



## **MICROBIOLOGY COLLECTION MANUAL**

### **Mercy One Laboratory- Clinton Iowa**

All specimens for cultures collected by nursing personnel are delivered to the laboratory immediately; orders must be placed into the computer. Please call delivery of specimen to laboratory personnel's attention.

#### **Proper Specimen Labeling**

Each specimen for culture must be labeled individually as to:

1. Patient identification with two identifiers
2. Date and time of collection
3. Body site
4. Collector initials

#### **Universal Specimen Rejection Criteria**

1. Mislabeled specimens/ Non-labeled specimens
2. Improper collection (dry swab, unsterile collection, Sputum with unacceptable criteria, Formed stool for *C. difficile* testing, insufficient quantity, etc.)
3. Improper specimen source
4. Delay in transit to the laboratory
5. Expired transport medium

#### **Critical/ Panic Values**

1. Positive Blood Cultures
2. Positive Culture of Joint, Bone, Pericardial, Peritoneal, or Pleural Fluid
3. Positive CSF Gram Stain or Culture
4. Positive stool for Salmonella or Shigella

5. Positive TB Acid Fast Stain or Culture

**Individual Culture Specifics**

**1. Anaerobic Cultures**

Anaerobic bacteria cause a variety of infections in humans. They can easily be overlooked or missed unless the specimen is properly collected and transported and subjected to appropriate procedures for isolation. Whenever possible, tissue samples or fluid aspirates should be collected rather than swab samples. Specimens should be placed in appropriate collection device and transported to the laboratory immediately. Some disadvantages of swabs are that they are easily contaminated, contain a relatively small volume and are easily subjected to air exposure leading to drying.

Anaerobes are a great proportion of the normal bacterial flora of the skin, oropharynx, gastrointestinal tract and genital tract; therefore specimens from these sites are not clinically relevant. Accordingly, specimens must be carefully selected and properly collected to prevent contamination by normal flora.

**Unacceptable Specimens**

|                        |                                |                        |
|------------------------|--------------------------------|------------------------|
| Saliva                 | Colostomy effluent             | Throat swabs           |
| Ileostomy effluent     | Expectorated sputum            | Skin swabs             |
| Nasotracheal aspirates | superficial wound swabs        | Bronchoscopy aspirates |
| Urine "clean catch"    | Vaginal secretions             | Decubitus ulcers       |
| Feces                  | Specimens kept in refrigerator |                        |

**Acceptable Specimens**

|                              |                  |                            |
|------------------------------|------------------|----------------------------|
| Normally sterile body fluids | Deep aspirates   | Sterile surgical specimens |
| Suprapubic bladder aspirates | Abscess contents | Trans tracheal aspirates   |
| Blood Cultures               |                  |                            |

The following guidelines are to be followed when submitting the above listed acceptable specimens:

- A. Fresh pus or tissue obtained from the active site of infection is superior to swab collected specimens.
- B. Since oxygen exposure must be minimal, one of the following collection methods should be employed:

- i. Specimens aspirated with a sterile syringe and needle may be transported without further transfer of specimen.
- ii. When swab collection must be performed, a special anaerobic collection device MUST be used.
- C. Deliver all specimens to the laboratory immediately. These must be delivered into the hands of a technologist.
- D. Anaerobic specimens must never be refrigerated.

## 2. Urine Cultures

For optimal recovery of bacteria and to reduce potential contamination, it is imperative that careful attention be paid to the proper collection of urine specimens.

### A. Clean Catch/ Midstream:

- i. The patient is provided a sterile wide mouth container with tightly fitted lid.
- ii. The periurethral area is cleansed with the cleansing wipe.
- iii. The first void of the urine is not collected and discarded.
- iv. The subsequent urine is voided directly into the sterile container and is then used for culture.

### B. Cath Specimen:

- i. This is often used if the patient cannot void and treatment needs to be started right away or done for diagnostic or therapeutic purposes.
  - a. The free flow from the mouth of the catheter should be obtained.
  - b. Urine from cath bags is generally unsuitable.

### C. Suprapubic/cystoscopy :

- i. This procedure is generally done by the physician who may collect up to three or four different samples from the right ureter, the left ureter, from the bladder as well as clean voided catch urine.

### D. Note that proper description of the collection method (Foley, straight cath, clean catch, etc.) impacts the examination of the urine culture and ultimately, the proper antimicrobial susceptibility testing.

## Urine Lab Policy for Culture Reflex

- A. Urine specimens for routine urinalysis will be screened for possible UTI.
- B. If the following criterion is met, cultures will be automatically reflexed.
  - i.  $\geq 11$  WBC's/hpf
  - ii.  $\leq 10$  squamous epithelial cells /hpf

## Contamination Policy for Cultures

- A. Mixed cultures with greater than 2 or 3 normal urogenital floras often consisting of gram positive cocci or gram positive rods. These are reported as mixed and if infection is suspected, recollection is recommended.

### 3. Sputum Cultures

Sputum is produced in the lungs and is normally sterile but/and if it has passed through the naso-oro-pharynx it cannot help but be contaminated by numerous types of microorganisms which normally colonize this area. Many pathogens also exist as part of this normal flora; therefore, sputum should be procured by aerosol induction; transtracheal aspiration; bronchoscopy; needle aspiration of the lung, or lung biopsy.

If sputum is coughed up, the patient should be instructed that upon awaking, he should brush his teeth, gargle and rinse mouth out with water. A deep cough specimen is then to be produced and collected. A gram stain will be performed to determine acceptability of specimen based upon neutrophil/squamous epithelial cell ratio. If necessary, the Respiratory Therapy department may be notified for assistance with repeat collection.

### 4. Bronchial Aspirate

The specimen should be delivered to the laboratory immediately. Also, if cytopathology is desired please supply requisition so personnel can correctly distribute specimen.

### 5. Tuberculosis Cultures (AFB)

This testing is sent out and performed by the Iowa State Hygienic Laboratory.

Tissue: Should not be placed in formalin prior to Microbiology processing.

Sputum: Three (3) morning specimens on separate days may be necessary.

Gastric Fluid: This fluid should only be examined for tubercle bacilli when a patient cannot cough up sputum, since it is much less reliable than sputum for acid fast work.

Urine: Collect the entire first morning specimen- NOT a 24 hour collection. Urine from 3 separate morning collections is strongly recommended.

CSF: Follow CSF procedure and alert the laboratory if TB meningitis is suspected.

## 6. Blood Cultures

This procedure is ordered on a STAT or timed basis. When nursing staff or physician performs the phlebotomy, bottles are available in the laboratory.

Since results of one blood culture cannot be considered reliable, two are to be ordered for the first draw set. The following schedule is widely accepted and recommended by the Pathologist and Sepsis Team at Mercy Medical Center-Clinton:

- A. A minimum of two sets of blood cultures are to be drawn on all adult patients in cases of suspected bacteremia.
- B. Cultures should be drawn from two different sites for a total of two blood culture sets.

### Blood Culture Collection

- A. Patient preparation: Extreme care must be exercised in preparing the site for venipuncture.
  - i. First scrub the site with alcohol.
  - ii. A second scrub with Chloroprep is applied, cleaning in a circular pattern from a minimum of 30 seconds and allowed to air dry.
  - iii. The venipuncture site should not be palpitated after this treatment unless a sterile glove is used.
- B. Specimen collection: 8-10 mL is to be collected per bottle using a sterile syringe. A lesser amount of blood volume may cause a lower bacterial recovery rate. Infant and children draws require less volume per bottle. The recommended volume for <18 years old is 1-3 mL per bottle.

## 7. Body Fluid/Joint Fluid Culture

(Ex. Pleural, Peritoneal, Pericardial, Joint, etc.)

- A. All specimens must be labeled clearly with date and time of collection, initials of collector, and body site/source and in a sterile container. Patient's name must be clearly labelled on the container.
- B. Bring the specimen to the laboratory immediately. Laboratory staff will distribute to Histology if necessary.
- C. If Joint Fluid is collected, a sterile screw top tube will be used for culture and Gram Stain and an EDTA tube and Lithium Heparin tube for cell count and crystal exam. These must be delivered immediately as they are at a great risk of clotting.

## 8. Cerebrospinal Fluid

The specimen should be collected in equal parts in three or four tubes which are included in the "spinal tap" tray obtained in central distribution. The tubes should be numbered in order of their collection. The properly labelled tubes should be immediately hand carried to the laboratory and given directly to a laboratory employee.

## 9. Wound Cultures

Samples are best when cultured deep within the wound. Please refrain from sampling surface pus and dried scabs as these will likely only exhibit normal skin flora. To minimize contamination of samples, attention must be made to carefully cleanse the exterior area of the site prior to collection.

## 10. Throat Cultures

Collection

- A. Using a sterile swab, ask the patient to open their mouth and say "ah".
- B. Depress the tongue with a tongue blade.
- C. Glide the swab gently into the posterior pharynx (without touching any other portion of the oral cavity).
- D. Swab the mucosa behind the uvula and between the tonsillar pillars and any inflamed area with a back and forth motion.
- E. Place the swab back into the sterile collection device and label appropriately.

## 11. Ear and Eye Cultures

Collection

- A. Eye: Wash exterior of the eye to avoid unnecessary contamination from normal skin flora. Collect the purulent material from the lower cul-de-sac and inner canthus of the eye onto a sterile culturette swab.
- B. Ear: Material from the ear, especially that obtained after the perforation of the ear drum is best collected by the otolaryngologist using sterile equipment. Tympanic membrane aspiration is rarely performed. In some cases of acute otitis media, the causative microorganism can be cultured from the posterior nasopharynx. If a gram stain is requested, two swabs should be obtained and submitted to the laboratory.

## 12. Fecal Specimens

Fecal specimens should be collected in sterile containers and covered with a tightly fitted lid. Whenever possible, multiple stool specimens should be examined. Also, choose portions that may display blood or mucus since these areas usually harbor a large number of organisms that are involved in disease process.

Rectal swabs may be acceptable when circumstances dictate, but the swab should be passed beyond the anal sphincter, carefully rotated and withdrawn.

Stool samples must be brought to the laboratory immediately as some pathogens may die if sample is kept at room temperature too long.

### 13. Ova and Parasites

**Giardia and Cryptosporidium are the two most common parasites in the Midwest and should be ordered instead,** as long as the patient has not had any foreign travel. This test is done in house and has about a 3 day quicker turnaround time.

For both tests, patient should defecate directly into the specimen container, but if not possible, specimen can be collected from clean bedpan. Specimen should NEVER be collected from toilet bowls, or bedpans mixed with urine. The entire stool passage should be submitted not just a portion.

No fecal specimen is acceptable for parasitological examination if contaminated with drugs or chemical compounds; and a period of 7-10 days must elapse if patient has had any of the following:

- A. Barium or bismuth
- B. Kaolin compounds
- C. Glycerin suppositories
- D. Epsom salts
- E. Antibiotics, if possible

The following purges are acceptable in obtaining fecal specimens:

- A. Fleet solution
- B. Citrate solution
- C. Warm saline enema

Specimens on patients who have been in the hospital for over 3 days are not recommended. Also, a total of three collections is best practice.

### 14. Pinworm Detection

- A. Special plastic pinworm paddles can be obtained from the Microbiology Department.
- B. The specimen is to be collected in the morning before the patient has bathed or defecated.
- C. Separate the buttocks and press the gummed surface of the tape against several areas of the perianal region.
- D. Return the tape into the holder and label the specimen with proper identification and return to the Laboratory.

| Powerchart Name     | Lab Department                                      | Specimen Collector    | Specimen Type   | Special Instructions   | Turn Around Time                                 | Reference Range                                   | Additional Comments  |
|---------------------|---|-----------------------|---|--|--|---|--|
| Culture, AFB        | Reference Laboratory<br>(State Hygienic Laboratory) | Physician/<br>Nursing | 3-5 mL bronchial fluid, sputum, gastric, body fluids, urine, tissue | Early morning specimens are preferred for expectorated sputum. Swabs are unacceptable.                                   | Smear: 2 to 3 days<br>Culture: 6 to 8 weeks      | No Acid Fast Bacilli isolated.                    | Specify possible exposure to reportable infectious organisms such as <i>Mycobacterium tuberculosis</i> when known. |
| Culture, Anaerobic  | Microbiology  | Physician/<br>Nursing | Preferably needle aspirate from abscess site.                       | If swabs must be submitted, clean the wound to eliminate surface contaminants and collect from the deepest area of site. | Preliminary: 24-36 hours.<br>Final: 48-96 hours. | No anaerobic organisms isolated.                  | Include relevant clinical data to aid in identification of suspect organisms.                                      |
| Culture, Blood      | Microbiology  | Laboratory            | Blood   | Cultures obtained from venous ports, arterial ports, and other sterile lines must be collected by nursing.               | Preliminary: 48 hours.<br>Final: 5 days.         | No growth at 2 days.<br>No growth at 5 days.      | Timing and number of cultures to be collected are dictated by physician order.                                     |
| Culture, Body Fluid | Microbiology  | Physician/<br>Nursing | 10 mL aspirated fluid in sterile tube or syringe.                   | Peritoneal fluid submitted on swabs will decrease sensitivity of culture and are   | Preliminary: 24-36 hours.<br>Final: 48-72 hours. | No growth at 2 days.<br>No growth at 2 or 3 days. | Include relevant clinical data to aid in identification of suspect organisms.                                      |



|                 |   |                       |   |   |   |   |  |
|-----------------|---|-----------------------|---|---|---|---|--|
|                 |   |                       |   | done at physician's discretion.   |   |   |  |
| Culture, CSF    | Microbiology  | Physician/<br>Nursing | 1.0 mL in sterile tube (#1 or #2 in sequential collection set).   | Not any other known infectious process or exposure.                             | Preliminary: 24-36 hours.<br><br>Final: 48-72 hours.                              | No growth at 2 days.<br><br>No growth at 2 or 3 days. | Bloody specimens may yield contaminant organisms.                    |
| Culture, Ear    | Microbiology  | Physician/<br>Nursing | Swab from external auditory canal. Aspirate from middle ear.  | Submit 2 swabs if Gram stain is requested.                                      | Preliminary: 24-36 hours.<br><br>Final: 48-72 hours.                              | No growth at 2 days.<br><br>No growth at 2 or 3 days. | Indicate suspected exposures or other relevant clinical information. |
| Culture, Eye    | Microbiology  | Physician/<br>Nursing | Conjunctiva:<br>Swab inside lower or upper lid.   | Submit 2 swabs if Gram stain is requested.                                      | Preliminary: 24-36 hours.<br><br>Final: 48-72 hours.                              | No growth at 2 days.<br><br>No growth at 2 or 3 days. | Indicate suspected exposures or other relevant clinical information. |
| Culture, Fungus | Reference Laboratory<br>(State Hygienic Laboratory) | Physician/<br>Nursing | Best specimens are aspirates, aseptically or surgically obtained tissue. Ideally, respiratory specimens are deep bronchial secretions | Submit in sterile container; avoid unnecessary exposure to air and desiccation. | Preliminary: If growth is observed. Yeast/Fungus is identified. Final: 4-6 weeks. | No fungus isolated.                                   | Indicate suspected exposures or other relevant clinical information. |

|                         |              |                       |  |   |  |  |  |
|-------------------------|--------------|-----------------------|--|---|--|--|--|
| Culture, GC             | Microbiology | Physician/<br>Nursing | Swab from male or female genitalia. Swab from newborn baby's eye.  | Do not expose to extreme temperature changes. Must be delivered to lab <2 hours.                                    | 24-72 hours                                      | No <i>Neisseria gonorrhoeae</i> isolated.  | Direct smears are only accepted on male patients.  |
| Culture, Sputum         | Microbiology | Physician/<br>Nursing | Expectorated, Induced, Trans tracheal, or suctioned.   | Follow procedure for clearing oral flora before collection.   | Preliminary: 24-36 hours.<br>Final: 48-72 hours. | Usual mixed respiratory flora isolated.  | Specimens are screened before set up. Recollection of rejected specimens is instructed and forms submitted to nursing units. |
| Culture, Stool          | Microbiology | Physician/<br>Nursing | Submit at least a walnut sized specimen in a sterile/ clean container. Pathogens are best preserved by using Cary-Blair enteric transport swab if any delay in set-up is expected. | Do not submit contaminated with urine or passed into toilet water. Specimen collected swabs are highly discouraged. | Preliminary: 24-36 hours.<br>Final: 48-72 hours. | No <i>Salmonella</i> or <i>Shigella</i> isolated. No <i>Campylobacter</i> antigen detected. No <i>E. coli</i> O157:H7 isolated. Shiga toxin1 and 2 not detected. | Recollection of QNS specimens will be directed to nursing unit by phone.   |
| Culture, Strep B Screen | Microbiology | Physician/<br>Nursing | Swab of the vaginal, endocervix or rectum.   | This is only a screen for <i>Streptococcus</i> Group B on women of child-bearing age.                               | Preliminary: 24-36 hours.<br>Final: 48-72 hours  | Negative for <i>Streptococcus</i> Group B  | Susceptibility testing for Strep Group B done only by request.   |

|                                    |                           |                       |  |  |   |   |   |
|------------------------------------|---------------------------|-----------------------|--|--|---|---|---|
| Culture, Throat                    | Microbiology              | Physician/<br>Nursing | Swab from oropharynx.  | Patient should abstain from food and drink for at least an hour before collection. | Preliminary:<br>24 hours.<br><br>Final: 48 hours          | Usual mixed Throat flora isolated.<br>Culture negative for Streptococcus Group A. | Note suspected presence of candidiasis (thrush).  |
| Culture, Urine                     | Microbiology              | Physician/<br>Nursing | 5 mL urine in sterile container.   | First morning specimens are generally preferred.                                   | Preliminary:<br>24 hours.<br><br>Final: 48 to 72 hours    | No growth at 2 days.  |   |
| Culture, Vaginal                   | Microbiology              | Physician/<br>Nursing | Swab Vaginal or endocervical secretions  | Specify suspected pathogens.   | Preliminary:<br>24-36 hours.<br><br>Final: 48 to 72 hours | Usual mixed Vaginal flora.  | Specify suspected pathogens to ensure proper processing.  |
| Culture, Wound                     | Microbiology              | Physician/<br>Nursing | Best specimens are aspirates or tissues from within the site. Submit in sterile container. | Swab collection is discouraged; if necessary submit 2 swabs.                       | Preliminary:<br>24-36 hours.<br><br>Final: 48 to 72 hours | No growth at 48 or 72 hours.  | Collect swab from deepest accessible area of the wound. Clean the external site with 70% alcohol or sterile saline prior to collection. |
| Chlamydia/<br>Gonorrhoeae<br>PCR   | Molecular<br>Microbiology | Physician/<br>Nursing | 7-10 mL urine for male or female.<br>Endocervical/<br>Vaginal swab for female.             | Refrigerate if transport delayed   | <24 hours   | Negative for Chlamydia<br>Negative for Gonorrhoeae                                | This is a combination test, but analytes will be reported.<br>Indicate suspected exposures or other relevant clinical information.      |
| Clostridium difficile<br>GDH/Toxin | Microbiology              | Physician/<br>Nursing | Stool in a clean container about the size of a walnut.                                     | Refrigerate if transport delayed   | <24 hours   | Negative  | Specimens submitted 7 days apart are considered duplicate specimens. After a  |

|   |                           |                       |   |   |                               |   |  |
|---|---------------------------|-----------------------|---|---|-------------------------------|---|--|
|   |                           |                       |   |   |                               |   | positive test, patient may not be retested for 30 days. Positive PCR will reflex to toxin testing. |
| Giardia/<br>Cryptosporidium<br>Ag               | Microbiology              | Physician/<br>Nursing | Stool in a clean container about the size of a walnut.      | Refrigerate if transport delayed                | <24 hours                     | Negative  | This is a combination test, but analytes will be reported.   |
| Gram Stain<br>Gram Stain, BF<br>Gram Stain, CSF | Microbiology              | Physician/<br>Nursing | Specimens as described in culture collection.               | Specify specimen type and body site.            | <24 hours or STAT as ordered. | #PMN's observed.<br>Bacteria and/or yeast observed. |  |
| Covid/Influenza<br>A/B/RSV PCR                  | Molecular<br>Microbiology | Physician/<br>Nursing | Nasopharyngeal collected in Universal Viral Transport Media | Refrigerate if transport delayed                | <24 hours                     | Negative  |  |
| KOH prep  | Microbiology              | Physician/<br>Nursing | Multiple specimen types and site. Do not submit a swab.     | Specify specimen type and body site.            | <24 hours or STAT as ordered. | No Fungal Elements seen.                            |  |
| Lactoferrin, stool                              | Microbiology              | Physician/<br>Nursing | Stool in a clean container about the size of a walnut.      | Refrigerate if transport delayed                | <24 hours                     | Negative  | Replaces Fecal Leukocytes or WBC smear testing.  |
| Mycoplasma                                      | Serology                  | Laboratory            | Venous- Serum (1 mL)  | None  | <24 hours                     | Negative  | Batched every 12 hours   |
| MRSA/ SA PCR                                    | Molecular<br>Microbiology | Physician/<br>Nursing | Bilateral Nares swab  | Collect double swab (Red) set in case physician | <24 hours                     | Negative for MRSA<br>Negative for SA                | This is a combination test, but analytes will be reported.   |

|   |  |                    |   |   |             |   |   |
|---|--|--------------------|---|---|-------------|---|---|
|   |  |                    |   | requests susceptibilities.  |             |   |   |
| Ova and Parasites                         | Reference Laboratory (State Hygienic Laboratory) | Physician/ Nursing | 1-3 grams of stool  | Specify suspected organisms. Transport to Lab ASAP.   | 3-5 days    | No Ova or Parasites seen.                         | Recollection of QNS specimens will be directed to the nursing unit by phone |
| Pertussis PCR or Bordetella Pertussis PCR | Reference Laboratory (State Hygienic Laboratory) | Physician/ Nursing | Nasopharyngeal swab dry in sterile tube   | Include clinical diagnosis and symptoms with order.   | 48-72 hours | <i>B pertussis</i> not detected.                  |   |
| Pinworm smear                             | Microbiology                                     | Physician/ Nursing | Skin around anal sphincter with pinworm paddle.   | Collect early morning before defecation or during the night 4 to 6 hours or sleep.                      | <24 hours   | No <i>Enterobius vermicularis</i> (pinworm) seen. | Contact Microbiology for paddle collection device.                          |
| Rapid Strep                               | Serology   | Physician/ Nursing | Throat swab- Liquid Stuarts Swab (Red Top Double Swab)                                  | Double swab must be collected. Lab performs back up strep culture on negatives.                         | <24 hours   | Negative  |   |
| Rotavirus                                 | Microbiology                                     | Physician/ Nursing | Stool in a clean container about the size of a walnut.                                  | Refrigerate if transport delayed  | <24 hours   | Negative  |   |
| Smear Wet Prep                            | Microbiology                                     | Physician/ Nursing | Vaginal/Cervical Drainage. Submit of swab in sterile saline supplied by the Laboratory. | Transport to the Laboratory ASAP. Do not refrigerate. Time delay will decrease sensitivity of the test. | <1 hour     | No Trichomonas<br>No Yeast<br>No Clue Cells       |   |