PRINCIPLES OF SPECIMEN COLLECTION

Affected Sites:  X Enterprise  ___Chandler  ___Good Samaritan

PRINCIPLES OF SPECIMEN COLLECTION

Moisture
Time of Collection
Labeling and Handling Containers
Effect of Temperature
Effect of Atmosphere

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PRINCIPLES OF SPECIMEN COLLECTION

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Body Fluid
Eye
Genital (female)
Genital (male)
Lower Respiratory Tract
Upper Respiratory Tract
Stool/Rectal
Surface Specimens
Surgical Specimens

COLLECTION BY SPECIMEN TYPE

URINE
SPUTUM
ABSCESSES, FISTULAS, PUS, ULCERS, WOUNDS
FAECES FOR PARASITOLOGY
SPINAL FLUID
FLUID, OTHER THAN SPINAL
NASOPHARYNGEAL
URETHRAL, VAGINAL, CERVICAL DISCHARGE
THROAT
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PRINCIPLES OF SPECIMEN COLLECTION

PRINCIPLES OF SPECIMEN COLLECTION

It is obviously a truism that the results of the test can be no better than the specimen on which it was made. Therefore, the laboratory must rely on the nurses to collect the specimen in an accurate and standardized manner. The welfare of the patient rests not only on the laboratory analysis and the physician's interpretation, but also on the way in which the specimen was obtained and transmitted to the laboratory. There are many variables involved in adequate handling of specimens and all must be considered separately to avoid critical errors. Factors that must be considered are moisture, time of collection, labeling and handling containers, transportation, temperature, and atmosphere.

Moisture

Dry swabs are of no value. Specimens must always be submitted moist to the laboratory. Most bacteria cannot survive in a dry environment, especially the pathogenic ones. The use of the COPAN E-swab helps eliminate the chance a dry swab is received in the lab. The E-swab is a swab submerged in liquid Amies media of viral transport medium depending upon the test request and is the preferred culture swab.

Time of Collection

Specimens must be taken if at all possible before antibiotics are administered. If antibiotics have already been started, then the requisition sheet must be so marked so that everyone is aware. The timing of blood specimens is very important. Detection of positive blood cultures of course depends on the pathogenic process of the organism. With some diseases the bacteremia occurs only in the early stages of the infection, while in other cases there is continuous presence of bacteria. In many cases the presence of bacteria in the blood is transient and can best be found after a chill when the patient spikes a fever. During a chill the bactericidal properties of blood are accentuated; the microvessels constrict and become clogged with cells and bacteria during the chill.

Labeling and Handling Containers

All containers used for specimen collection must be sterile. The patient should be instructed to handle the container as aseptically as possible, i.e., not to touch the inside of the container, laying the lid down in such a way as to contaminate it, leaving the lid
off for an excessive length of time, etc. If any of the specimens is spilled on the outside, it should immediately be cleaned with a disinfectant. The lid should be secured tightly and the container transported with care to insure against spillage. From outside facilities all containers must be labeled clearly with the patient’s name, hospital number, room number, and the source of specimen. Specimens from the Enterprise should have a barcoded Container ID number (CID) that prints from Collection Manager. All specimens are to be sent in ziploc bags to the laboratory. It is absolutely necessary that the specimen be accompanied by a requisition sheet completely filled out. The requisition sheet should include the information on the specimen container as well as the physician, examinations requested, time specimen was collected, clinical diagnosis, current antibiotic therapy. Specimens and sheets improperly identified can and should be refused by the laboratory.

Effect of Temperature

Most of the microorganisms found in clinical specimens have an optimal temperature of 37°C. Most have a broad range of temperature tolerance; however, some very important pathogens die rapidly when subjected to temperatures below their optimal requirements. Therefore, it is best never to refrigerate any specimen, especially spinal fluids and vaginal and urethral discharges, but deliver them immediately to the laboratory after collection.

Effect of Atmosphere

The atmosphere plays a very important role in isolating and identifying pathogenic bacteria. The two principal gases that affect metabolism of the bacteria are oxygen and carbon dioxide. Some bacteria require oxygen, some require small amounts with varying concentrations of carbon dioxide, and some, the anaerobes, must have an atmosphere completely devoid of any trace of oxygen. Again, it is most important to get the specimen to the laboratory immediately. Anaerobic organisms must be placed in an oxygen-free environment within 30 minutes after collection. Port-A-Cul vials and Vacutainer Brand anaerobic swabs are available from Central Supply for anaerobic transport.

The following guidelines is designed to be used by the laboratory and all other medical personnel responsible for collecting and transporting specimens to the bacteriology laboratory. The Clinical Microbiology Laboratory plays a critical role in patient care, but the value of its results is dependent upon specimen handling. Specimen handling involves proper selection, appropriate collection, and timely transportation of the specimen to the Microbiology Laboratory. In the final
PRINCIPLES OF SPECIMEN COLLECTION

Analysis, the Clinical Microbiology Laboratory can be of little value to the physician and thus offer only minimal service to patient care if specimens are improperly handled.

Laboratory policies are formulated with the patient in mind. Laboratories should recognize that many patients cannot be expected to do exactly what is asked of them. The specimens received may be less than optimal but should not be accepted if the specimen is obviously inappropriate. The guidelines in the following pages are not meant to be inflexible. The very nature of both patient and organism variability necessitates intelligent decisions and appropriate measures to provide significant information to the physicians. What may be “normal flora” in a “normal” individual may be potential pathogen in an immune-compromised host.

SECTION I

SPECIMEN SELECTION AND COLLECTION

The ONE WHO COLLECTS THE SPECIMEN may hold in his or her hand the course of the patient’s recovery.

General Considerations

1. Collect before antibiotic therapy whenever possible.

2. Collect material from where the suspected organism will most likely be found.

3. Observe asepsis in collection of all specimens.

4. Consider stage of disease.

5. Instruct patients clearly.

6. Use proper containers and/or transport media.

7. Deliver specimen promptly.

8. Provide sufficient information to the laboratory.
PRINCIPLES OF SPECIMEN COLLECTION

A. Requisition

When using a manual requisition the Laboratory Requisition (#J352) is to include the following information:

a. Patient name
b. Patient age and sex
c. Patient room number or location
d. Physician’s name (and address if outside the Hospital)
e. Specific anatomic culture site
f. Date and hour of specimen collect.
g. Clinical diagnosis, special culture request, relevant patient history where necessary
h. Special procedures used in obtaining specimen if appropriate.
i. Name of individual collecting specimens.
j. Antimicrobials, if any, patient is receiving.
k. Matching Tag Identification number.

This information prints on the Sunrise Clinical Manager requisition, along with any additional information entered in SCM at the order level.

Rationale

The requisition form should provide as much information as needed for correct interpretation of laboratory results. The need for the patient’s name and location is obvious. The patient’s age may be important in certain instances; e.g., if special culture techniques are required or pathogens considered. The physician’s name and location is essential so that interim reports can be given. The exact anatomical culture site, clinical diagnosis, and special collection procedures used are essential for the microbiologist in selecting appropriate culture media. The name of person collecting the specimen is needed should problems concerning the culture request arise. The date and hour of collection should be indicated so that culture results can be properly interpreted.

B. Label

Each specimen should have a barcoded Container ID blaster label firmly attached to the specimen container.
PRINCIPLES OF SPECIMEN COLLECTION

If the specimen is coming from an outpatient facility that does not have access to the internal information systems, the label on the outside of the specimen container should have the following information clearly written:

- Patient Full Name
- Hospital Number
- Date of Birth
- Date of Collection

Rationale

Unfortunately, many specimen containers are received in the laboratory without labels or with labels that are not properly completed. All entries on the label MUST be legibly PRINTED. Patient’s first and last names should be used to prevent mix-up of specimens from individuals with the same surnames. The hospital number or other designator is a valuable crosscheck on the name.

C. Collection

1. The optimal times for specimen collection must be based upon both the type of infectious disease process and the ability of the laboratory to expertly process samples.

2. Twenty-four hour specimen collections for culture are not accepted.

3. The first early morning sputum and urine samples are optimal for recovery of acid-fast bacteria, fungi, and other pathogens. Samples collected at other times are acceptable.

4. The timing of blood cultures* should be determined by the clinical condition of the patient. Physicians should always indicate the collection
PRINCIPLES OF SPECIMEN COLLECTION

schedule. Except in acute cases of septicemia, blood cultures should not be drawn more frequently than ½ hour apart. A total of three cultures per 24 hours is usually sufficient to diagnose most cases of septicemia.

*A “blood culture” is defined as a draw of at least 16-20 ml of blood divided between two 25 ml bottles, one incubated aerobically and one anaerobically. For children, 0.5-3 ml of blood per bottle is acceptable. Smaller volume bottles (Peds Plus bottles) are available on the Pediatric floors. Collect 0.5-3 mL of blood per bottle submitted.

Rationale

1. The Microbiology laboratory may not be well staffed during evening and late night hours to perform certain tests. However, provisions must be made to handle urgent samples during “off” hours, and consultation with supervisory personnel is highly recommended.

2. Pathogens, in highest concentration, in first morning collections, will be diluted by added secretions. There is a high likelihood that samples stored after collection may become overgrown with contaminants. Improved laboratory extraction techniques preclude the need for large volumes of samples.

3. Early morning secretions are more concentrated and more likely to contain large numbers of etiologic agents.

4. In Endocarditis, Typhoid Fever Brucellosis and other uncontrolled infections, the bacteremia is continuous, thus making timing of collection less critical. In other infections, bacteremia is intermittent and may precede the onset of fever by an hour, making collection timing important. In acute febrile episodes, two draws of 10 ml blood each, obtained from separate venipuncture sites, will allow immediate initiation of therapy. Samples drawn within ½ hour may reflect the same bacteremic episode and sequential positive cultures may not be as valid as those spaced at longer time intervals. The recovery rate after three negative cultures per 24 hours is extremely low, except in cases where a sudden fever spike is observed, then drawing of an additional blood culture may be indicated.

5. The following specimens should be collected only after consultation with the Microbiology Director, CMT or Supervisor.
PRINCIPLES OF SPECIMEN COLLECTION

a. MIC and MBC’s
b. Special blood cultures for recovery of fungi of cell-wall deficient “L” forms
c. Recovery of Corynebacterium diphtheriae, Vibrio, Rickettsia, Leptospira, or other unusual organisms.
d. Darkfield examination assays are no longer available at UK HealthCare.

D. Collection Procedures

1. All specimens must be collected in appropriate sterile containers. If samples are to be delayed in processing or are sent to reference laboratories, a transport medium must be used.

2. Anaerobic cultures are best collected by aspirating abscess fluid with a sterile syringe and needle. Tissue biopsies are another acceptable specimen time for anaerobic culture. If swabs are used, they must be placed immediately into gassed tubes or suitable anaerobic containers. Specimens must be delivered within 30 minutes.

3. Sputum samples must contain lower respiratory secretions. Patients must be instructed to cough deeply. Habitual smokers understand well what a deep cough means. The mouth should be rinsed with water or patient should gargle, and dentures should be removed immediately before collection.

4. Bronchial washings should be processed as soon as possible after they are collected.

5. The collection of clean-catch urine samples must not be left to chance. Ideally, the specimen should be collected by the patient after specific instructions by a nurse or aide.

6. Stool specimens submitted for the recovery of acid-fat bacilli should not be processed, except in the cases of Bone Marrow Transplant patients or AIDS patients.

7. Surface lesions (wounds) must be sampled carefully. It is imperative that the surface lesion be opened and the advancing edge of the lesion firmly
PRINCIPLES OF SPECIMEN COLLECTION

sampled. Pus must be expressed onto the swab. Surface lesions are unsuitable for anaerobic studies.

8. Wound specimens submitted for anaerobic work-up must be submitted in an appropriate anaerobic transport medium or in the syringe used to collect as aspirate.

Rationale

1. If the container is not sterile, results may be erroneous. It is the laboratory’s responsibility to see that sterile containers of suitable construction are made available to physicians or ward personnel. Containers for stool culture should be clean but need not be sterile.

2. It is important to protect species of anaerobic bacteria from the killing effect of atmospheric oxygen. The greatest chance for recovery is by protecting the specimen from any contact with atmospheric oxygen before inoculation in the laboratory.

3. All sputum samples are contaminated to varying degrees with oropharyngeal secretions. Mechanical rinsing of the mouth immediately before expectoration will reduce the number of contaminating bacteria. Induced specimens or transtracheal aspirations are recommended for patients who cannot produce sputum.

4. Some microorganisms, which may infect the respiratory tract, such as *Haemophilus influenzae*, are susceptible to drying to or low temperature.

5. There is a high potential for contamination of the periurethral area in females from vaginal or bowel flora. Since this laboratory performs routine colony counts on all urine samples, meticulous care must be taken in specimen collection if valid results representative of bladder urine are to be obtained. If patients are to collect specimens unattended, specific verbal and written instructions will help to ensure collection of a good specimen. It may be well to actually read the instructions to the patient, particularly if there is a language barrier. It is recommended that these instructions be printed on a card from the patient to retain during the collection procedure. Instructions should be available in the predominant languages of this area.
PRINCIPLES OF SPECIMEN COLLECTION

6. It is virtually impossible to recover acid-fast bacilli from fecal material because of the inability to prevent heavy overgrowth with bowel flora.

7. Pus, alone, may not reveal growth upon plating since the encased organisms may be dead. The **REPRESENTATIVE** specimen is at the advancing margin of the wound. Never submit a dry swab that has been carelessly rubbed over a surface lesion. Anaerobes are abundant on skin surfaces and are common surface wound contaminants. Scrub the area around the wound carefully before sampling.

8. Anaerobic transport media are designed to protect the strictest anaerobe. Other methods of transport may preserve some anaerobes for a time but may not allow optimal recovery of anaerobes. The physician’s need for complete anaerobic data is no less than that of the laboratory for a properly selected and submitted specimen in anaerobic transport.

SPECIMENS AND POTENTIAL PATHOGENS ASSOCIATED WITH CERTAIN DISEASES

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>SPECIMEN COLLECTION</th>
<th>POTENTIAL PATHOGENS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Meningitis</td>
<td>1. Spinal fluid</td>
<td>1. Adults + Children &gt;1 months</td>
</tr>
<tr>
<td></td>
<td>2. Blood</td>
<td>a. <em>Neisseria meningitidis</em></td>
</tr>
<tr>
<td></td>
<td>3. Wounds</td>
<td>b. <em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>4. Subdurals (infant)</td>
<td>c. <em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td></td>
<td>5. Respiratory Tract</td>
<td>d. <em>Streptococcus &amp; others</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. <em>Listeria monocytogenes</em> (elderly)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Neonates/neurosurgical patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Enterobacteriaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. <em>Streptococcus agalactiae</em> (Group B Strep)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d. <em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>e. <em>Listeria monocytogenes</em></td>
</tr>
</tbody>
</table>
## PRINCIPLES OF SPECIMEN COLLECTION

| Eye | 1. Purulent discharge  
2. Lower cul-de-sac  
3. Inner canthus | 1. *Haemophilus influenzae* subgroup aegyptius  
2. *Staphylococcus aureus*  
3. *Moraxella* sp.  
4. *Streptococcus pneumoniae*  
5. *Neisseria gonorrhoeae*  
6. *Pseudomonas aeruginosa* (reported STAT)  
7. *Bacillus cereus* (reported STAT) |
|---|---|---|
| Ear | 1. Acute  
   a. Nasopharynx  
   b. Tympanic membrane aspirate  
2. Chronic  
   a. Drainage | 1. Acute  
   a. *Streptococcus pneumoniae*  
   b. *Haemophilus influenzae*  
   c. *Streptococcus pyogenes*  
   d. Others  
2. Chronic  
   a. *Pseudomonas aeruginosa*  
   b. *Proteus species*  
   c. Others |
| Sinusitis | 1. Acute  
   a. Nasopharynx  
2. Chronic  
   a. Nasopharynx  
   b. Surgical Aspirate | 1. Acute  
   a. *Streptococcus pneumoniae*  
   b. *Staphylococcus aureus*  
   c. *Haemophilus* sp.  
   d. *Klebsiella* sp. and other Enterobacteriaceae  
2. Chronic  
   a. As above  
   b. Anaerobes  
   c. Mold/Fungi |
| Wounds/Abscesses | 1. Purulent drainage  
2. Tissue affected  
3. Body fluids  
4. Ulcers | 1. *Staphylococcus aureus*  
2. Anaerobes (deep wounds, aspirates)  
3. Enterobacteriaceae |
## PRINCIPLES OF SPECIMEN COLLECTION

**Throat/Pharynx (Bacterial)**

<table>
<thead>
<tr>
<th>Source</th>
<th>Types of Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharynx &amp; both fauces</td>
<td>1. <em>Streptococcus pyogenes</em>, <em>Streptococcus dysgalactiae</em></td>
</tr>
<tr>
<td>Nasopharyngeal swab recommended possible pertussis</td>
<td>2. <em>Arcanobacterium haemolyticum</em></td>
</tr>
<tr>
<td></td>
<td>3. <em>Corynebacterium diphtheria</em> (send out testing by culture)</td>
</tr>
<tr>
<td></td>
<td>4. <em>Fusobacterium necrophorum</em></td>
</tr>
<tr>
<td></td>
<td>5. <em>Neisseria gonorrhoeae</em></td>
</tr>
<tr>
<td></td>
<td>6. <em>Bordetella pertussis</em> (send out testing by PCR)</td>
</tr>
<tr>
<td></td>
<td>7. <em>Neisseria meningitidis</em> (colonization, does not cause infection in this site)</td>
</tr>
</tbody>
</table>

**Pulmonary**

<table>
<thead>
<tr>
<th>Source</th>
<th>Types of Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transtracheal aspirate</td>
<td>1. <em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td>Lung aspirate/biopsy</td>
<td>2. <em>Haemophilus sp.</em></td>
</tr>
<tr>
<td>Bronchoscopy (BAL)</td>
<td>3. <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Sputum</td>
<td>4. <em>Klebsiella sp.</em></td>
</tr>
<tr>
<td>Blood</td>
<td>5. Other Enterobacteriaceae</td>
</tr>
<tr>
<td>PAL (for patients in the ICU)</td>
<td>6. <em>Mycobacterium sp.</em></td>
</tr>
<tr>
<td>Streptococcus pneumoniae and Legionella urinary antigen testing</td>
<td>7. <em>Legionella sp.</em> (PCR on BAL or <em>Legionella</em> urinary antigen testing)</td>
</tr>
<tr>
<td>BAL for PCP and Legionella PCR.</td>
<td>8. <em>Mycoplasma pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>9. <em>Pneumocystis jiroveci</em> (Detected by GMS staining in Cytology laboratory for BAL specimens. PCR is also available as a referred test on BAL specimens)</td>
</tr>
<tr>
<td></td>
<td>10. Almost any organism in pure culture.</td>
</tr>
</tbody>
</table>

**Septicemia**

<table>
<thead>
<tr>
<th>Source</th>
<th>Types of Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1. <em>Staphylococcus sp.</em></td>
</tr>
<tr>
<td></td>
<td>2. <em>Escherichia coli</em></td>
</tr>
<tr>
<td></td>
<td>3. <em>Klebsiella sp.</em></td>
</tr>
<tr>
<td></td>
<td>4. <em>Pseudomonas sp.</em></td>
</tr>
</tbody>
</table>
PRINCIPLES OF SPECIMEN COLLECTION

Endocarditis

| 1. 2 or 3 blood cultures on 1st day |
| 2. Repeat next day if initial culture negative |
| 3. Interval 1-6h |

Diarrheal Disease

| 1. Stool |
| 2. Rectal mucous sp. |
| 3. Blood |

**Bacterial Infection**

1. *Salmonella* sp. 
2. *Shigella* sp. 
3. *Escherichia coli* 0157:H7 and other toxigenic *E. coli* subspecies. 
4. *Vibrio* sp. 
5. *Yersinia* sp. 
6. *Campylobacter* sp. 
7. *Plesiomonas* sp. 
8. *Aeromonas* sp. (cultured only by specific request). 
9. *Edwardsiella tarda* (cultured only by specific request). 
10. *Bacillus cereus* (sent to State lab for toxin testing by specific request only)

**Viral Infection**

1. *Norovirus* 
2. *Astrovirus* 
3. *Adenovirus* 
4. *Sapovirus* 
5. *Rotavirus*

**Parasitic Agents**
## PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Genital Tract</th>
<th>UTI</th>
<th>Bone/Joint</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cervix</td>
<td>1. Neisseria gonorrhoeae (PCR preferred, culture by special request)</td>
<td>1. Bone</td>
</tr>
<tr>
<td>2. Urethral discharge</td>
<td>2. Treponema pallidum (serology, no culture)</td>
<td>2. Joint aspirate</td>
</tr>
<tr>
<td>3. Rectum</td>
<td>3. Haemophilus ducreyi (cultured by special request only)</td>
<td>3. Overriding skin lesions</td>
</tr>
<tr>
<td>5. Serum</td>
<td>5. Candida sp.</td>
<td>2. Propionibacterium acnes</td>
</tr>
<tr>
<td></td>
<td>7. Chlamydia trachomatis (PCR preferred, culture by special request)</td>
<td>4. Salmonella sp.</td>
</tr>
<tr>
<td></td>
<td>10. Herpes simplex</td>
<td>7. Streptococcus pneumoniae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. Pseudomonas sp.</td>
</tr>
</tbody>
</table>

1. Giardia lamblia
2. Cyclospora sp.
3. Cryptosporidium sp.
4. Entamoeba histolytica
5. Other Parasites

1. Neisseria gonorrhoeae (PCR preferred, culture by special request)
2. Treponema pallidum (serology, no culture)
3. Haemophilus ducreyi (cultured by special request only)
4. Trichomonas vaginalis (antigen detection, no culture)
5. Candida sp.
6. T-Mycoplasma
7. Chlamydia trachomatis (PCR preferred, culture by special request)
8. Group B Streptococcus – OB/GYN
9. Listeria monocytogenes – OB/GYN
10. Herpes simplex

1. Clean catch midstream
2. Suprapubic aspirate
3. Catheterization
4. Infants – bag catheter tips unacceptable for culture

1. Escherichia coli
2. Klebsiella sp.
3. Proteus mirabilis
4. Pseudomonas sp.
5. Enterococcus sp.
6. Corynebacterium urealyticum
7. Aerococcus urinae
8. Others
PRINCIPLES OF SPECIMEN COLLECTION

Skin

1. Impetiginous lesion
2. Cellulitis margins
3. Petechial lesions
4. Bullae
5. Pustules
6. Ulcers

1. *Staphylococcus aureus*
2. *Streptococcus/Enterococcus*
3. Enterobacteriaceae
4. Dermatophytes and other mycotic agents
5. *Mycobacterium marinum*
6. Varicella zoster
7. *Herpes simplex* I and II
8. Skin manifestations of syphilis are not suitable for culture or direct exam. Submit serum for serologic testing.

Burns

1. Tissue
2. Aspirate of fluid or pus beneath eschar
3. Blood

1. *Staphylococcus sp.*
2. *Streptococcus/Enterococcus*
3. *Pseudomonas aeruginosa*
4. Enterobacteriaceae
5. *Candida sp.*
6. Aspergillus sp.
7. *Mucor sp.*
8. *Fusarium sp.*

Newborn Systemic infection

1. Blood
2. Spinal fluid
3. Urine
4. Respiratory tract
5. Skin-umbilicus
6. Skin-ear
7. Consider: wounds, eye etc.

1. Enterobacteriaceae – *Escherichia coli, Klebsiella* sp.
2. *Staphylococcus aureus*
3. *Streptococcus* – Groups A, B,
4. *Enterococcus* sp.
5. *Haemophillus* sp.
6. *Listeria monocytogenes*
7. *Streptococcus pneumoniae*
8. Others including *Neisseria gonorrhoeae, Chlamydia trachomatis* and *Herpes simplex* viruses.

SUITABILITY OF VARIOUS CLINICAL MATERIALS FOR ANAEROBIC CULTURE STUDIES

<table>
<thead>
<tr>
<th>SUITABLE</th>
<th>UNSUITABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Properly collected abscess material</td>
<td>Throat or nasopharyngeal swab samples, unless you are specifically</td>
</tr>
</tbody>
</table>
PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>looking for Lemierre's disease <em>(Fusobacterium necrophorum)</em></td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>Sputum, tracheotomy aspirate, bronchoscopic washings</td>
</tr>
<tr>
<td>Lung aspirate</td>
<td>Voided or bladder catheterization urine samples</td>
</tr>
<tr>
<td>Suprapubic urine tap</td>
<td>Vaginal or cervical swabs</td>
</tr>
<tr>
<td>Endometrial or endocervical material collected by</td>
<td>Specimens contaminated with feces (draining fistulae, colostomy, bowel contents, rectal/</td>
</tr>
<tr>
<td>direct visualization through a speculum</td>
<td>perianal abscess)</td>
</tr>
<tr>
<td>Ascetically collected tissue</td>
<td>Feces (there are a few exceptions: infant botulism, <em>C. perfringens</em> foodborne disease)</td>
</tr>
<tr>
<td>Body fluids (ascetic, cerebrospinal, pericardial,</td>
<td></td>
</tr>
<tr>
<td>pleural, synovial)</td>
<td>Specimens contaminated with feces (draining fistulae, colostomy, bowel contents, rectal/</td>
</tr>
<tr>
<td></td>
<td>perianal abscess)</td>
</tr>
<tr>
<td></td>
<td>Body fluids (ascetic, cerebrospinal, pericardial, pleural, synovial)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BLOOD CULTURE COLLECTION

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Collection Protocol</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADULTS AND ADOLESCENTS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe Septicemia</td>
<td>2 culture sets prior</td>
<td>Two 20 mL samples from each arm (4 bottles, 8-10cc in each)</td>
</tr>
<tr>
<td></td>
<td>to therapy</td>
<td></td>
</tr>
<tr>
<td>Subacute endocarditis</td>
<td>3 cultures sets</td>
<td>Space each collection at least 1 hour apart. Two should be collected at</td>
</tr>
<tr>
<td></td>
<td>within 24 hours</td>
<td>the beginning of fever spikes.</td>
</tr>
<tr>
<td>Low-grade intravascular</td>
<td>3 culture sets</td>
<td>Specimens collected at least 1 hour apart. Two should be collected at</td>
</tr>
<tr>
<td></td>
<td>within 24 hours</td>
<td>first sign of febrile episodes.</td>
</tr>
<tr>
<td>Bacteremia of unknown origin (Patient on Therapy)</td>
<td>4-6 culture sets</td>
<td>Take specimen just before next dose of antimicrobial agent.</td>
</tr>
<tr>
<td></td>
<td>within 48 hours</td>
<td></td>
</tr>
</tbody>
</table>
## PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>YOUNG CHILDREN:</th>
<th>Febrile Episodes</th>
<th>Bacteremia may precede episodes of fever and chills by about 1 hour.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No more than 3 total culture sets</td>
<td>Bacteremia may precede episodes of fever and chills by about 1 hour.</td>
<td></td>
</tr>
<tr>
<td>0.5-3 ml in Peds-plus Aerobic bottle only</td>
<td>Two culture sets usually suffice for diagnosing bacteremia in the newborn</td>
<td></td>
</tr>
</tbody>
</table>

When collecting the initial blood culture, consider collecting one tube of blood as an acute-phase serum for tests, which may be needed in later studies with a convalescent-phase serum.

1. Single set of blood culture is defined as 20 cc of blood collected at a single phlebotomy, divided into 10 cc each into an aerobic and an anaerobic blood culture bottle.
2. Sets of blood cultures is defined as 40 cc of blood collected 20 cc at a time from 2 distinct phlebotomy sites with each phlebotomy being divided into 2 x 10 cc aliquots into an aerobic and an anaerobic blood culture bottle.

### Site #1

- [Image of blood collection](image1.png)

### Site #2

- [Image of blood collection](image2.png)
PRINCIPLES OF SPECIMEN COLLECTION

- 2 distinct phlebotomies
- 20cc each site
- 10cc each bottle
- Aerobic + Anaerobic bottles from each site
- Total 40cc blood collected
- Collected prior to antibiotics!

SECTION II

SPECIMEN TRANSPORT

Microorganisms are living things – rapidly they grow, they reproduce, they die. Transport media are designed to prevent or slow all three processes. Incomplete or misleading laboratory data may result if ANY of the three occur before the specimen can be culture in the laboratory. Please hurry; the work can’t be started until the specimen arrives!

SPECIMEN TRANSPORT

Specimen Transport

1. It is important that culture specimens be processed as soon as possible after collection, preferably within 1 hour. If longer delays are unavoidable, a suitable transport medium must be used. If urine samples will be delayed, they should be refrigerated or urine vacutainer with preservative before transport.

Rationale

1. Many species of bacteria are vulnerable to delays in processing, temperature changes, and decreased moisture; during prolonged transport, rapidly growing bacteria may overgrow the more fastidious pathogens. Colony counts on urine samples are not valid if not processed within 1 hour of receipt because of rapid doubling time of many urinary tract pathogens. If the urine is not cultured within 1 hour, refrigerate the specimen. Refrigerated transport is recommended if the specimen is to be sent by a private office to a private laboratory.

Specimen Transport
PRINCIPLES OF SPECIMEN COLLECTION

2. If a delay in transport is anticipated, or if cultures are sent to a reference Laboratory; use an E-swab or Carey Blair transport (stool) medium should be used.

Rationale

2. Transport medium is formulated to maintain the viability of bacteria with only a slow rate of replication. Fastidious strains, however, may not survive the nutritionally poor medium. Some bacterial populations may double within 1 hour if body fluids are present.

Specimen Transport

3. When possible, specimens should be delivered directly to the microbiology laboratory, bypassing central collection areas or other departments in the laboratory if someone is not there to receive specimen.

Rationale

3. We are not measuring the chemicals, enzyme levels, or body cells. We grow living, replicating organisms that cannot be expected to conform to our schedules of convenience, no matter how busy we may be.

Specimen Transport

4. See topic of “Specimen Refrigeration”.

TRANSPORT MEDIA

STUART’S (1954)

1. Originally formulated for transport of Neisseria gonorrhoeae.
2. Used charcoal-impregnated swabs.
3. “Non-nutritive” medium.
4. Good for most specimens.
5. Charcoal caused difficulty in Gram stain interpretation
6. Some Gram-negative rods can utilize glycerophosphate in the medium, thus overgrowing the culture.

Amies (1965)

1. Modified Stuart’s medium.
PRINCIPLES OF SPECIMEN COLLECTION

2. Replaced glycerophosphate with a balanced salt solution.
3. Better transport system for most specimens.
4. Distributed as the bacteria support medium with Eswabs

Cary Blair (1964)
1. Similar to Stuart's but modified for fecal specimens.
2. pH increased from 7.4 to 8.4.
3. Good for stool specimens.
4. Recommended for fecal specimens that are being tested by the Comprehensive GI panel by PCR (but not for use with the Clostridium difficile specific PCR which requires a raw (undiluted) stool specimen)

COPAN Eswab
1. Liquid based multi-purpose collection and transport system.
2. Maintains aerobic, anaerobic and fastidious bacteria for up to 48 hours at room temperature or refrigerated.
3. Comprises 1 mL of liquid Amies and a FLOQSwab™

BBL Anaerobic Transport
1. Transport swab that provides anaerobic Swab atmosphere for transport to the laboratory.

Universal Viral Transport Media (VTM)
1. Liquid based collection and transport system for viruses, chlamydiae, mycoplasmas and ureaplasmas.
2. Maintains organism viability at room temperature or refrigerated.
3. Includes antimicrobials to suppress bacterial and fungal contamination.
4. May be used to store specimens frozen with the presence of a cryoprotectant.

Urine Vacutainer
1. Collection and transport container with buffered boric acid formula that maintains urine for up to 48 hours at room temperature.
2. Prevents overgrowth without causing toxicity to existing pathogens.

Total-Fix® Preservative
PRINCIPLES OF SPECIMEN COLLECTION

1. Stool collection kit used for untrained personnel to properly collect and preserve stool specimens for parasitological studies.
2. Contains mercury, formalin and PVA-free fixative to preserve parasite morphology.

Transgrow Bottle

1. Bottled transport media. Thayer-Martin filled medium for the isolation of *N. gonorrhoeae* and *N. meningitidis*.

Probetec ET and Abbott M2000 CT/NG

1. Special collection tubes must be used for the transport of specimens for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by PCR.

Specimens that **CAN** be refrigerated before inoculation of media:
- Urines

Specimens that **CANNOT** be refrigerated before inoculation of media:
- Spinal fluids and other body fluids (spinal fluids should be held at 35°C)
- Genital/cervical for gonococcus isolation
- Blood
- Stool/Feces
- Wounds/exudates
- Respiratory

ENVIRONMENTALLY FRAGILE ORGANISMS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Most Likely Specimen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella sp.</em></td>
<td>Stool</td>
<td>Immediate processing</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>Genital</td>
<td>Sensitive to cold, needs 5-10% CO₂ soon after collection</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>Spinal Fluid</td>
<td>Do not refrigerate; process soon after receipt in laboratory</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>CSF, eye, ear</td>
<td>Sensitive to cold</td>
</tr>
</tbody>
</table>
**PRINCIPLES OF SPECIMEN COLLECTION**

**COLLECTION AND TRANSPORT OF CLINICAL SPECIMENS FOR BACTERIOLOGIC EXAMINATION**

<table>
<thead>
<tr>
<th>SPECIMENT</th>
<th>COLLECTION</th>
<th>TRANSPORT</th>
<th>INSTRUCTION/COMMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobe special request</td>
<td>Syringe Vacutainer Brand Anaerobic Specimen Collector Eswab – Amies medium with flocked swab</td>
<td>No refrigeration Use anaerobic transport method</td>
<td>1. Avoid all O\textsubscript{2} exposure 2. Expel air from syringe 3. Label properly 4. Hold needed supply of media in anaerobic atmosphere for better initial growth. (pre-reduced)</td>
</tr>
<tr>
<td>Blood</td>
<td>Commercial Kit Aerobic, Aerobic FAN, Peds-plus and Anaerobic</td>
<td>Culture broth in bottles 25mL/bot. 10mL/blood Bottles; 2 bottles – one Aerobic + one Anaerobic DO NOT REFRIGERATE. Pediatric bottle available on floor (0.5mL – 3.0mL)</td>
<td>1. Decontaminate puncture site with alcohol/iodine 2. Do no palpate disinfected site 3. 10% (vol:vol) blood:broth 4. Decontaminate bottle stopper with alcohol/iodine 5. Always allow iodine to dry.</td>
</tr>
<tr>
<td>CSF</td>
<td>Surgical prep and collection by physician. Sterile screw-cap or snap-cap tubes</td>
<td>Transport in collection tube. Do no refrigerate</td>
<td>1. Surgical prep of puncture site 2. Obtain “as much as possible”, 4-5mL is optimal for adults. 0.5-1.0mL in children. 3. Handle as EMERGENCY specimens hand carry to lab</td>
</tr>
</tbody>
</table>
## PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Collection Method</th>
<th>Transport Method</th>
<th>Instructions</th>
</tr>
</thead>
</table>
| EAR      | Flocked ESwab with Amies | Liquid collection-transport system | 1. Clean external ear surface  
2. CAREFULLY take representative area  
3. Label properly |
| EYE      | Corneal scraping (by physician). Solid media inoculated at bedside. Eswab in Amies | Amies Transport medium (no charcoal) | 1. Do not touch external skin  
2. Obtain maximum material  
3. Label properly  
4. Prepare smears for Giemsa and/or Gram stain. |
| FECES    | Cary-Blair (yellow lid in stool collection kit is preferred) for CGIPCR. Raw, undiluted stool for CDPCR. Clean or sterile collection cup (white lid in stool collection kid acceptable) for CDPCR. Swab (only if necessary) for special tests – Infection control surveys, | Transport within 1 hour to lab | 1. Best specimen is diarrheal stool  
2. Insert swab beyond anal sphincter.  
3. Swab must show feces. |
### PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Collection Method</th>
<th>Transport Medium</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOSE</td>
<td>ESwab</td>
<td>Amies Transport Medium</td>
<td>1. Swab anterior nares only 2. Culture quickly</td>
</tr>
<tr>
<td>SINUS</td>
<td>BBL CultureSwab Plus</td>
<td>Amies Transport Medium</td>
<td>1. Insert and remove carefully 2. Prepare slide for stain using second swab or after inoculating media</td>
</tr>
<tr>
<td>SPUTUM</td>
<td>Sterile cup</td>
<td>Transport in collection container within 1 hour</td>
<td>1. Carefully instruct patient to cough deeply (not to spit) 2. First morning specimen is best (no 2 hour collection)</td>
</tr>
</tbody>
</table>
# PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Collection Method</th>
<th>Transport Method</th>
<th>Notes</th>
</tr>
</thead>
</table>
| **THROAT** | ESwab | Liquid collection-transport system | 1. Use tongue blade  
2. Sample ONLY back of throat between and around the tonsillar area thoroughly.  
3. Avoid cheeks, teeth, etc. |
| **URINE (midstream)** | Urine Vacutainer or Sterile screw-cap cup | Transport in urine preservative (grey top vacutainer) at room temperature or if collection container use; Refrigerate if delayed more than 1 hour | 1. Give patient clear and detailed instructions.  
2. Clean with soap, not disinfectant  
3. A 1 hour delay before culturing is too long  
4. Refrigerate no longer than 6 hours prior to culture  
5. Seal container securely |
| **URINE (catheter)** | Sterile screw-cap tube Needle and syringe | Sterile tube (grey top) | 1. Collect using an in-out catheter  
2. Do not culture Foley tip or bag contents  
3. If collecting from a foley, collect from the line, not the bag  
4. Decontaminate line as for venipuncture |
| **WOUND (surface)** | ESwab | Amies Transport Medium | 1. Decontaminate surrounding skin  
2. Open lesion and express pus into swab; sample advancing margin of lesion.  
3. Label properly |
| **WOUND (deep)** | Syringe Eswab | Transport aspirate in the collecting syringe or collect deep | 1. Maintain anaerobic conditions  
2. Label properly |
SECTION III

CRITERIA FOR REJECTION OF MICROBIOLOGICAL AND MOLECULAR SPECIMENS

1. Unlabeled or improperly labeled specimen.

2. Prolonged transport to the lab or improper or inadequate processing of specimen, i.e., serum or plasma not separated from cells or improper storage temperature.

3. Improper or damaged/leaking container.

4. Improper specimen type for requested test. (ie. formed stool for *C. difficile* testing).

5. Oropharyngeal-contaminated sputum.

6. Obvious contamination with foreign materials.

7. Duplicate specimens collected within a 24 hour period:
   - Multiple stool samples for PCR and/or Ova and Parasite testing
   - Multiple sputa samples for culture. Exceptions: multiple sputa following Bronchoscopy, up to 3 sputa to rule-out MTB infection
   - Multiple PCR requests on same target pathogen unless indicated
   - Multiple urine samples from the same source and collect time

8. *C. difficile* by PCR and Comprehensive GI Panel by PCR duplicated and rejection criteria:
   - Specimens received within one week of a previous negative PCR result.
   - Specimens received within one month of a previous positive PCR result.
PRINCIPLES OF SPECIMEN COLLECTION

- Formed or semi-formed stool received for *C. difficile* testing or CGIPCR testing.

9. Specimen unsuitable for culture request; i.e., routine vaginal for anaerobe workup or Foley catheter tip.

10. Specimen unsuitable for molecular testing, i.e., serum submitted instead of plasma.

11. Quantity not sufficient for testing.

12. Viral load not sufficient for genotyping, i.e., HIV RNA value of <500 copies/ml.

13. Viral load not sufficient for HCV genotyping, i.e., HCVRNA <500 copies/ml.

ACTION ON REJECTED SPECIMENS

1. Unlabeled or improperly labeled specimen: Contact the collector (physician and/or nurse) and inform of unlabeled or improperly labeled specimen. The sample may only be run if the nurse or physician comes to the laboratory and positively identifies the sample. The nurse or physician must complete a mislabeled specimen form and must physically re-label the specimen. The mislabeled specimen form is attached to the patient requisition. If the sample is not positively identified, it is placed in the specimen storage container and kept for at least 24 hours before discarding.

2. Prolonged transport to the lab or improper or inadequate processing of specimen, e.g., serum or plasma not separated from cells or improper storage temperature: notify ward nurse or physician and ask for a recollected sample. Document the full name, date and time of the person notified.

3. Improper or damaged/leaking container: notify ward nurse or physician and ask for a recollected sample. Document the outcome and the full name of the person notified and the date and time.

4. Oropharyngeal-contaminated sputum: the following statement is entered in the gram stain result “Inadequate sputum. Please recollect. A credit has been issued.”
PRINCIPLES OF SPECIMEN COLLECTION

5. Obvious contamination with foreign materials: Notify ward nurse or physician and ask for a recollected sample. Document the outcome and the full name of the person notified and the date and time.

6. Duplicate stool, sputa (except sputa following bronchoscopy), urine (from the same source) and PCR requests on the same target pathogen unless indicated within a 24 hour period: physician and/or nurse notification is not required. The ordering technologist completes a voided sample form which is submitted to the senior tech or supervisor. The senior tech or supervisor documents the information in the patient’s electronic medical record and issues a credit.

7. *C. difficile* PCR duplicates (see “CRITERIA FOR REJECTION OF MICROBIOLOGICAL AND MOLECULAR SPECIMENS”): physician and/or nurse notification is not required. The ordering technologist completes a voided sample form which is submitted to the senior tech or supervisor. The senior tech or supervisor documents the information in the patient’s electronic medical record and issues a credit.

8. Specimen unsuitable for culture request; e.g., routine vaginal for anaerobe workup or Foley catheter tip: The ordering technologist notifies ward nurse or physician (documents the full name, date and time) and completes a voided sample form which is submitted to the senior tech or supervisor. The senior tech or supervisor documents the information in the patient’s electronic medical record and issues a credit.

9. Specimen unsuitable for molecular testing, i.e., serum submitted instead of plasma: The ordering technologist notifies ward nurse or physician (documents the full name, date and time) and completes a voided sample form which is submitted to the senior tech or supervisor. The senior tech or supervisor documents the information in the patient’s electronic medical record and issues a credit. Formed or semi-formed stools for *C. difficile* by PCR are resulted using the stick test as evidence of an inadequate specimen.

10. Viral load not sufficient for genotyping, i.e., HIV RNA value of <500 copies/ml: Physician and/or nurse notification is not required. The ordering technologist completes a voided sample form which is submitted to the senior tech or supervisor. The senior tech or supervisor documents the information in the patient’s electronic medical record and issues a credit.
PRINCIPLES OF SPECIMEN COLLECTION

11. Quantity not sufficient for testing: The ordering physician is notified and asked to recollect and/or prioritize testing. The full name of the person contacted, date and time must be documented on the patient requisition. Tests that are not performed due to insufficient quantity will have a specimen void form completed and given to the senior tech or supervisor for crediting.

12. If processing of inadequate or improper specimens is necessary, explain the discrepancy on the report to the physician and indicate that the results may not be valid or complete on the final report.

SPECIMEN PROCESSING GUIDELINES

Specimens for molecular diagnostics, Virology, AFB, Mycology, Parasitology, and Routine Bacteriology are received and initially checked for appropriate volume, appropriateness of test requested and matched to patient name and identification number on requisition and specimen label. Specimens are also checked for integrity and signs of contamination. Test requests deemed questionable or inappropriate are referred to the supervisor, chief technologist or Director. The ordering physician is then consulted regarding the test ordered.

Specimens meeting test criteria are processed individually to prevent cross contamination and mixing of specimens. Specimens may be processed and incubated immediately, or sorted and stored for processing the next regular workday as appropriate for the test requested.

When processing, specimens should be handled separately and no specimen aliquot is to be returned to the original container once it has been removed. Separate specimens must not be mixed. Specimens must come from original tube (not previously open) to maintain specimen integrity. All specimens should be aliquoted aseptically to preserve integrity.

Testing personnel run a daily log of all specimens that need to be tested to assure that no specimens are missed. If a specimen cannot be located, the appropriate personnel are contacted for a recollect.
PRINCIPLES OF SPECIMEN COLLECTION

DIRECT EXAMINATION BY GRAM STAIN

A. Specimens received in the laboratory on which a direct Gram stain should be performed. (*results given to the physician.)

1. Spinal fluid and other body fluids*
2. Positive blood culture smears*
3. Peritoneal fluid
4. Eye/Ear
5. Any purulent discharge
6. Sputum, transtracheal aspirate
7. Surgical aspirates
8. Tissue
9. Urethral exudates from males (for *N. gonorrhoeae*)
10. Vaginal “bacterial vaginosis” specimens
   Report Gram morphology and exudate characteristics (cells such as WBC’s epithelial cells, RBC’s).

B. Disease states in which a direct Gram stain may prove helpful.

1. Meningitis
2. Brain, spinal, epidural abscess
3. Epiglottitis
4. Severe pneumonia
5. Endocarditis
6. Peritonitis
7. Gas gangrene
8. Necrotizing fasciitis
9. Potential postoperative sequelae of heart valve replacement, Intra-abdominal infection, etc.
10. Gonorrhea
11. Diphtheria
12. Vincent’s angina
13. Staphylococcal enterocolitis
14. Vaginitis with suspected bacterial vaginosis
PRINCIPLES OF SPECIMEN COLLECTION

POSITIVE LACTOFERRIN ASSAYS REFLECTING FECAL LEUKOCYTES IN STOOL SPECIMENS FROM PATIENTS WITH DIARRHEAL DISEASE

A positive lactoferrin test on stool may be indicative of such diarrheal diseases: Campylobacteriosis, Shigellosis, Salmonellosis, Invasive E. coli, and C. difficile infection.

The UK HealthCare Microbiology laboratory performs a lactoferrin test using a rapid 10 minute immunochromatographic test.

This test is not indicated for breast-fed infants, false positive results may be attained.

NOTE: due to the lack of sensitivity of direct stains for WBC, a direct exam is no longer offered.

CRITERIA FOR GRADING SPUTUM SPECIMENS

The following criteria indicate oropharyngeal contamination and suggest that the sputum may not be representative of the lower respiratory tract.

1. Greater than 10 squamous epithelial cells per low power field.
2. Less than 25 white blood cells (WBCs) per low power field.
3. Greater than 10 epithelial cells and less than 25 WBCs per lower power field.

This laboratory uses only the first criterion for grading sputum specimens.

HANDLING STOOL SPECIMENS

Stool cultures are performed using the Biofire FilmArray Comprehensive GI Panel by PCR. The following analytes are tested using this panel:

Bacteria

- Campylobacter sp. (C. jejuni, C. coli and C. upsaliensis)
PRINCIPLES OF SPECIMEN COLLECTION

• *Clostridium difficile* (toxin A/B)*
• *Plesiomonas shigelloides*
• *Salmonella* sp.
• *Yersinia enterocolitica*
• *Vibrio* sp. (*V. parahaemolyticus, V. vulnificus, and V. cholerae*)
• Enteroaggrevative *E. coli* (EAEC)
• Enteropathogenic *E. coli* (EPEC)
• Enterotoxigenic *E. coli* (ETEC) stx1/stx2
• Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2
• *E. coli* O157
• *Shigella/Enteroinvasive E. coli* (EIEC)

*A positive* *C. difficile* result may not be clinically significant in children under 2 years of age. Infectious Disease consult is recommended

Parasites

• *Cryptosporidium*
• *Cyclospora cayetanensis*
• *Entamoeba histolytica*
• *Giardia lamblia*

Viruses

• *Adenovirus* F 40/41
• *Astrovirus*
• *Norovirus* GI/GII
• *Rotavirus* A
• *Sapovirus* (I, II, IV and V)

This comprehensive assay has a rapid 1 hour resulting time (workload dependent) and is available 24h a day.

Physician notification occurs for Shiga-like toxin producing *E. coli* and *E. coli* 0157.

Targeted culturing occurs for:

• *Campylobacter sp.*
• *Salmonella* sp.
PRINCIPLES OF SPECIMEN COLLECTION

- Yersinia enterocolitica
- Vibrio sp.
- E. coli O157
- Shigella sp. (susceptibilities performed)

These organisms are reportable diseases that are chronicled at the State Laboratory for epidemiological purposes.

Physician request for a test of cure may be requested. A stool sample should be submitted along with a manual requisition with indication of a test-of-cure, along with what the previous positive pathogen detected. This will be performed using a targeted culture method.

Previous positive patients using the Comprehensive GI Panel will not have a repeated PCR performed anytime < 1 month from last previous positive. A false positive may be detected due to latent DNA within the stool sample.

Targeted Culturing:

<table>
<thead>
<tr>
<th>GENERAL PURPOSE</th>
<th>ORGANISM</th>
<th>SELECTIVE/DIFFERENTIAL MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targeted Culturing</td>
<td>Salmonella/Shigella</td>
<td>Xylose-Lysine-Desoxycholate (XLD), Selenite Broth (TCBS) - <em>Vibrio</em></td>
</tr>
<tr>
<td>Yersinia sp.</td>
<td>Yersinia (CIN) agar</td>
<td></td>
</tr>
<tr>
<td>Campylobacter sp.</td>
<td>Campy Agar</td>
<td></td>
</tr>
<tr>
<td>Vibrio sp.</td>
<td>Thio Sulfate-Citrate-Bile Salts (TCBS) Agar</td>
<td></td>
</tr>
<tr>
<td>E. coli O157</td>
<td>MacConkey with Sorbitol</td>
<td></td>
</tr>
</tbody>
</table>

GEITAL SPECIMENS

Female Genital Specimens

Not Cultured for Anaerobes
- Vaginal
- Urethra

Cultured for Anaerobes
- Placenta, C-section
- Uterus (endometrial)
PRINCIPLES OF SPECIMEN COLLECTION

**Not Cultured for Anaerobes**  
Placenta (Vaginal sampling)  
Vulva  
Lachia  
Perineum  
Cervical

**Cultured for Anaerobes**  
Culdocentesis  
Fallopian tube  
Endocervical  
Ovary  
Bartholin’s gland

Females of child-bearing age (14-45 years) should have targeted culturing selected for genital specimens based on the suspected infection. Infection due to *Trichomonas vaginalis* and Candidiasis should have their proper tests ordered (*Trichomonas* antigen, Mycological Evaluation). Genital cultures suspected of Bacterial Vaginosis will be evaluated based using the BV Blue Assay. The presence of Beta hemolytic Streptococcus Group B will be identified no matter the amount.

Prepubescent females (<14 years) have specific guidelines for such bacteria as Non-lactose fermenting Gram negative rods and *Pasteurella* sp.

Any specimens from the outside genital region (male/female) will be processed and worked up like a wound.

**Male Genital Specimens**

**Not Cultured for Anaerobes**  
Urethral  
Prostatic Fluid  
Seminal Fluid

**INCUBATION CONDITIONS**

SPECIMENS TO BE INCUBATED UNDER 5%-10% CO₂  
Genital – Blood, MacConkey, Chocolate, Modified Thayer-Martin, V Agar  
Wounds – Blood, MacConkey, Chocolate  
Anaerobes – Anaerobic PEA, Laked KV, Schaedler (Anaerobic jar/hood)  
Respiratory – Blood, Chocolate, MacConkey (CNA for Bronchoscopy specimens)  
Blood – sub culture to Chocolate, Blood, MacConkey (Anaerobic BAP in anaerobic jar)  
Throat – Blood  
Surveillance – VACC agar
PRINCIPLES OF SPECIMEN COLLECTION

SPECIMENS TO BE INCUBATED IN O$_2$

Urine – Blood, MacConkey  
Surveillance – Chromagar MRSA II  
Cystic Fibrosis Respiratory – OFPBL (30°C)

SPECIMENS TO BE INCUBATED UNDER REDUCED O$_2$
Targeted culturing from positive Comprehensive GI Panels (Stool)  
Note: Campylobacter sp. To be placed in Microaerophilic conditions.

SELECTED EXAMPLES OF MEDIA FOR PRIMARY ISOLATION OF MICROORGANISMS

<table>
<thead>
<tr>
<th>General Purpose Media</th>
<th>Primary Isolation Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (5% Sheep)</td>
<td>Enriched medium used for primary recovery of most commonly encountered microorganisms</td>
</tr>
<tr>
<td>Chocolate Agar</td>
<td>Enriched medium for primary recovery with hemolyzed blood or hemoglobin which for fastidious organisms such as Neisseria gonorrhoeae and Haemophilus influenzae.</td>
</tr>
<tr>
<td>MacConkey</td>
<td>Selective and Differential medium for primary recovery of Gram-negative bacilli. Lactose and crystal violet are added to the agar to differentiate Lactose fermenting Gram negative bacteria by producing pink colonies.</td>
</tr>
<tr>
<td>Blood Agar (Anaerobic)</td>
<td>Non-selective blood agar used to recover anaerobic bacteria from clinical specimens. Contains sheep blood supplemented with yeast extract, hemin, Vitamin K and L-cystine.</td>
</tr>
<tr>
<td>Colistin-Nalidixic Acid (CAN)</td>
<td>Selective medium to promote the growth of Gram-positive bacteria and yeast. Gram negative growth is stunted.</td>
</tr>
<tr>
<td>Laked KV</td>
<td>Selective anaerobic blood agar with Kanamycin and Vancomycin for the</td>
</tr>
</tbody>
</table>
**PRINCIPLES OF SPECIMEN COLLECTION**

<table>
<thead>
<tr>
<th>Special Purpose Media</th>
<th>Isolation Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylethyl Alcohol (PEA)</td>
<td>Selective anaerobic blood agar for the isolation of Gram positive anaerobes. Good for inhibiting swarming colonies such as <em>Proteus</em> species.</td>
</tr>
<tr>
<td>Thioglycollate Broth</td>
<td>Tubed broth medium that is enriched designed to test the aerotolerance of bacteria. Good for the recovery of anaerobic bacteria</td>
</tr>
</tbody>
</table>

**SELECTED EXAMPLES OF SPECIAL PURPOSE MEDIA**

<table>
<thead>
<tr>
<th>Special Purpose Media</th>
<th>Isolation Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacConkey w/Sorbitol</td>
<td>Selective and Differential for E. coli O157</td>
</tr>
<tr>
<td>MacConkey w/Cipro</td>
<td>Selective media in aid of the identification of Fluoroquinolone resistant Gram-negative rods</td>
</tr>
<tr>
<td>TCBS</td>
<td>Selective and differential media for the isolation of <em>Vibrio</em> species.</td>
</tr>
<tr>
<td>CIN</td>
<td>Selective and differential media for the isolation of <em>Yersinia</em> species.</td>
</tr>
<tr>
<td>XLD</td>
<td>Selective and differential media for the isolation of <em>Shigella</em> and <em>Salmonella</em> species.</td>
</tr>
<tr>
<td>Campy</td>
<td>Special media with a variety of antibiotics which are designed to inhibit normal fecal flora but not effect growth of <em>Campylobacter</em> species. This media is meant to be incubated at 42°C in microaerophilic conditions.</td>
</tr>
<tr>
<td>Selenite</td>
<td>Selective broth in aid of the recovery of <em>Salmonella</em> and <em>Shigella</em> species.</td>
</tr>
<tr>
<td>CHROMagar MRSA II</td>
<td>Chromogenic selective and differential media for the isolation and identification of MRSA.</td>
</tr>
<tr>
<td>VACC</td>
<td>Selective medium for the recovery of multi-drug resistant bacteria such as...</td>
</tr>
</tbody>
</table>
PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL, KPC, VRE etc.</td>
<td>The blood agar is embedded with Vancomycin, Amphotericin B, Ceftazidime and Clindamycin. Further identification and susceptibilities required.</td>
</tr>
<tr>
<td>OFPBL</td>
<td>Selective and differential medium for the isolation of <em>Burkholderia cepacia</em>.</td>
</tr>
<tr>
<td>Sabouraud Dextrose/Inhibitory Mold Agar</td>
<td>Selective and inhibitory medium for the isolation of dermatophytes, yeasts and fungi.</td>
</tr>
<tr>
<td>V agar</td>
<td>Selective medium for the isolation and presumptive identification of <em>Gardnerella vaginalis</em>.</td>
</tr>
<tr>
<td>Modified-Thayer Martin</td>
<td>Selective and enriched medium used for the isolation of <em>Neisseria gonorrhoeae</em> and <em>Neisseria meningitidis</em>.</td>
</tr>
</tbody>
</table>

SELECTED EXAMPLES OF TRANSPORT MEDIA

<table>
<thead>
<tr>
<th>Transport Media</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eswab (with liquid Amies Medium)</td>
<td>Transport system for maintaining the viability of anaerobes, aerobes and fastidious microorganisms.</td>
</tr>
<tr>
<td>Viral culturette in Viral Transport Media</td>
<td>A transport medium for maintaining the viability of viruses to the laboratory.</td>
</tr>
<tr>
<td>Transgrow</td>
<td>Modified Thayer-Martin medium with 5% CO₂ designed to maintain viability of <em>Neisseria gonorrhoeae</em>.</td>
</tr>
<tr>
<td>BBL Anaerobic Transport</td>
<td>Transport swab that provides anaerobic atmosphere for transport to the laboratory.</td>
</tr>
<tr>
<td>Urine (grey top) Vacutainer</td>
<td>Used in the transport of urine with a preservative (boric acid) to discourage the proliferation of bacteria while in transport.</td>
</tr>
</tbody>
</table>
## SPECIMEN PROCESSING IN BACTERIOLOGY

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Media/ Special Conditions</th>
<th>Normal Flora</th>
<th>Common Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat</td>
<td>BAP (CO₂ 35°C)</td>
<td>Alpha and gamma strep, commensal Neisseria spp., S. epidermidis, Diphtheroids, S. pneumoniae, N. meningitidis.</td>
<td>S. pyogenes (Group A Strep), S. dysgalactiae (Group C, G Strep), Arcanobacterium haemolyticum</td>
</tr>
<tr>
<td>Respiratory</td>
<td>BAP, CHOC, MAC (all CO₂ 35°C)</td>
<td>Larynx, trachea, sinus. Sputum: S. epidermidis, non-beta streptococci, Diphtheroids, Alpha Streptococcus, Haemophilus sp., commensal Neisseria sp.</td>
<td>S. pyogenes (Group A Strep), H. influenzae, S. aureus, Enterobacteriaceae, Pseudomonas sp., S. pneumoniae, Neisseria meningitidis</td>
</tr>
<tr>
<td>Transtracheal</td>
<td>Same as Respiratory</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>BAP, MAC (all O₂ 35°C)</td>
<td>None</td>
<td>Escherichia coli and other enterics, Enterococci, Pseudomonas sp., Staphylococci</td>
</tr>
<tr>
<td>Stool (targeted culturing)</td>
<td>MAC/SOR XLD Selenite F CIN TCBS (all O₂ 22°C)</td>
<td>Enterobacteriaceae Enterococcus sp. Yeast</td>
<td>Salmonella sp., Shigella sp., Campylobacter sp., Aeromonas sp., Plesiomonas sp., Yersinia</td>
</tr>
</tbody>
</table>
### PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Culture Media</th>
<th>Microbes</th>
<th>Additional Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campy (42°C O₂)</td>
<td>enterocolitica, Vibrio sp., Bacillus cereus.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genital for GC only</td>
<td>Modified Thayer-Martin (MTM) CHOC (all CO₂ 35°C)</td>
<td>Lactobacilli, Diphtheroids, Alpha streptococcus (inhibited by MTM) Enterobacteriaceae, Enterococci, Yeast</td>
<td>Neisseria gonorrhoeae</td>
</tr>
<tr>
<td>Vaginal, Cervical, Routine Genital</td>
<td>BAP CHOC MAC MTM V (all CO₂ 35°C)</td>
<td>Lactobacilli, Diphtheroids Enterococci, Enterobacteriaceae, non-Beta streptococi</td>
<td>N. gonorrhoeae, C. albicans, Gardnerella vaginalis, Listeria monocytogenes, S. pyogenes (Group A Strep), S. agalactiae (Group B strep)</td>
</tr>
<tr>
<td>Genital – surgical aspirates</td>
<td>BAP CHOC MAC MTM (all CO₂ 35°C) ANPEA LKV ANBAP (all anaerobic 35°C)</td>
<td>None</td>
<td>Same as above plus anaerobic pathogens</td>
</tr>
<tr>
<td>Sterile body fluids, CSF, joint fluid, pleural fluid, peritoneal fluid.</td>
<td>BAP CHOC MAC Thio Broth (all CO₂ 35°C)</td>
<td>None</td>
<td>Identify all isolates. S. pneumoniae, N. meningitis, H. influenzae, Gram-negative rods, S. aureus, Pseudomonas sp., anaerobic bacteria.</td>
</tr>
</tbody>
</table>

*Gram Stain (Nugent scoring for Vaginal specimens)*
## PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Culture Media</th>
<th>Stains/Tests</th>
<th>Antimicrobial Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td>ANBAP (all anaerobic 35°C)</td>
<td>None</td>
<td>Any isolate potentially significant.</td>
</tr>
<tr>
<td>After flagged positive by analyzer plate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic: BAP MAC CHOC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic: add ANBAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram stain Wayson Stain Acridine Orange Stain (for false positive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Wound</strong> (superficial, includes eye and ear)</td>
<td>BAP CHOC MAC (all CO₂ 35°C)</td>
<td>S. epidermidis, Diphtheroids, commensal Neisseria sp., other skin flora</td>
<td>S. aureus, Beta-hemolytic streptococcus, P. aeruginosa, H. aegyptius (eye), H. influenzae, S. pneumoniae, Enterobacteriaceae, Enterococci, Bacillus sp. (eye)</td>
</tr>
<tr>
<td>Gram Stain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Wound (deep surgical or aspirate)</strong></td>
<td>Same as above plus anaerobic Thio Broth ANBAP LKV ANPEA (all anaerobic 35°C)</td>
<td>None</td>
<td>Same as above plus anaerobes. Potentially any isolate.</td>
</tr>
<tr>
<td>Gram Stain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tissue Specimens</strong></td>
<td>Same as wound superficial and/or deep</td>
<td>Potentially skin flora depending on type of tissue</td>
<td>Same as Wound deep.</td>
</tr>
</tbody>
</table>
SECTION IV

ALTERNATIVE AVENUES TO CONSIDER IN LABORATORY DIAGNOSIS

When should Mycobacterium sp. be considered?

1. In tissues demonstrating granulomas

2. Smears reveal poorly stained or diphtheroid-like organisms, but routine bacteriologic culture fails to grow anything within 48-72 hours.

3. Cases of cervical lymphadenitis.

4. Cultures on routine bacteriology media fail to yield growth.

5. Cultures in thioglycollate broth are still negative after several days of incubation.

6. The patient fails to respond to treatment with common anti-bacterial drugs.

7. Serological tests fail to reveal a rise in antibody titer to the suspected pathogen(s).

When should fungal cultures be considered?

1. Spinal fluid with increased lymphocytes has a negative Gram stain and acid-fast stain.

2. A Gram stain of aspirated pus is negative.

3. Sputum culture and Gram stain repeatedly fail to yield anything significant bacteriologically in a compromised host.

4. A Gram stain (and acid-fast stain, depending on tissue) of a surgical specimen is negative. Fungal cultures can be made of some specimens following examination of Gram stain.

5. Blood cultures are negative for bacteria in a compromised host who is deteriorating for unknown reasons.
PRINCIPLES OF SPECIMEN COLLECTION

6. Any mold of encapsulated yeast appears on blood agar.

7. The specimen is a skin or other tissue biopsy with granulomatous inflammation.

A DICTIONARY OF CLINICAL SPECIMENS

BODY FLUIDS

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid</td>
<td>Fluid produced by the innermost layer of the placenta early in gestation and contained within the amniotic sac surrounding the embryo in utero.</td>
</tr>
<tr>
<td>Ascites Fluid</td>
<td>Serous fluid aspirated from the abdominal cavity (the peritoneum).</td>
</tr>
<tr>
<td>Bile</td>
<td>Brown-green fluid secreted by the liver and either poured into the intestine or concentrated in the gallbladder.</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>The soft, highly cellular, blood-forming tissue that fills bone cavities.</td>
</tr>
<tr>
<td>Joint (synovial) fluid</td>
<td>Alkaline, thick fluid contained in joint cavities, bursae, and tendon sheaths serving as a lubricant.</td>
</tr>
<tr>
<td>Pericardia fluid</td>
<td>Fluid contained within the membranous sac that encases the heart.</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>Same as ascites fluid</td>
</tr>
<tr>
<td>Pleural Fluid</td>
<td>Fluid contained within the membranous coverings of the lungs.</td>
</tr>
<tr>
<td>Spinal fluid (CSF)</td>
<td>Fluid contained within the membranous coverings of the spinal cord and brain within the space known as the subarachnoid space.</td>
</tr>
<tr>
<td>Blood</td>
<td>The red liquid that circulates in the arteries and veins of humans and other vertebrate animals carrying oxygen to</td>
</tr>
</tbody>
</table>
PRINCIPLES OF SPECIMEN COLLECTION

and carbon dioxide from the tissues of the body.

EYE

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>The mucous membrane covering the anterior surface of the eyeball and the</td>
</tr>
<tr>
<td></td>
<td>under surfaces of the eyelids.</td>
</tr>
<tr>
<td>Inner Canthus</td>
<td>The inner (nasal) angle formed by the union of the upper and lower eyelids.</td>
</tr>
<tr>
<td>Lid</td>
<td>Folds of skin that protect the anterior eyeball surface.</td>
</tr>
</tbody>
</table>

GENITAL (FEMALE)

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartholin’s gland</td>
<td>One of two, small mucous scripting glands on either side of the vaginal</td>
</tr>
<tr>
<td></td>
<td>orifice.</td>
</tr>
<tr>
<td>Cervical aspirate</td>
<td>Mechanical withdrawal of material from the cervix.</td>
</tr>
<tr>
<td>Culdocentesis</td>
<td>Aspiration of fluid from recto-uterine excavation by puncture of the vaginal</td>
</tr>
<tr>
<td></td>
<td>wall.</td>
</tr>
<tr>
<td>Endocervical</td>
<td>From the interior of the cervix</td>
</tr>
<tr>
<td>Endometrium</td>
<td>The mucous membrane comprising the inner lining of the uterine cavity.</td>
</tr>
<tr>
<td>Fallopian Tube</td>
<td>Tube from uterus to ovary.</td>
</tr>
<tr>
<td>Female genital</td>
<td>Nondescript term generally taken to mean a vaingal/cervical specimen</td>
</tr>
<tr>
<td>Lochia</td>
<td>The final vaginal discharge occurring 1-2 weeks after childbirth</td>
</tr>
<tr>
<td>Ovary</td>
<td>Reproductive egg-forming gland within the pelvis in the female, lying lateral to the uterus.</td>
</tr>
</tbody>
</table>
### PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perineum</td>
<td>The space between the anus and the scrotum of the male and between the anus and vulva of the female.</td>
</tr>
<tr>
<td>Placenta</td>
<td>Highly vascularized organ of pregnancy, composed of multiple layers within the gravid uterus, supplying nutrients and gas exchange to the fetus.</td>
</tr>
<tr>
<td>Placenta (C-section)</td>
<td>Collected from above the vagina as a result of a cesarean section.</td>
</tr>
<tr>
<td>Urethral</td>
<td>From the membranous canal conveying urine from the bladder to the exterior of the body.</td>
</tr>
<tr>
<td>Uterus</td>
<td>Hollow, muscular organ in the female in which the fetus develops.</td>
</tr>
<tr>
<td>Vaginal</td>
<td>From the canal that extends from the vulva to the cervix.</td>
</tr>
<tr>
<td>Vulva</td>
<td>The region of the external female genitalia.</td>
</tr>
</tbody>
</table>

### GENITAL (MALE)

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion</td>
<td>A more or less circumscribed pathologic or traumatic injury to tissue</td>
</tr>
<tr>
<td>Penile exudate</td>
<td>Exudate expressed through the urethra</td>
</tr>
<tr>
<td>Prostate</td>
<td>A gland which, in the male, surrounds the neck of the bladder and the urethra.</td>
</tr>
<tr>
<td>Urethral</td>
<td>See above</td>
</tr>
</tbody>
</table>

### LOWER RESPIRATORY TRACT

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchial</td>
<td>Referring to the large air passages which dichotomously branch within the lungs.</td>
</tr>
<tr>
<td>Bronchial aspirate</td>
<td>Material collected from the bronchi by means of instrumentation</td>
</tr>
</tbody>
</table>
PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiberoptic</td>
<td>Collection of material with an instrument designed for visualization of the lower respiratory area and for specimen collection.</td>
</tr>
<tr>
<td>Sputum</td>
<td>Matter ejected from the lungs, bronchi, and trachea through the mouth.</td>
</tr>
<tr>
<td>Tracheal transtracheal aspirate</td>
<td>The tube from the larynx to the bronchi. Material obtained by surgical passage of a catheter through the tracheal wall and into the lower respiratory area.</td>
</tr>
</tbody>
</table>

UPPER RESPIRATORY TRACT

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear</td>
<td>Unless specified, refers to the external ear</td>
</tr>
<tr>
<td>Mouth and dental</td>
<td>Gums, gingivae, teeth, root canals, tongue etc.</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>That part of the pharynx above the soft palate.</td>
</tr>
<tr>
<td>Nose (nasal/nares)</td>
<td>In Microbiology the term usually refers to culture obtained from about 1-2cm deep within the nostril.</td>
</tr>
<tr>
<td>Sinus</td>
<td>Any body cavity, hollow space, or open channel</td>
</tr>
<tr>
<td>Throat</td>
<td>That area within the deep oral cavity between and including the tonsillar pillars.</td>
</tr>
</tbody>
</table>

STOOL/RECTAL A term referring to the fecal discharge from the bowels

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool</td>
<td>A term referring to the fecal discharge from the bowels</td>
</tr>
<tr>
<td>Rectal/Perianal</td>
<td>Opening to the bowel from the buttock</td>
</tr>
</tbody>
</table>

SURFACE SPECIMENS
## PRINCIPLES OF SPECIMEN COLLECTION

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn</td>
<td>A traumatic lesion caused by contact of tissue with heat.</td>
</tr>
<tr>
<td>Cyst</td>
<td>Any liquid or exudate containing sac.</td>
</tr>
<tr>
<td>Decubitus ulcer</td>
<td>Ulceration due to prolonged pressure of lying down or sitting (bed sores).</td>
</tr>
<tr>
<td>Exudate</td>
<td>Fluid-containing protein, cells or solid material escaped from blood vessels as a reaction to injury or inflammation.</td>
</tr>
<tr>
<td>Laceration</td>
<td>A cut</td>
</tr>
<tr>
<td>Lesion</td>
<td>Any break in the integrity of the skin, mucous membrane or organ surface.</td>
</tr>
<tr>
<td>Paronychia</td>
<td>Inflammation around the folds of skin around the fingernails</td>
</tr>
<tr>
<td>Skin</td>
<td>External body covering</td>
</tr>
<tr>
<td>Stoma</td>
<td>Any small opening or orifice on a free surface (ie. the opening from a colostomy or ileostomy site)</td>
</tr>
<tr>
<td>Suture</td>
<td>A surgical “stitch”</td>
</tr>
<tr>
<td>Ulcer</td>
<td>A loss of integrity of a cutaneous or mucous surface lining resulting from the sudden or gradual sloughing of necrotic tissue.</td>
</tr>
<tr>
<td>Vesicle</td>
<td>A small blister containing a serous fluid.</td>
</tr>
</tbody>
</table>

## SURGICAL SPECIMENS

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess</td>
<td>Localized collection of pus in a cavity formed by disintegration of tissue.</td>
</tr>
<tr>
<td>Aspirate</td>
<td>Removal of fluids from a cavity by suction.</td>
</tr>
<tr>
<td>Biopsy</td>
<td>Surgical removal of small portions of tissue from a living body for the purpose of establishing a precise diagnosis.</td>
</tr>
<tr>
<td>Bone</td>
<td>Mineralized connective tissue that comprises the skeleton of vertebrates.</td>
</tr>
<tr>
<td>Clot</td>
<td>A semisolid mass, usually of blood or lymph.</td>
</tr>
</tbody>
</table>
PRINCIPLES OF SPECIMEN COLLECTION

| Drain | An artificially placed device used to create a channel by which fluid or pus can be exited from a cystic space or body cavity. |
| Exudate | Fluid-containing protein, cells or solid material escaped from blood vessels as a reaction to injury or inflammation. |
| Fistula | An abnormal passage or communication between two organs to the outside. |
| Hematoma | A tumor of effused blood – a bruise |
| IV Catheter | Tubing used to infuse sterile material into the veins |
| Prosthesis | An artificial body part |
| Pus | A liquid inflammatory product of leukocytes and fluid. |
| Stone | A very hard mass or calculus usually composed of mineral salts. |
| Tissue | A surgically removed mass of body cells |
| Wound | A more or less circumscribed pathologic or traumatic injury to tissue. |

URINE

<table>
<thead>
<tr>
<th>Specimen Site</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Catheterized Urine aspirated from a urinary catheter</td>
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<tr>
<td>Midstream/Clean Catch Urine collected in a container after the first few milliliters of urine has been passed.</td>
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</tr>
<tr>
<td>Suprapubic Urine surgically aspirated with syringe and needle by direct puncture into the bladder.</td>
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COLLECTION BY SPECIMEN TYPE

URINE
PRINCIPLES OF SPECIMEN COLLECTION

1. Cleansing Procedures

   a. **Females**: After the patient has thoroughly washed her hands, instruct her to wash her introitus from the front to the back with each of 4 separate 4” x 4” sterile gauze sponges that have been soaked in 10% green soap solution or some appropriate cleansing agent. She is then to spread the labia with the fingers of one hand (using 2 sterile dry gauze pads) and void a midstream portion of urine directly into a sterile container, which is held in the other hand. The lid should be placed on the container and the specimen taken to the laboratory immediately. Make sure the patient is instructed on proper ways of handling the lid and container and that the inside of the lid does not come in contact with any item.

   b. **Males**: After thorough hand washing, the patient should be instructed to retract the foreskin and wash the tip of the glans penis with 4 separate 4” x 4” gauze sponges which have been soaked in 10% green soap or other appropriate cleansing agents. The urethra is then flushed by the first portion of urine and a midstream specimen is collected in a sterile container. The lid should be placed on the container and the specimen transported to the laboratory.

2. Time of Collection

   The best specimen for culturing is the first morning voided urine. If this is not possible, the urine should be allowed to incubate in the bladder a minimum of 2 hours before collection. This is an important point to remember for patients with indwelling catheters.

   Urine makes a nice growth medium for bacteria; therefore, if skin contaminants or feces contaminants happen to get into the specimen (which they usually do in small numbers) and are allowed to grow, they cause erroneous results. The average bacteria double in number every 18 to 20 minutes. It can easily be seen that if a urine sample is allowed to stand in the patient’s room or at the nurse’s station the results are meaningless. It is therefore important that the time of specimen collection is marked on the patient’s requisition sheet.

3. Urine Specimens for Tuberculosis Culture
PRINCIPLES OF SPECIMEN COLLECTION

Collect the first voided morning specimen ONLY. Twenty-four hour specimens will not be processed. At least 3 first morning specimens should be submitted. Time of specimen collection must be marked on the requisition sheet.

SPUTUM

1. Specimens for Tuberculosis, Fungus, and Routine Culturing

Most pathogens causing upper respiratory tract infections are found in the sputum; therefore, carefully instruct the patient of the importance of sputum expectoration from the lungs as opposed to expectoration of saliva and nasopharyngeal secretions. A first morning specimen of sputum is best for culturing. The nurse should look at the specimen and decide if it is of the right quality before sending it to the laboratory. If the patient cannot expectorate sputum, then the doctor should be notified instead of sending saliva to the laboratory.

Make sure the patient has the proper instructions for handling the container aseptically. Anaerobe cultures are never performed on bronchial washings or sputum specimens. Trans-tracheal aspirations are needed for anaerobe studies.

2. Post-bronchial Sputum Specimens

The first three (3) specimens of sputum produced by the patients following bronchoscopy are considered excellent for diagnostic purposes. These are NOT to be collected together but sent separately to the laboratory immediately after collection. Twenty-four hour specimens will not be accepted.

ABSCESSES, FISTULAS, PUS, ULCERS, WOUNDS

1. If at all possible, and aspirate should be obtained from the area with a syringe. If this is impossible, at least two sterile swabs should be used, and the entire area of the wound must be swabbed since microbial flora can vary in different parts of the same wound. The swabs, as moist as possible, must be placed in a sterile test tube and delivered to the laboratory immediately. The swabs must not be allowed to dry out. The time of collection must be on the requisition sheet as well as the nature of the lesion and the diagnosis. Special anaerobic transport vials are available in the Bacteriology Laboratory.
PRINCIPLES OF SPECIMEN COLLECTION

FECES FOR PARASITOLOGY AND DIARRHEAL DISEASE IDENTIFICATION

1. Fecal specimens should be collected in a clean container and delivered to the laboratory immediately. Collection containers for stool are available in 3-tube kits which provide specific preservation conditions required for the test or culture being ordered. Specimens MUST be collected prior to barium enemas. The time of collection and the diagnosis must be on the requisition sheet.

<table>
<thead>
<tr>
<th>CONTAINER TYPE</th>
<th>USES</th>
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</table>
| Clean Vial (white/clear lid) | • *Helicobacter pylori* antigen test  
  • *C. difficile* PCR (standalone test)  
  • Follow up PCR for *Entamoeba* spp.  
  • Viral stool culture |
| Cary Blair (yellow lid)   | • Comprehensive GI Panel by PCR (see HANDLING OF STOOL SPECIMENS for complete list of analytes)  
  • Targeted culturing  
  • Test of Cure |
| Total-Fix™ Tube (black lid) | – Routine O&P  
  • Helminth eggs and larvae  
  • *Entamoeba* spp., *Iodamoeba* spp., *Blastocystis hominis*, *Endolimax*, *Giardia*, *Chilomastix*, *Dientamoeba fragilis*, *Balantidium coli*  
  • *Cystisospora belli* oocysts  
  – Need to order separately:  
    • DFA for *Cryptosporidium* and *Giardia*  
    • Modified Acid fast for *Cryptosporidium*  
    • *Cyclospora* and *Cystisospora* |
PRINCIPLES OF SPECIMEN COLLECTION

FLUIDS, SPINAL

1. All spinal fluids should be collected in sterile tubes and brought to the laboratory IMMEDIATELY and handed to a technologist in the Primary Processing area of the Microbiology laboratory. Time of collection must be marked on the requisition sheet, with a diagnosis.

FLUIDS, OTHER THAN SPINAL

1. Pleural, synovial, pericardial, and peritoneal fluids should be collected in sterile tubes and brought to the laboratory before clotting. Time of collection as well as diagnosis MUST be on the requisition sheet.

NASOPHARYNGEAL

1. A swab on the end of a 28 gauge nichrome wire is preferred for the collection of this kind of specimen. Many fastidious microorganisms can be found in the nasopharynx and are usually not overgrown by normal flora when plated from the original swab. A separate swab should be used for each nostril and placed in the sterile test tube after collection of the specimen. The swab must be delivered to the laboratory while still moist. The diagnosis and time of specimen collection must be on the sheet.

2. If the specimen is for a viral or PCR assay for respiratory pathogens it must be placed into Universal Viral Transport Media.

URETHRAL AND VAGINAL OR CERVICAL DISCHARGES

1. It is important that the specimen be collected when the patient is actually showing a discharge. Urethral and vaginal discharges should be collected only from a patient who has not voided for a minimum of two hours. If at all possible, more than one swab should be used and transgrow bottles inoculated immediately. Inoculate specimens on the surface of transgrow medium as follows.

2. Remove cap of bottle only when ready to inoculate medium.
3. Soak up any excess moisture in the bottle with specimen swab and then roll swab from side to side across medium, starting at the bottom of the bottle.
4. Tighten the cap immediately to prevent loss of CO₂.

CAUTION: Keep neck of the bottle in an upright position to prevent CO₂ loss. Desiccation or a change in temperature can easily kill the fastidious
PRINCIPLES OF SPECIMEN COLLECTION

Gonococcus. The time of specimen collection must be on the requisition sheet.

THROAT CULTURES

1. The purpose of collecting a throat specimen is usually to recover Beta-hemolytic Streptococcus and *Arcanobacterium haemolyticum*.

MATERIALS

ESwab, Sterile tube or Amies Transport Medium, Tongue Depressor, bright light

PROCEDURE

Ask the patient to open the mouth widely and phonate an “ah”. This will lift the uvula and help to reduce the gag reflex. Gently depress the tongue with a tongue blade, and taking care not to touch the lateral walls of the buccal cavity, extent the swab between the tonsillar pillars and behind the uvula. Sweep the swab back and forth across the mucosa to the posterior pharynx to obtain an adequate sample.

For a Strep-screen:
- Place Eswab into Amies medium & break off swab. Seal tube tightly and label.
- This Strep screen will look for Group A Strep, Group C strep, Group G strep and *A. haemolyticum*.

For evaluation of Gonococcus:
- Place Eswab into Amies medium as above.
- Order culture for *Neisseria gonorrhoeae* screen.

Place the swab immediately posterior pharynx to obtain an adequate sample. Place the swab immediately into a sterile tube or transport tube and deliver to the laboratory.

Special media and techniques are required if the physician suspects *Fusobacterium necrophorum*, Yeast, and Methicillin-resistant *Staphylococcus aureus*

*Bordetella pertussis* and *Corynbacterium diphtheria* are send-out tests and not performed by culture in the Microbiology laboratory.

INTERPRETATION
PRINCIPLES OF SPECIMEN COLLECTION

The following microbial flora may occur in the throat: *Neisseria* species, Alpha hemolytic *Streptococci*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium* species, *Haemophilus* species, non-hemolytic *Streptococci*, *Streptococcus pneumoniae* and many specimens of anaerobic bacteria.

SKIN OR NAIL SCRAPINGS

1. Clean the area with 70% alcohol to remove external contaminants and body oils. With a sterile knife blade, obtain only diseased portions of the nail and only the periphery of the skin lesion since fungi grow out from the center of the lesion. Viabile fungi are usually not found in the center. Send the specimen to the laboratory in a sterile tube or sterile petri dish. Include time of collection as well as diagnosis on the requisition sheet. **DO NOT PLACE IN SALINE.**

IV CATHETER TIPS

1. Clean the exposed external surface around the catheter with sterile gauze sponges soaked in 4% green soap or other appropriate cleansing agents. Remove the catheter with sterile gloves. With sterile scissors cut the end of the tube to be cultured and collected in a sterile test tube. The specimen must be delivered to the laboratory immediately, and the diagnosis and time of specimen collection must be on the requisition sheet. Foley catheter tips are unacceptable for culture.

BLOOD SPECIMENS FOR CULTURE

1. Administration of antibiotics should be withheld until the blood has been collected, if possible. The blood sample should be collected immediately after the chill and before or during the subsequent rise in temperature.
PRINCIPLES OF SPECIMEN COLLECTION

Refer to the Blood Culture Collection portion of this procedure for more information.

SUPRAPUBIC BLADDER PUNCTURE

PURPOSE

The purpose of a suprapubic bladder puncture is to obtain a valid urine specimen for culture. This is particularly useful in young children.

MATERIALS

Sterile Tray including antiseptics, local anesthetic, syringe, and sterile container.

PROCEDURE

Place the patient on his back with knees elevated. The superapubic skin over the bladder area is cleaned with antiseptics as in preparation for surgery and an anesthetic is injected at the site of the needle puncture. Direct the needle into the urinary bladder just the symphysis pubis. Aspirate the urine with a syringe, transfer to an appropriate transport container, and deliver to the laboratory immediately.

The urine is then placed on a Blood Agar plate using a calibrated platinum loop (0.01m) for a colony count. A Blood agar (BAP) Plate and McConkey Agar plates are streaked for isolation.

INTERPRETATION

Any pure culture growth is considered significant and is identified and antibiotic susceptibility testing. Mixed cultures will be worked up per lab policy.

is performed.
SECTION V

VIRAL SPECIMEN COLLECTION

PRINCIPLE

Proper collection of specimens is highly important to the success of any subsequent laboratory examination for viruses. The type of specimen collected depends on the nature of the illness, and because of the wide range of agents responsible for similar syndromes, more than one specimen is often required. Infectivity is usually the first property of viruses to be lost in the face of adverse environmental conditions. For this reason it is especially important that specimens for virus isolation contain virus in highest concentration (the acute phase) or as soon as possible after the onset of symptoms.

PATIENT INFORMATION REQUESTED

Minimum data to be supplied with a specimen:

1. Name of patient, age and sex
2. Viral disease suspected
3. Specimen type, date and time collected
4. Antibiotic treatment and chemotherapy
5. Relevant Immunization
6. Brief Clinical History
7. Identifying Patient Number (i.e., Hospital number, Social Security or Birth date)

COLLECTION OF SPECIMENS/BASIC INFORMATION
PRINCIPLES OF SPECIMEN COLLECTION

1. Collect specimens promptly, preferably within 3 days and generally as soon as possible, after the onset of symptoms.

2. Collect postmortem specimens aseptically, as soon as possible after death.

3. Immediately transport all specimens on wet ice to the laboratory.

4. With a few exceptions, specimens to be tested within 24 hours may be held at 4°C or on wet ice; for longer intervals, freeze specimens at –70°C. (Avoid freezing specimens at –20°C as many viruses are labile at this temperature.)

5. Do not use any preservatives or fixatives. For certain types of specimens, the laboratory will supply viral transport media.

6. Label each specimen with the patient’s name, type of specimen, and date of collection.

TYPES OF SPECIMENS AND METHODS OF COLLECTION FOR ALL VIRUS ISOLATION EXCEPT CHLAMYDIA

1. Nasal Excretions
   a. Collect with a sterile cotton swab and place in Virocult Transport Tube supplied by the laboratory.
   b. Nasal washings can be obtained by instilling 4-5 ml of sterile saline into each nostril with the head tilted back slightly; the head is then brought forward and the saline is allowed to flow into a small container held beneath the nose.
   c. Transport to the laboratory immediately on wet ice.

2. Throat Washings
   a. The patient should gargle with approximately 10 ml of saline solution and expectorate into a sterile container.
   b. Transport to the laboratory immediately on wet ice.

3. Throat Swabs
   a. Rub a sterile, flocked swab that comes with the viral transport medium on the tonsils and the back of the pharynx. Insert into the Universal Viral
PRINCIPLES OF SPECIMEN COLLECTION

Transport Media supplied by materials management. **DO NOT USE CALGISWABS. DO NOT USE WOODEN SHAFT SWABS.**

b. Transport to the laboratory immediately.

4. **Vesicular Fluids or Skin Scrapings**
   a. Vesicular fluids and cellular material from the base of lesions should be collected for viral isolation attempts during the first 3 days of the eruption; fluids collected later rarely yield virus.
   b. Vesicles are first washed gently with ordinary soap and water and rinsed. The cleansed vesicles may be opened and the exudate absorbed onto a dry, sterile flocked swab that comes with the viral transport medium. Rub the swab on the cultures surface to collect cellular material. Break off swab into the viral transport medium tube.
   c. Transport to the laboratory immediately.

5. **Spinal Fluids**
   a. Collect 3-5 ml in a sterile screw-capped viral or tube and transport at room temperature to the laboratory immediately.
   b. It is important that spinal fluid either be inoculated soon after collection or else be frozen and maintained at –70°C until tested, as many viruses are very labile in spinal fluid.

6. **Body Fluids**
   a. “Sterile” fluids: pleural, peritoneal, pericardial, joint, collect as above for spinal fluids.
   b. Transport to the laboratory immediately.

CHLAMYDIA ISOLATION

1. Specimen Collection and Transport
   a. A good specimen should ideally contain host cell scrapings. Therefore the infected area should be vigorously swabbed. Use a flocked swab in viral transport medium and **NOT** calgiswabs or cotton swabs for optional isolation of *Chlamydia*.

2. Genital Specimens are best when taken from the transitional zone of the cervix or endourethra (4-6 cm from meatus). Discharges and urine specimens are usually inadequate.
PRINCIPLES OF SPECIMEN COLLECTION

3. Sputums or throat washings are suitable for Isolations.

4. Eye cultures should be swabs of the conjunctival surface NOT the purulent discharge.

5. Specimens must be promptly transported on ice in special transport media (available from the lab) to the laboratory. They can be held up to 24 hours in the refrigerator but for prolonged storage freezing in transport media at –70°C is recommended.

If you have questions regarding specimen handling, call 323-5411 for a supervisor.

SPECIMEN HANDLING FOR VIROLOGY

1. GENERAL COMMENTS

   a. All paperwork must match specimen label, and complete information must be given

   b. ONLY Universal Viral Transport Media should be used for specimen submitted via swab.

   c. Other specimen types should be submitted in sterile container.

   d. When multiple requests are on the same specimen, a minimum of 0.5 cc per request is needed, except stools for CDPCR – need 1 cc minimally. Otherwise, allocate as much as you can.

   e. Tissue can be homogenized and split for Virology, if multiple requests are ordered. If only for Virology, leave intact.

   f. Only FRHD and CHLM are frozen. Serum submitted for FRHD must be separated from red cells and put into a labeled tube before freezing.

2. SPECIMEN TO BE FROZEN AT –70°C: CHLM, FRHD

   Chlamydia Culture
PRINCIPLES OF SPECIMEN COLLECTION

a. Place computer label on tube, after matching paperwork.

b. Any specimen for Chlamydia culture must be received and held in the Chlamydia transport medium. Exceptions are bronchial washings or similar specimens obtained by an invasive procedure. These can be placed in transport after receipt in lab if not in transport.

c. No other test request can be performed from a Chlamydia specimen.

d. There is generally a 48-hour turnaround time once the culture is set up.

Freeze and Hold Specimen (FRHD)

a. Red-stoppered tubes – after clotting, centrifuge, remove serum and place into a correctly labeled plastic tube.

b. Other body fluids may be frozen in the tube received or transferred to a sterile plastic tube.

3. OTHER VIRUSES (TO BE REFRIGERATED)

Cytomegalovirus

a. Any specimen type may be submitted for CMV culture. Whole blood must be in a heparinized tube (green top). Refrigerate specimen.

Adenovirus, Enterovirus, Herpes Simplex Virus

a. Various specimen types may be submitted for culture. Refrigerate specimen.

Influenza

a. Specimen type should be NP swabs in VTM. Notify the Virology laboratory during working hours when received. Refrigerate specimen.

Unknown Viral Requests

a. Any specimen type except whole blood may be submitted for culture. Refrigerate specimen.
PRINCIPLES OF SPECIMEN COLLECTION

Respiratory Battery

a. NP swab or aspirate in viral transport media

CLOSTRIDIUM DIFFICILE BY PCR

a. Test is performed on liquid stools only. Formed or semi-formed stools will be rejected.

b. Minimum of 1 cc/1 gram of stool is needed.

c. The test is set up daily.

d. Only one sample is to be tested per week. No test of cure is to be performed.

e. Patients with previously positive stools in whom diarrhea has recurred, DNA may persist for up to 1 month following the return of symptoms. For this reason, repeat testing is not performed within 3 weeks to 1 month of a prior positive PCR test result.
# PRINCIPLES OF SPECIMEN COLLECTION

Replaces: MIC.SCP202.03  
Changes: Hyperlink glossary added

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<th>Sue Overman</th>
<th>Date: 1993</th>
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<td>Jennifer Simpson</td>
<td>Date: 2/2/2017</td>
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<td>Approved by: Julie A. Ribes</td>
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<td>Chief Technologist</td>
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