. Push cap to bring swab in contact with moistened pledget.
   F. Transport to Laboratory as soon as possible.
IV. **Aerobic/Anaerobic Culturette (Culturette II® collection and transport system)**

A. Peel open one-third length. Remove culturette from package.

B. Remove cap/swab from tube.

C. Take sample, being careful not to touch surrounding areas that may contaminate culturette with normal/other flora.

D. Return cap/swab to tube. Return to package.

E. Push cap to bring swab into contact with moistened pledget.

F. Transport to Laboratory as soon as possible.

V. **Containers for Stool Specimens**

There are three different types of containers used in collection of stool specimens:
- Yellow Parapak Vial (SAF)
- Peach Parapak Vial (C&S)
- Gray cup (clean/sterile vial)

For optimal recovery of parasites, stools should be collected once a day for three consecutive days. Multiple specimens on one day will not be accepted. If the physician orders more than one stool specimen, they should be collected on separate days.

Stools for culture on inpatients should only be collected within the first 3 days of admission. No more than 2 stools will be accepted for culture.

The stool specimen should be passed into a clean dry container. Stool should then be placed into each vial or container provided. Fill the Parapak vials to the red line and do not discard any liquid. Shake the vials after capping them. If a gray cup only is provided, just place some of the stool in the cup.

When using the Parapak vials the patient may collect stool specimens for three consecutive days then transport all specimens back to the Laboratory at the same time as long as it is within 72 hours (3 days) of the collection time of the first specimen. These must be labeled with the patient's name and date and time collected. If the date and time collected are not specified the specimen will not be accepted. If the specimen is collected in a gray cup, it must be transported within two hours to the Laboratory; prolonged transport time decreases the likelihood of isolating the causative agent. Refrigeration is not necessary.

The following tests are ordered on stools, the appropriate collection containers are also listed. If a specimen is submitted in the improper container, the test procedure cannot be done and the specimen will be rejected.
<table>
<thead>
<tr>
<th>TEST</th>
<th>CONTAINER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ova and parasites (O&amp;P, OCP)</td>
<td>Yellow Parapak vial or gray cup</td>
</tr>
<tr>
<td>WBC (leukocytes)</td>
<td>Yellow Parapak vial or gray cup</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Yellow Parapak vial or gray cup</td>
</tr>
<tr>
<td>C&amp;S (culture &amp; sensitivity enteric pathogens)</td>
<td>Peach Parapak vial or gray cup</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>Gray cup</td>
</tr>
<tr>
<td>Blood</td>
<td>Gray cup or Hemocult card</td>
</tr>
<tr>
<td>pH</td>
<td>Gray cup</td>
</tr>
<tr>
<td>Reducing Substance</td>
<td>Gray cup</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Gray cup</td>
</tr>
<tr>
<td>Fecal fat (qualitative)</td>
<td>Gray cup</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Rectal swab or gray cup</td>
</tr>
</tbody>
</table>

Patients should be provided with a copy of the patient's instructions. If there are none available, copies may be obtained from the Microbiology Supervisor, or ordered from the print shop (Sacred Heart Hospital form 701-007).

VI. Procedure for Collection of Rectal Swab
(Culture or Rotavirus Assay)

Rectal swabs are the specimen of choice when a physician orders a stool culture or a rotavirus assay on an infant. They are preferable since they can be collected quickly and easily without having to wait for the patient to have a bowel movement. They are also preferable over dirty diapers especially when stools are runny.

1. Patient should be positioned on his/her stomach.
2. Separate anal opening with left hand.
3. Insert the swab end of an aerobic culturette slowly (about 2 inches deep).
4. Slowly remove the culturette and insert it back into the culturette transport tube.

The use of gloves while collecting a specimen in this manner is recommended.

**This method may be used for adults when a stool specimen cannot be obtained.

VII. Blood Culture Specimen Collection

A. Type of containers:

1. BacTAlert Tryptic Soy Broth - Aerobic (blue capped bottle)
2. BacTAlert Tryptic Soy Broth - Anaerobic (purple capped bottle)
3. BacTAlert Pediatric - Aerobic (yellow capped bottle)

B. Volume:

1. No less than 5 ml and no more than 10 ml of whole blood in BacTAlert bottles.
NOTE: Volume of blood collected is critical for recovery of organisms. At least 5 ml of blood should be collected in each bottle unless extraordinary collection difficulties are present.

2. 0.5 - 4 ml of whole blood in Pediatric bottles (yellow capped).

3. Collect blood cultures in sets of two (2 venipunctures). Limit routine blood cultures to twice per day (4 venipunctures).

C. Collection time:

1. Per request of physician.

2. If physician does not specify:
   a. Blood cultures times two - draw from different sites.
   b. Blood cultures times three - draw one hour apart.
   c. Blood cultures from same site - draw 15 minutes apart.

D. Procedural Notes:

1. Clean the rubber tops of the BacTAlert bottles with an alcohol pad. DO NOT USE IODINE.

2. If a pediatric patient (under five years of age) has a blood culture ordered draw 0.5 to 4 mls of blood with 4 mls being the preferred volume. Pediatric bottles (yellow capped) must only be used on children 5 years and under, and preferably only on children under two years of age.

3. Sterile collection technique must be used. Please follow the current guidelines for blood culture specimen collection.

VIII. GC and Chlamydia Sample Collection & Transportation

A. PCR for Neisseria gonorrhoeae and Chlamydia trachomatis is designed to detect the presence of Neisseria gonorrhoeae and/or Chlamydia trachomatis in specimens obtained from the male urethra and the female endocervical canal It may be used to detect Chlamydia trachomatis from conjunctiva as well.

B. The M4 collection kit contains the transport tube and 2 swabs. Only polyester, dacro or rayon swabs with plastic or aluminum shafts may be submitted in a M4 transport tube. Swabs with cotton tips and/or wooden shafts are not acceptable.

Collect swab samples as follows:

1. Cervical swab specimens
   a. Remove excess mucus from the cervical os and surrounding mucosa using one of the swabs provided in the cervical collection kit and discard the swab.
   b. Insert the second swab from the collection kit into the endocervical canal.
c. Rotate the swab for 10 - 30 seconds in the endocervical canal to ensure adequate sampling.

d. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.

e. Insert the swab into the M4 Transport Tube.

f. **Break off the swab shaft** to fit inside the tube and cap the tube. Do not use any instrument to cut the shaft.

2. Urethral swab specimens

   a. Patient should not have urinated for at least 1 hour prior to sample collection.

   b. Collect the urethral exudate or insert the swab 2-4cm into the urethra using a rotating motion to facilitate insertion.

   c. Once inserted, rotate the swab gently using sufficient pressure to ensure that the swab contacts all urethral surfaces. Allow the swab to remain inserted for 2 to 3 seconds.

   d. Withdraw the swab.

   e. Insert the swab into the M4 Transport Tube.

   f. Break off the swab shaft to fit the tube and cap the tube.

3. Conjunctival swab specimens (For Chlamydia trachomatis detection ONLY: **Not approved for Neisseria gonorrhea**)

   a. If pus or discharge is present, use a sterile, untreated dacron cleaning swab to clean the area.

   b. Do not scrape the conjunctiva while cleaning the eye.

   c. If both eyes are affected, first swab the least affected eye then swab the most affected eye.

   d. Thoroughly swab the lower then the upper conjunctiva 2-3 times each using the male urethral/conjunctival swab provided.

   e. Immediately place the swab into the M4 Transport Tube.

   f. Break the swab shaft to fit the tube and cap the tube tightly.

   g. Transport the tubes to the laboratory for testing. If transportation is delayed till the next day or later, refrigerate the sample at 2-8°C.

**Normal Microbial Flora**
Microorganisms normally residing on body surfaces or in various cavities of the body without invasion or harm to the host are referred to as "normal" flora. The type and numbers vary according to the environments of the surfaces and cavities. These organisms help prevent colonization, invasion and infection by pathogenic microorganisms. Some of the normal flora in the alimentary tract help synthesize vitamin K, aid in nutrient absorption and help convert bile pigments and acids in the intestines. Although harmless in their usual sites, normal flora may produce disease if introduced into other areas (especially those cavities which are normally sterile) as opportunists, or when certain organism(s) become predominant in quantity.

The following is a compilation of microorganisms which constitute normal flora encountered in various body sites. The "*" indicates organisms which when in predominating quantity, may cause disease.

A. **Normal flora of the mouth and oropharynx**

1. Viridans group streptococci  
2. Coagulase-negative Staphylococcus species  
3. Veillonella spp  
4. Fusobacterium spp  
5. Treponema spp  
6. Bacteroides spp  
7. Neisseria spp  
8. Moraxella catarrhalis *  
9. Streptococcus pneumoniae *  
10. Beta-hemolytic streptococci (not group A) *  
11. Candida spp  
12. Haemophilus spp *  
13. Diphtheroids  
14. Actinomyces spp  
15. Eikenella corrodens  
16. Staphylococcus aureus *

B. **Normal flora of the nose:**

1. Coagulase-negative Staphylococci  
2. Viridans group Streptococci  
3. Staphylococcus aureus *  
4. Neisseria spp  
5. Haemophilus spp *  
6. Streptococcus pneumoniae *

C. **Normal flora of the outer ear:**

1. Coagulase-negative Staphylococci  
2. Diphtheroids  
3. Pseudomonas spp *  
4. Enterobacteriaceae (occasionally) *

D. **Normal flora of the conjunctiva:**

1. Coagulase-negative Staphylococci  
2. Haemophilus spp *  
3. Staphylococcus aureus *  
4. Streptococci (various species)
E. **Normal flora of the skin:**

1. Coagulase-negative Staphylococci
2. Diphtheroids (including Propionibacterium acnes)
3. Staphylococcus aureus *
4. Streptococci (various species)
5. Bacillus spp
6. Malassezia furfur
7. Candida spp *
8. Mycobacterium spp

F. **Normal flora of the urethra:**

1. Coagulase-negative Staphylococci
2. Diphtheroids
3. Streptococci (various species)
4. Mycobacterium spp
5. Bacteroides spp and Fusobacterium spp
6. Peptostreptococcus spp

G. **Normal flora of the vagina**

1. Lactobacillus spp
2. Peptostreptococcus spp
3. Diphtheroids
4. Streptococci (various) *
5. Clostridium spp
6. Bacteroides spp
7. Candida spp
8. Gardnerella vaginalis *

H. **Normal flora of the gastrointestinal tract:**

1. Small intestine
   a. Lactobacillus spp
   b. Bacteroides spp
   c. Clostridium spp
   d. Mycobacterium spp
   e. Enterococci
   f. Enterobacteriaceae

2. Large intestine
   a. Bacteroides spp
   b. Fusobacterium spp
   c. Clostridium spp
   d. Peptostreptococcus spp
   e. Enterobacteriaceae
f. Lactobacillus spp

g. Enterococci

h. Streptococci (various species)
i. Pseudomonas spp

j. Acinetobacter spp

k. Coagulase-negative Staphylococci

l. Staphylococcus aureus *
m. Mycobacterium spp

n. Actinomyces spp

MICROBIOLOGY/MISCELLANEOUS
SIGNIFICANT RESULTS

The following Microbiology findings are considered significant and will be called to the appropriate unit, doctor, or office and documented as outlined in the "Critical Values" policy.

1. Positive body fluid gram stain or initial culture (CSF, Peritoneal, Pleural, Synovial, Pericardial, etc.)

2. Positive blood cultures

3. Positive blood culture ID of Staph. Aureus or MRSA by culture or PCR

4. Positive AFB smear or initial culture

5. Culture positive for Histoplasma, Coccidioides, or Blastomyces.

6. Organisms listed as Category A agents (see attached)

7. Cultures or EIA positive for E.coli 0157/H7 or other shigatoxin producing E.coli.

8. Organisms listed as Telephone Reportable on the current State of Florida Reportable Disease list (see attached)

9. Positive Cryptococcus AG test

10. Positive PCR test on CSF for any virus or bacteria

11. Positive Legionella Urinary AG

12. Positive Strep. pneumo. CSF AG

13. Culture positive for Neisseria meningitides

14. Initially positive malaria films

15. Unusual result that the Tech finds exceptional or critical not listed above.
Criteria for Rejection

* Inappropriate or leaking container
* Specimen received in a non-sterile container
* Multiple urine, sputum, or stool specimens received within a 24 hour period.
* Dried out swab
* Foley catheter tip received for culture
* Sputum specimen contaminated with excess saliva
* Specimen received in alcohol or formalin
* Anaerobic culture requested on the following: sputum, urine, vaginal, feces, gastric aspirates, placenta, respiratory sources, or skin.
* Specimen containers with gross external contamination by blood or body fluid material
* Midstream clean catch urines on inpatients received in the laboratory more than two hours after collection
* Inpatient stools for culture after the 3rd day of admission. No more than 2 stools will be accepted for culture on inpatients.
* Formed stool for culture.
When To Expect Microbiology Result

**GRAM STAINS:** Gram stains and KOH preps will be resulted the same day that they were received.

**ROUTINE CULTURES:** Most cultures take from 48 to 72 hours before resulted as "final". A preliminary report is usually generated at 24 hours.

**ANAEROBIC CULTURES:** The microbiology department determines whether a specimen is suitable for anaerobic culture based on information provided. All anaerobic cultures are held for a minimum of 5 days before a final report is released.

**ACID FAST SMEARS:** Acid Fast Smears are resulted within 24 hours of receipt in the laboratory. All positive (AFB Smear) AFB smears are called to the physician or unit.

**ACID FAST CULTURES:** All AFB cultures are held for 6 weeks before a final report is released. No (Mycobacterium) preliminarily negative reports, except for the smear report, will be sent out. Any positive smear or culture results will be phoned to the floor or doctor immediately by a microbiologist and a preliminary report will be sent out with that information.

**FUNGUS CULTURES:** All fungus cultures are held for 4 weeks before a final report is released. The smear report is sent out on the day the specimen is received. A preliminary report is sent at one week.

**BLOOD CULTURES:** All blood cultures are examined continuously for 5 days. Preliminary reports are generated at 1, 2 and 3 days with a final report at 5 days. All positive cultures are phoned immediately to the floor or doctor.

**WET PREPS:** Wet preps will be done within one hour from receipt in the laboratory.
Specimen Requirements for Ova and Parasite Examination

1. **Amount**
   - A. Submit at least two tablespoons of fresh stool, or
   - B. Fill Para-Pak preservative containers per package insert.

2. **Transport Time**
   - A. Fresh specimens must be received in lab within one hour after passage or be preserved in a Para-Pak preservative container.
   - B. Para-Pak preserved specimens are not adversely affected by lengthy transport time, but should be transported to the lab within 72 hours.

3. **Multiple specimens**
   Only one specimen per day will be accepted. It is ideal to collect specimens every other day when multiple specimens are ordered. However, one per day on consecutive days is acceptable.

4. **Interfering Substances**
   - A. Do not use anti-diarrhea compounds or oil-based cathartics prior to specimen collection.
   - B. Barium, Bismuth, Mineral Oil, must be withheld for 10 days prior to specimen collection.
   - C. Antibiotics should be withheld for 2-3 weeks prior to specimen collection. Antibiotics cause a decrease in the number of parasites excreted.
   - D. A delay of 3 weeks should be observed following administration of gallbladder dyes.
   - E. Stools contaminated with urine are not accepted.

   * Ideal specimens should be loose or liquid in consistency
   * Only specimens for ova, cyst, and parasite exam and cryptosporidium test may be submitted in formalin/PVA preservatives. All other test specimens must be unpreserved.

**When to Expect Parasitology Results**

Parasitology tests are performed Monday through Friday. All inpatient parasitology tests received on Fri-Sat will be resulted by Sunday at 4PM.