PRINCIPLES OF SPECIMEN COLLECTION

The Specimen Collection and Handling Procedure Manual (online) has been reviewed by the following individuals and found to be complete and representative of procedures performed in the Microbiology section of the Clinical Laboratory of the University Hospital, Albert B. Chandler Medical Center, and University of Kentucky. The following is a printout of the online manual.

3/18/08 Date
Julie A Ribes, M.D., Ph.D.
Director, Clinical Microbiology

3/18/08 Date
Sue B. Overman, SM (AAM)
Chief Technologist

Specimen Collection and Handling in Clinical Microbiology
Table of Contents

Principles of Specimen Collection.................................................................i

Section I
Specimen Selection and Collection..........................................................2

General Considerations.............................................................................3

Specimen and Potential Pathogens Associated with Disease.....................9

Anaerobe Cultures, Suitability of Various Clinical Material.......................12
Blood Culture Collection.................................................................13

Section II
Specimen Transport.............................................................................14

Rationale for Specimen Transport........................................................15

Transport Media..................................................................................16

Section III
Specimen Processing............................................................................21

Criteria for Rejection of Bacteriological Specimens.............................28

Action on Rejected Specimens.............................................................28

Direct Examination by Gram Stain.......................................................29

Fecal Leucocytes in Stool Specimens from patients with Diarrheal Disease.....30

Criteria for Genital Specimens.............................................................31

Alternative Avenues to Consider in Lab Diagnosis.................................32

Collection of various Specimens..........................................................38
Urine, Abscesses, Fistulas, Pus, Wounds, Stool, Fluids, Nasopharyngeal,
Urethral and Vaginal, Throat, Skin, IV Catheter Tips, Blood specimens

Collection of Urine from Suprapubic Bladder Puncture..........................43

Collection of Throat Specimens............................................................44
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**

Specimens must always be submitted moist to the laboratory. Most bacteria cannot survive in a dry environment, especially the pathogenic ones. Dry swabs are of no value, so when a specimen is taken the nurse must be sure the swab is moist and then delivered to the laboratory immediately before it dries out.

**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. All containers must be labeled clearly with the patient’s name, hospital number, room number, and the source of specimen. All specimens are to be sent in ziplock 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 Pharmacy 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 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 immunocompromised 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.
A. **Requisition**

The Laboratory Requisition (#J352) is to include the following information:

1. Patient name
2. Patient age and sex
3. Patient room number or location
4. Physician’s name (and address if outside the Hospital)
5. Specific anatomic culture site
6. Date and hour of specimen collect.
7. Clinical diagnosis, special culture request, relevant patient history where necessary
8. Special procedures used in obtaining specimen if appropriate.
9. Name of individual collecting specimens.
10. Antimicrobials, if any, patient is receiving.
11. Matching Tag Identification number.

A. **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 label firmly attached to the specimen container bearing the following information:

PATIENT NAME: ____________________________________________
B. **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 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 20 ml of blood divided between two 25 ml bottles, one incubated aerobically and one anaerobically. For children, .5-3 ml of blood per bottle is acceptable. Smaller volume bottles (Teddy Bear bottles) are available on the Pediatric floors. A 1:10 dilution is recommended.
C. **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.

   a. MIC and MBC’s
   b. Schlichter
   c. Darkfield examinations for spirochetes or other bacteria
   d. Special blood cultures for recovery of fungi of cell-wall deficient “L” forms
   e. Recovery of *Corynebacterium diphtheriae*, Vibrio, Rickettsia, Leptospira, or other unusual organisms.
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. Needles may be inserted in a sterile stopper and submitted for 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 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.

D. 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.

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

DISEASE: Bacterial Meningitis

SPECIMEN COLLECTION:

1. Spinal fluid
2. Blood
3. Wounds
4. Subdurals (infant)
5. Respiratory Tract

POTENTIAL PATHOGENS

1. Adults
   a. Neisseria meningitidis
   b. Streptococcus pneumoniae
   c. Haemophilus influenzae
   d. Streptococcus & others

2. Infants, neuosurgical patients
   a. Enterobacteriaceae
   b. Staphylococcus aureus
   c. Streptococcus-Group B Beta
   d. Haemophilus influenzae

DISEASE: Bacterial Eye Infections

SPECIMEN COLLECTION:

1. Purulent discharge
2. Lower cul-de-sac OR
3. Inner canthus

POTENTIAL PATHOGENS

1. *Haemophilus influenzae*, biotype III. (*H. aegyptius*)
2. *Staphylococcus aureus*
3. *Moraxella* sp.
4. *Streptococcus pneumoniae*
5. *Streptococcus* sp.
6. *Neisseria gonorrhoeae*
7. *Pseudomonas aeruginosa*  
   (Reported STAT)
8. Others

**SPECIMEN COLLECTION**

1. Acute
   a. Nasopharynx
   b. Tympanic membrane aspirate
2. Chronic
   a. Drainage

**POTENTIAL PATHOGENS**

1. Acute
   a. *Streptococcus pneumoniae*
   b. *Haemophilus influenzae*
   c. *Streptococcus pyogenes*
   d. *Neisseria lactamica*
   e. Others
2. Chronic
   a. *Pseudomonas aeruginosa*
   b. *Proteus* sp.

**DISEASE:** Bacterial Sinusitis

**SPECIMEN COLLECTION**

1. Acute
   a. Nasoharynx
2. Chronic
   a. Nasopharynx
b. Surgical aspirate

**POTENTIAL PATHOGENS**

1. Acute
   a. *Streptococcus pneumoniae*
   b. *Streptococcus* sp.
   c. *Staphylococcus* sp.
   d. *Haemophilus*
   f. *Klebsiella* sp. and other
      Enterobacteriaceae

2. Chronic
   a. As above
   b. Anaerobes
DISEASE: Wounds, Abscesses

SPECIMEN COLLECTION

1. Purulent drainage
2. Tissue affected
3. Body fluids
4. Ulcers
5. Wound margins

POTENTIAL PATHOGENS

1. Staphylococcus aureus
2. Anaerobes (deep wounds, aspirates
3. Enterobacteriaceae
4. Streptococcus sp.
5. Clostridium sp.
6. Enterococcus
7. Pseudomonas aeruginosa

DISEASE: Bacterial Throat-Pharynx Infections

SPECIMEN COLLECTION

1. Pharynx & both fauces
2. Nasopharyngeal swab recommended for possible pertussis instead of “cough plate”. Must use Bordet-Gengou plates and streak immediately.

POTENTIAL PATHOGENS

1. *Streptococcus pyogenes*
2. *Haemophilus influenzae*
3. *Corynebacterium diphtheriae*
4. *Neisseria meningitidis*
5. *Neisseria gonorrhoeae*
6. *Bordetella pertussis*
**DISEASE:** Bacterial Pulmonary Infections

**SPECIMEN COLLECTION**

1. Transtracheal aspirate
2. Lung aspirate/biopsy
3. Bronchoscopy (?)
4. Sputum (?)
5. Blood

**POTENTIAL PATHOGENS**

1. *Streptococcus pneumoniae*
2. *Haemophilus* sp.
3. *Staphylococcus* sp.
4. *Klebsiella* sp.
5. Other enterobacteriaceae
6. Mycobacterium sp.
7. Legionella sp.
8. *Mycoplasma pneumoniae*
9. Pneumocystis carinii
10. Almost any organism in pure culture

**DISEASE:** Possible Septicemia

**SPECIMEN COLLECTION**

1. Blood

**POTENTIAL PATHOGENS**

1. *Staphylococcus* sp.
2. *Escherichia coli*
3. *Klebsiella* sp.  
4. *Pseudomonas* sp.  
5. *Bacteroides* sp.  
6. *Enterococcus*  
7. *Streptococcus pneumoniae*  

**DISEASE:** Bacterial Endocarditis  

**SPECIMEN COLLECTION**  

Cultures  

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

**POTENTIAL PATHOGENS**  

1. Viridans group *Streptococcus*  
2. *Streptococcus-Enterococcus*  
3. *Staphylococcus* sp.  
4. Enterobacteriaceae  
5. Candida/fungi  
6. Anaerobes  

**DISEASE:** Bacterial Diarrhea
SPECIMEN COLLECTION

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

POTENTIAL PATHOGENS

*1. Salmonella sp.
*2. Shigella sp.
*3. Escherichia coli
*4. Vibrio sp.
5. Staphylococcus aureus
6. Yersinia sp.
*7. Campylobacter fetus ssp Jejuni (special media required)
8. Clostridium difficile

*Minimum for routine culture

DISEASE: Genital Tract

SPECIMEN COLLECTION

1. Cervix
2. Urethral discharge
3. Rectum
4. Lesions
5. (Darkfield)

POTENTIAL PATHOGENS

1. *Neisseria gonorrhoeae*
2. *Treponema pallidum*
3. *Haemophilus ducreyi*
4. *Trichomonas vaginalis*
5. Candida sp.
6. T-Mycoplasma
7. Chlamydia trachomatis
8. Gardnerella vaginalis
9. Group B Streptococcus – ob/gyn
10. Listeria monocytogenes – ob/gyn
11. Herpes simplex

DISEASE: Urinary Tract Infections

SPECIMEN COLLECTION

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

POTENTIAL PATHOGENS

1. *Escherichia coli*
2. *Klebsiella sp.*
3. *Proteus mirabilis*
4. *Pseudomonas sp.*
5. *Enterococcus*
6. Others
DISEASE: Bacterial Bone and Joint Infections

SPECIMEN COLLECTION

1. Bone
2. Joint aspirate
3. Overriding skin lesions

POTENTIAL PATHOGENS

1. Staphylococcus sp.
2. Haemophilus influenzae
3. Streptococcus sp.
4. Salmonella sp.
5. Neisseria gonorrhoeae
6. Enterobacteriaceae
7. Streptococcus pneumoniae
8. Pseudomonas sp.

DISEASE: Skin Infections

SPECIMEN COLLECTION

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

POTENTIAL PATHOGENS

1. Staphylococcus aureus
2. *Streptococcus* sp.
3. *Neisseria meningitidis*
4. Enterobacteriaceae
5. Dematophytes and other mycotic agents
6. Treponema pallidum
7. Mycobacterium marinum
8. Varicella zoster
9. Herpes simplex I and II

**DISEASE:** Bacterial Burn Infections

**SPECIMEN COLLECTION**

1. Tissue
2. Beneath eschar
3. Blood

**POTENTIAL PATHOGENS**

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

**DISEASE:** Newborn systemic Infection
SPECIMEN COLLECTION

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

POTENTIAL PATHOGENS

1. Enterobacteriaceae – Escherichia coli, Klebsiella sp.
2. Staphylococcus aureus
3. Streptococcus – Groups A, B, D
4. Haemophilus sp.
5. Listeria monocytogenes
6. Streptococcus pneumoniae
7. Others

SUITABILITY OF VARIOUS CLINICAL MATERIALS FOR ANAEROBIC CULTURE STUDIES

SUITSABLE

1. Properly collected abscess material.

UNSUITABLE

1. Throat or nasopharyngeal swab samples.
2. Blood
3. Bone marrow
4. Lung aspirate and transtracheal aspirate.
5. Suprapubic urine aspirate
6. Endometrial or endocervical material collected by direct visualization through a speculum
7. Aseptically collected tissue
8. “Sulfur granules” from sputum or other materials
10. Bile

2. Sputum, tracheotomy aspirate, bronchoscopic washings.
3. Voided or bladder catheterization urine samples
4. Vaginal or cervical swabs
5. Material from superficial abscesses or lesions improperly collected
7. Feces* or rectal swab samples

* There are a few exceptions: example when botulism (especially infant botulism), C. perfringens foodborne disease, or antibiotic associated pseudomembranous colitis are suspected, it is appropriate to test stool specimens.
## BLOOD CULTURE COLLECTION

<table>
<thead>
<tr>
<th>CLINICAL CONDITION</th>
<th>COLLECTION PROTOCOL</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults and adolescents:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe septicemia</td>
<td>Two cultures* prior to therapy</td>
<td>Two 20 ml samples from each arm</td>
</tr>
<tr>
<td>Subacute bacterial endocarditis</td>
<td>Three cultures within 24 hours</td>
<td>Space each collection at least 1 hour apart. Two should be collected at the beginning of fever spikes.</td>
</tr>
<tr>
<td>Low-grade intravascular infection</td>
<td>Three cultures within 24 hours</td>
<td>Specimens collected at least 1 hour apart. Two should be collected at first sign of febrile episodes.</td>
</tr>
<tr>
<td>Bacteremia of unknown origin</td>
<td>Four to six cultures within 48 hours</td>
<td>Take specimen just before next dose of antimicrobial Agent</td>
</tr>
<tr>
<td>Patient on therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Febrile episodes</td>
<td>No more than three total cultures</td>
<td>Bacteremia may precede episodes of fever and chills by about 1 hour.</td>
</tr>
<tr>
<td>Young children:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 to 3 ml</td>
<td>Two cultures usually suffice for diagnosing bacteremia in the newborn</td>
</tr>
</tbody>
</table>

*A “blood culture” is defined as a draw of at least two 10 ml samples of blood (except in small children) divided between an aerobic and/or anaerobic bottle.

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.
## ENVIRONMENTAL SAMPLING

<table>
<thead>
<tr>
<th>RECOMMENDED FOR ROUTINE SAMPLING</th>
<th>FREQUENCY OF SAMPLING ACCEPTABLE</th>
<th>ACCEPTABLE RESULTS</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilizers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steam</td>
<td>Weekly (or monthly)*</td>
<td>No growth</td>
<td>Spore test every load if it contains implantable objects <em>Bacillus stearothermophilus</em> spores.</td>
</tr>
<tr>
<td>Hot Air</td>
<td>Monthly (or weekly)</td>
<td>No growth</td>
<td><em>Bacillus subtilis</em> spores</td>
</tr>
<tr>
<td>Gas</td>
<td>Weekly (or monthly)</td>
<td>No growth</td>
<td><em>Bacillus subtilis</em> spores - test every load if implantable object included</td>
</tr>
<tr>
<td>Infant formulas manufactured in the hospital</td>
<td>Weekly</td>
<td>Less than 25 Organisms/ml</td>
<td>Commercially prepared formulas need not receive an in-house check unless sterile bulk formula is transferred to individual bottles</td>
</tr>
<tr>
<td>High-level disinfected Items (not sterilized)</td>
<td>At least quarterly more often if previous results dictate</td>
<td>No organisms grown, No viable vegetative pathogens</td>
<td></td>
</tr>
<tr>
<td>Anesthesia equipment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory therapy Apparatus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoscopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water used to prepare Dialysate fluid</td>
<td>At least monthly</td>
<td>Less than 200 organisms/ml</td>
<td></td>
</tr>
<tr>
<td>Dialysate during use</td>
<td>At least monthly</td>
<td>Less than 2000 organisms/ml</td>
<td></td>
</tr>
<tr>
<td><strong>Laminar flow hoods pharmacy</strong></td>
<td>Optional (perhaps)</td>
<td>No growth or rarely growth on open</td>
<td>Compounded materials should not be tested</td>
</tr>
</tbody>
</table>

*Note: The table above outlines the recommended sampling frequencies and acceptable results for various environmental sampling scenarios. The comments section provides additional details and considerations for each category.*
<table>
<thead>
<tr>
<th>Laminar flow hoods</th>
<th>Open blood agar plates left at back of hood for 1 hour during operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(routine checks with “DOP smoke”) Semiannually OR when hood is moved OR obvious accident</td>
<td>As per manufacturers specifications Filters, air flow, etc should be on regularly scheduled preventive maintenance program.</td>
</tr>
</tbody>
</table>

*Federal laboratory standards require at least monthly testing with spore strips.

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 cultured 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 either be refrigerated, inoculated to primary isolation medium before transport, or transported in preservative solution. Agar paddle devices may be used from distant laboratories.

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
2. If a delay in transport is anticipated, or if cultures are sent to a reference laboratory, Stuart’s Amies or Carey Blair transport medium should be used.

Rationale
2. Transport medium is formulated to maintain the viability of bacteria but allow 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, but living, replicating organisms 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.
2. Replaced glycerophosphate with a balanced salt solution.
3. Retained charcoal but incorporated it into the medium rather than the swab.
4. Better transport system for most specimens.

Carey Blair (1964)

1. Similar to Stuart’s but modified for fecal specimens.
2. pH increased from 7.4 to 8.4.
3. Removed charcoal from formula.
4. Good for stool specimens.
5. Recommended for fecal specimens suspicious for *Campylobacter* sp.

Buffered glycerol saline
1. Designed for stool specimens only.
2. Good for mailing fecal specimens.
3. High pH to favor fecal pathogens.
4. Not for transport of fecal specimens in which *Campylobacter* sp. is suspected.
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
- Genital/cervical for gonococcus isolation
- Blood
- Stool/Feces
- Wounds
- Respiratory
- Exudates

If processing is delayed, spinal fluids should be held at 35°C.

**ENVIRONMENTALLY FRAGILE ORGANISMS**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Most Likely Specimen</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella</em> sp.</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, throat</td>
<td>Sensitive to cold</td>
</tr>
<tr>
<td>SPECIMEN</td>
<td>COLLECTION EQUIPMENT</td>
<td>TRANSPORT</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Anaerobe special request</td>
<td>Port-A-Cul vials</td>
<td>No refrigeration</td>
</tr>
<tr>
<td></td>
<td>Needle and syringe</td>
<td>Use anaerobic</td>
</tr>
<tr>
<td></td>
<td>Vacutainer Brand Anaerobic Specimen collector</td>
<td>transport method</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>Commercial kit Needle and syringe</td>
<td>Culture broth in bottles 25 ml/bot, 10 ml/blood Bottles; 2 bottles – one Anaerobic DO NOT REFRIGERATE. Ped-</td>
</tr>
<tr>
<td></td>
<td>available on floor iodine</td>
<td>(0.5 to 3 ml – Teddy Bear)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></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 not refrigerate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAR</td>
<td>Calgi Swab</td>
<td>Transport medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EYE</td>
<td>Calgi Swab Corneal scraping (by physician)</td>
<td>Transport medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FECES
Clean or sterile
Collection cup
Swab (only if necessary)
Transport within 1 hour to lab
1. Best specimen is diarrheal stool
2. Swab is satisfactory in acute cases but not for routine specimens or surveys
3. Insert swab beyond anal sphincter
   Swab must show feces

Carey-Blair acceptable for *Campylobacter* sp.

---

**Collection and transport of clinical specimens for bacteriology examination continued:**

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>COLLECTION EQUIPMENT</th>
<th>TRANSPORT</th>
<th>INSTRUCTION (COMMENTS)</th>
</tr>
</thead>
</table>
| Genital  | Swab                  | Do not refrigerate immediate CO₂ for GC | 1. Collect cervicals with a swab inserted through a speculum  
2. Avoid touching swab to uninfected mucosal surfaces.  
3. Clean external urethra before taking urethral specimen  
4. For GC, inoculate a modified Thayer-Martin plate, at bedside if possible  
5. Prepare slide for staining using a second swab.  
6. Label properly |
|          | Transport media       |           |                        |
| Nasopharynx | Cotton-tipped nichrome or Stainless wire/28 ga. (Calgi Swab) | Do not refrigerate Transport medium | 1. Unwrap nasopharyngeal  
2. Pass through nose into Nasopharynx  
3. Allow to remain for a few seconds  
4. Carefully withdraw  
5. Label properly |
| Nose     | Swab                  | Transport Medium | 1. Swab anterior nares only  
2. Culture quickly |
| Sinus    | Calgi Swab            | Transport Medium | 1. Insert and remove carefully  
2. Prepare slide for stain using second swab or after inoculate- |
## Sputum

**Specimen**: Sterile cup  
**Collection Equipment**: Transport in collection  
**Transport**: Container within 1 hour

1. Carefully instruct patient to cough deeply (not to spit)  
2. First morning specimen is best (no 2 hour collection)  
3. Transport immediately: seal container securely  
4. Consider sputum potentially contaminated with M. tuberculosis

## Throat

**Specimen**: Swab (tongue blade)  
**Collection Equipment**: Transport medium if more than 2 hour delay to lab

1. Use Tongue Blade  
2. Sample ONLY back of throat between and around the tonsillar are thoroughly  
3. Avoid cheeks, teeth, etc.

---

### Collection and transport of clinical specimens for bacteriologic examination continued:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Collection Equipment</th>
<th>Transport</th>
<th>Instructions (comments)</th>
</tr>
</thead>
</table>
| Urine (midstream) | Sterile screw-cap cup | Transport in collection container  
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 |
<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Collection Method</th>
<th>Container</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine (catheter)</td>
<td>Collect from catheter</td>
<td>Sterile tube</td>
<td>1. Collect from catheter</td>
</tr>
<tr>
<td></td>
<td>Do not culture Foley tip</td>
<td></td>
<td>2. Do not culture Foley tip</td>
</tr>
<tr>
<td></td>
<td>Decontaminate line as venipuncture</td>
<td></td>
<td>3. Decontaminate line as venipuncture</td>
</tr>
<tr>
<td>Wound (surface)</td>
<td>Decontaminate surrounding skin</td>
<td>Swab</td>
<td>1. Decontaminate surrounding skin</td>
</tr>
<tr>
<td></td>
<td>Open lesion and express pus into swab; sample</td>
<td>Transport medium</td>
<td>2. Open lesion and express pus into swab; sample</td>
</tr>
<tr>
<td></td>
<td>advancing margin of lesion</td>
<td></td>
<td>3. Label properly</td>
</tr>
<tr>
<td>Wound (deep)</td>
<td>Decontaminate</td>
<td>Syringe anaerobic</td>
<td>1. Maintain anaerobic conditions</td>
</tr>
<tr>
<td></td>
<td>swab kit</td>
<td>Anaerobic transport</td>
<td>Transport aspirate in the collecting syringe OR place</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aspirate into anaerobic broth OR collect pus onto swab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and place directly into anaerobic transport (not</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>recommended)</td>
</tr>
</tbody>
</table>

- Refrigerate no longer than 6 hours prior to culture.
- Seal container securely.
SECTION III

SPECIMEN PROCESSING

A properly processed specimen provides only a certain amount of information. Interpreted properly, the generated data becomes useful.

A physician’s initial diagnosis is based upon observation of,
and symptoms from, his patient.

A microbiologist’s interpretation of that patient’s specimen results requires no less pertinent information.
SPECIMEN PROCESSING

A. General

1. Specimens should not be processed until the requisition slip and the label are correctly prepared.

Rationale

1. The person transcribing the orders of the doctor should be called by the laboratory to complete the requisition or verify questionable responses.

General

2. Specimens should not be processed if received in inappropriate containers or improper transport medium, or if received after a prolonged delay. Call physician in charge of patient to see if a second specimen can be conveniently obtained. (A nurse may be contacted for specimens noninvasively obtained such as urines or stools).

Rationale

2. Insignificant information may mislead the attending physician. Outpatients or difficult collection procedures must be considered, then appropriate decision made based on a specific case. Report should clearly indicate specimen inadequacy and the results might not be valid or complete.

General

3. The second specimen obtained from the same site within 24 hours should not be processed unless there are specific orders by the physician.

Rationale

3. Duplicate orders most commonly represent clerical errors which are costly to the patient and take up unnecessary laboratory time. There are few instances when two cultures within 24 hours are clinically indicated.
B. SPUTUM SPECIMENS

General

1. Sputum samples of less than 2 cc in volume should not be processed unless the material is obviously purulent.

Rationale

1. With the exception of Legionellosis, most respiratory bacterial infections cause copious amounts of sputum to be expectorated. Small quantities of a clear, thin material usually represent saliva.

General

2. Only one sputum sample per 24 hours should be submitted, except for post-bronchoscopy specimens. If more than one specimen is received in series, the samples can be combined and treated as one.

Rationale

2. One sputum sample per 24 hours is usually adequate to reflect the respiratory secretion pool. Post-bronchoscopy specimens usually represent the most ideal, deep cough specimens that can be obtained.

General
3. A direct Gram stain should be performed on all routine sputum specimens to assess their quality as representative of lower respiratory secretory secretions. A scoring system must be used (see page 31).

**Rationale**

3. Samples which are representative of “spit” rather than true lower respiratory secretions produce insignificant results. The reporting of a potentially pathogenic organism in a nonrepresentative sputum sample could be misleading, particularly in cases of clinical pneumonitis.

**General**

4. Each smear is Gram stained and at least 10 fields of view are examined and evaluated.

**Rationale**

4. Squamous epithelial cells are derived from the oral mucosa and their presence in sputum samples represents contamination with “spit”. If a contaminating organism from the oropharynx is incorrectly considered the pathogen causing the pneumonitis, therapy may be misdirected. For this reason, it is recommended that the Gram stain results be reported along with the culture results. Grading criteria are not applicable to sputum specimens submitted for isolation of Mycobacterium tuberculosis or mycotic agents.

**C. Urine Specimens**

**General**

1. Quantitative colony counts should be performed routinely on all urine samples.

**Rationale**
1. It is generally agreed that greater than 100,000 organisms/ml in a clean catch urine specimen and greater than 10,000 in a catheter specimen are indicative of a urinary tract infection. There ARE instances when less than 100,000 organisms/ml are isolated from infected patients.

**General**

2. Anaerobic cultures are not to be set up on routine clean-catch urine specimens.

**Rationale**

2. Urinary tract infections are rarely caused by anaerobic bacteria. If an anaerobic infection is suspected, a suprapubic bladder aspiration should be performed.

**General**

3. Foley catheter tips will not be accepted for culture.

**Rationale**

3. It is impossible to remove a catheter without contaminating it with microorganisms inhabiting the urethra.

**General**

4. Urine samples collected from indwelling catheter bags will not be accepted for culture. If cultures are to be taken from indwelling catheters, a needle and syringe is used for urine aspiration through the rubber connector or the catheter line.

**Rationale**

4. Stagnant urine in a catheter bag will be overgrown with bacteria, making culture results misleading and insignificant. More fastidious pathogens also may be overgrown with more rapidly growing coliforms.

**D. Wound Specimens**
General

1. In order to determine if wound samples represent a superficial or deep specimen, direct Gram stains can be performed to evaluate the relative numbers of neutrophils and squamous epithelial cells.

Rationale

1. Clinically infected wounds almost always produce a pyogenic reaction and many polys should be seen in Gram stains. The presence of abundant epithelial cells indicates a superficial sample or contamination from the skin of the wound margins.

General

2. Anaerobic cultures should not be routinely set up on wound cultures which are not submitted in an appropriate anaerobic container, or if delayed more than 30 minutes in a non-anaerobic transport system.

Rationale

2. Physicians should use clinical judgment when ordering anaerobic cultures. They are quite expensive and require considerable technical expertise. Gas production, foul order, and copious pus production are clinical indications of anaerobic wound infections.

E. Spinal Fluids

General
1. Direct Gram stains are performed on the sediments of all spinal fluids submitted for culture. All positive results must be immediately called to the physician.

Rationale

1. Immediate results may represent life-saving information in some cases of meningitis. Even a report of no bacteria may be important information in the assessment of clinical cases of meningitis. A simple methylene blue stain of spinal fluid (Wayson’s) also gives rapid information on the presence or absence of bacteria.

General

2. If specimen processing is to be delayed, spinal fluids should be placed in a 35°C incubator until they can be inoculated to culture media. In no instance should samples be stored in the refrigerator.

Rationale

2. Spinal fluid itself is a good innate culture medium. In most instances, infections are caused by one species or overgrowth with contaminants is not a concern. Organisms such as Haemophilus influenzae and Neisseria meningitidis, common causes of meningitis, are sensitive to chilling and may die in refrigerated samples.

E. Throat and Nasopharyngeal Cultures

General

1. Throat cultures are processed for the recovery of all pathogens.

Rationale

1. Organisms other than beta hemolytic streptococci do not cause primary acute Staphylococci may cause tonsillar abscesses, H. influenzae constrictive epiglottitis and Corynebacterium diphtheriae will cause a membranous pharyngitis.
General

2. Attempts to routinely recover *H. influenzae* from throat cultures are the prerogative of each hospital or laboratory.

Rationale

2. A high percentage of healthy adults and children harbour *Haemophilus* species in their oropharynx. Physicians must inform the laboratory if *Haemophilus* infection is suspected.

General

3. Antibiotic susceptibility testing should not be performed on beta hemolytic streptococci recovered from throat cultures.

Rationale

3. Beta hemolytic streptococci are universally susceptible to penicillin.

General

4. Coliform bacilli in throat cultures are not generally significant unless predominant or present in immunocompromised patients.

Rationale

4. Coliform bacilli do not normally cause pharyngitis but can colonize the throat and serve as a reservoir for lower respiratory infections. Hospitalized patients tend to colonize with coliform organisms that are resistant to many antibiotics used in that hospital.

G. Vaginal and Endometrial Cultures
General

1. In general, vaginal cultures are of minimal value. Cultures for gonorrhea should be obtained directly from the uterine cervix and submitted on appropriate transport media. Anaerobic cultures should not be performed except on abscess fluid aspirated by syringe and needle from a para-vaginal abscess. Other infections such as trichomonas, candidiasis, or that caused by Gardnerella vaginalis may be diagnosed by direct mounts of smears.

Rationale

1. The normal vaginal flora includes a wide variety of aerobic and organisms. Anaerobic cultures set up on vaginal swab specimens are impossible to interpret in light of the normal background flora. Direct Gram stains of vaginal secretions for an assessment of bacterial flora, particularly in search for Neisseria gonorrhoeae in female patients may be misleading because of the various resident organisms that can mimic or simulate organisms known to be pathogens.

General

2. Endometrial cultures should be collected by direct vision through the endocervical canal and placed in an anaerobic container. These cultures should always be processed for aerobic and anaerobic organisms.

Rationale

2. Cause the environment of the endometrial cavity is relatively anaerobic, it is not uncommon for anaerobic infections to take place. In cases of postpartum infection, organisms such as Group B streptococci and Listeria monocytogenes should be specifically cultured for.

General

3. Culdocentesis cultures are preferred where possible.

Rationale
3. Fluids accumulate in this area in PID.

H. Miscellaneous Cultures

General

1. In processing of eye cultures, enriched chocolate agar should be used to detect fastidious organisms such as *Haemophilus* sp., *Neisseria* sp. or slow growing Gram negative bacilli. A direct Gram stain should always be examined at the time cultures are set up.

Rationale

1. Fastidious organisms often infect the eye and may be missed if an enriched culture medium is not used. The presence of Gram negative bacilli that morphologically resemble Pseudomonas species should be made immediately known to the physician because the organism can cause a rapidly blinding ophthalmitis.

General

2. Tissue biopsy specimens should be processed only after processing through a stomacher. Anaerobic cultures should be set up on request or if gas or foul odor is detected by the technologist. Direct gram stains should be performed on the ground eluate and any positive findings reported immediately to the physician.

Rationale

2. Anaerobic cultures may be valid only if the tissue has been submitted in a container protected from exposure to atmospheric oxygen although in larger specimens the reducing properties of the tissue proteins tend to maintain a relative anaerobic environment, *Clostridium perfringens*, may be immediately suspected by Gram stain.
General

3. Gastric specimens in general do not reveal meaningful results, except perhaps in septic infants or in older individuals with high intestinal obstruction. Bacterial colony counts on gastric secretions are of questionable value.

Rationale

3. Anaerobic bacteria may inhibit the normal gastric secretions and interpretation of culture results may be difficult. The presence of large numbers of bacteria in gastric secretions usually indicates an alkaline pH shift from regurgitation of duodenal secretions in cases of intestinal obstruction. The recovery of certain species of mycobacteria may be significant. Gastric aspirates for TB should be neutralized before holding for processing. Preferential time of receipt is between 7-9 a.m.

General

4. Body fluids, such as joint fluids, pleural fluids, peritoneal fluids, etc., should be inoculated to enriched media. Direct Gram stains may be very helpful in deriving a presumptive diagnosis and may also be helpful in selecting the proper culture media. It may be necessary to use an anticoagulant with some specimens.

Rationale

4. Body fluids effusions and transudates often contain proteins and clotting factors, which produce a clot or gel. Bacteria may be trapped in these clots and not appear in cultures of the fluid itself. It is recommended that body fluids be allowed to settle or undergo centrifugation in order to concentrate any bacteria present. This may not be possible if the specimen is allowed to clot. Enriched media should always be used since fluids often have smaller numbers of organisms per ml.
General

5. Fecal specimens should be routinely cultured for *Salmonella-Shigella* and E. coli 0157:H7, and Campylobacter. Media for *Vibrio* and *Yersenia* are available depending on physician’s diagnosis.

Rationale

6. *Yersinia enterocolitica* serotypes 05 and 08 are common in the U.S. *Yersinia* selective medium is used for the initial isolation. Selective media for Salmonella-Shigella are often inhibitory to Campylobacter. If swabs are submitted, one alone should be used for Campylobacter isolation.

CRITERIA FOR REJECTION OF MICROBIOLOGICAL SPECIMENS

1. Unlabelled or improperly labeled specimen.
2. Prolonged transport.
3. Improper or damaged/leaking container.
4. Oropharyngeal-contaminated sputum.
5. Obvious contamination with foreign materials.
6. Duplicate specimens (two stools, two sputum) within a 24 hour period (exception – multiple sputa following Bronchoscopy).
7. Specimen unsuitable for culture request; i.e., routine vaginal for anaerobe workup or Foley catheter tip.

**ACTION ON REJECTED SPECIMENS**

1. Alert physician and/or ward nurse of discrepancy.
2. Request a repeat specimen.
3. Hold first rejected specimen in refrigerator until physician is contacted.
4. If processing of inadequate or improper specimen 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.
5. If specimen is voided, indicate physician name who agreed to repeat on requisition form.
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 can not be located, the appropriate personnel is contacted for a recollect.

Prepared By: Doug Eley          Date: 6/2/00
Approved By Julie A. Ribes     Date: 6/2/00
Reviewed By: Julie A. Ribes    Date: 3/18/02, 5/16/02,
                                3/18/03, 3/18/04,
                                3/18/05
Revised By: Julie A. Ribes      Date: 3/18/05
Reviewed By: Julie A. Ribes     Date: 11/29/05

Reviewed By:____________________________  Date:______________

Reviewed By:____________________________  Date:______________
DIRECT EXAMINATION BY GRAM STAIN

Specimens received in the laboratory on which a direct gram stain should be performed and results given to the physician.

1. Spinal fluid and other body fluids
2. Peritoneal fluid
3. Eye
4. Any purulent discharge
5. Sputum, transtracheal aspirate
6. Surgical aspirates
7. Tissue
8. Urethral exudates from males (for \textit{N.gonorrhoeae})
9. Stools for white cells
10. Vaginal 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 fascitis
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 *Gardnerella vaginalis*
### FECAL LEUKOCYTES IN STOOL SPECIMENS FROM PATIENTS WITH DIARRHEAL DISEASE

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>PREDOMINANT CELL TYPE IN FECES (ACUTE ILLNESS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacteriosis</td>
<td>Polymorphonuclear</td>
</tr>
<tr>
<td>Shigellosis</td>
<td>Polymorphonuclear</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>Polymorphonuclear</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>Mononuclear</td>
</tr>
<tr>
<td>Cholera</td>
<td>None</td>
</tr>
<tr>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
<td>None</td>
</tr>
<tr>
<td>Invasive <em>E. coli</em></td>
<td>Polymorphonuclear</td>
</tr>
</tbody>
</table>
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 SPECIMENS

<table>
<thead>
<tr>
<th>General Purpose</th>
<th>Enrichment</th>
<th>Differential Media</th>
<th>Selective Media</th>
<th>One-Purpose Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar Plate</td>
<td>Selenite F</td>
<td>MacConkey</td>
<td>Xylose-Lysine-Medium</td>
<td>Campylobacter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Desoxycholate</td>
<td>Desoxycholate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PEA Plates</td>
<td>PEA Plates</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thio sulfate Citrate</td>
<td>Thio sulfate Citrate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bile Salts (Vibrio) Yersina Selective</td>
<td>Bile Salts (Vibrio) Yersina Selective</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agar</td>
<td>Agar</td>
<td></td>
</tr>
</tbody>
</table>
NOTE: C. difficile Agar, PEA, and Yersina Selective are plated only on request.

## GENITAL SPECIMENS

### Female Genital Specimens

<table>
<thead>
<tr>
<th>Not Cultured for Anaerobes</th>
<th>Cultured for Anaerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal</td>
<td>Placenta, C-section</td>
</tr>
<tr>
<td>Urethra</td>
<td>Uterus (endometrial)</td>
</tr>
<tr>
<td>Placenta, Vaginal</td>
<td>Culdocentesis</td>
</tr>
<tr>
<td>Vulva</td>
<td>Fallopian tube</td>
</tr>
<tr>
<td>Lachia</td>
<td>Endocervical</td>
</tr>
<tr>
<td>Perineum</td>
<td>Ovary</td>
</tr>
<tr>
<td>Cervical</td>
<td>Bartholin’s gland</td>
</tr>
</tbody>
</table>

### Male Genital Specimens

<table>
<thead>
<tr>
<th>Not Cultured for Anaerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethral</td>
</tr>
<tr>
<td>Prostatic Fluid</td>
</tr>
</tbody>
</table>
Seminal Fluid

**INCUBATION CONDITIONS**

**SPECIMENS TO BE INCUBATED UNDER 5%-10% CO₂**

Genital – Blood, MacConkey, Modified Thayer-Martin, V Agar  
Wounds – Blood, MacConkey (Cocolate and/or anaerobe screen if appropriate)  
Respiratory – Blood, Chocolate, MacConkey  
Blood – sub culture to Chocolate, Blood – incubate second BAP anaerobically  
Throat – Blood, Chocolate, MacConkey

**SPECIMENS TO BE INCUBATED UNDER REDUCED O₂**

Feces (for Campylobacter) – Campylobacter – one purpose medium  
5% O₂, 10% CO₂, 85%N₂ – (Campy Gas)
## SELECTED EXAMPLES OF MEDIA FOR PRIMARY ISOLATION OF MICROORGANISMS

### General Purpose Media – Enriched

<table>
<thead>
<tr>
<th>Media</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (Sheep-5%)</td>
<td>Enriched medium used for the primary recovery of most commonly encountered microorganisms</td>
</tr>
<tr>
<td>Chocolate Agar (Enriched)</td>
<td>Hemolyzed blood or hemoglobin agar with supplements which serves as an enriched medium for recovery of such fastidious organisms as <em>Neisseria gonorrhoeae</em> and <em>Haemophilus influenzae</em>.</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 K₁, and L-cystine. Kanamycin and Vancomycin may be added for selective purposes. May be held anaerobically before inoculation.</td>
</tr>
</tbody>
</table>

### Enteric Media

<table>
<thead>
<tr>
<th>Media</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacConkey Agar</td>
<td>Differential medium for the recovery of Xylose Lysine Gram-negative bacilli. Most Gram-positive bacteria are inhibited. Media more selective (XLD) than MacConkey or eosin Methylene Blue, designed specifically to recover species of Salmonella or Shigella from feces or other body fluids or secretions. S-S Agar is not recommended for the isolation of S.<em>sonnie</em> but may be helpful for the isolation of <em>Y. Enterocolitica</em>.</td>
</tr>
<tr>
<td>Desoxycholate Agar</td>
<td></td>
</tr>
<tr>
<td>Desoxycholate Agar</td>
<td></td>
</tr>
<tr>
<td>MacConkey or eosin Methylene Blue</td>
<td></td>
</tr>
<tr>
<td>Selenite Broth</td>
<td>Enrichment broths for concentrations of Pathogenic Salmonella or Shigella from contaminated clinical specimens, particularly feces.</td>
</tr>
</tbody>
</table>

### Inhibitory Media – Special Use
### Blood Agar
*(Phenylethyl Alcohol)*
Selective medium for the isolation of Gram-positive organisms, especially for specimens heavily contaminated with Gram-negative organisms, such as Proteus sp. which are inhibited by this medium.

### Modified Thayer-Martin Agar (MTM)
A modified chocolate agar containing enrichments and the antibiotics Vancomycin, Colistin, and Nystatin to inhibit growth of bacteria other than *Neisseria* species. In the modified formula, trimethoprim lactate has been added and the agar concentration increased to 2% to inhibit the spreading of Proteus species. The carbohydrate concentration has also been increased to 0.25% to enhance growth. Other modifications are available.

### Broth Media

<table>
<thead>
<tr>
<th>Broth Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioglycollate Broth</td>
<td>Broth designed for recovery of anaerobes from clinical materials.</td>
</tr>
<tr>
<td>(enriched with hemin and Vitamin K)</td>
<td></td>
</tr>
<tr>
<td>Trypticase Soy Broth With Fildes Enrichment</td>
<td>General nutrient broths used for recovery of fastidious bacteria.</td>
</tr>
<tr>
<td>Trypticase Soy Broth</td>
<td>For subculturing colonies of bacteria or for preparing the standard inoculum for the Kirby-Bauer susceptibility test.</td>
</tr>
</tbody>
</table>

### Transport Media

<table>
<thead>
<tr>
<th>Transport Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port-A-Cul Vial</td>
<td>Transport system for maintaining the viability of anaerobes in transit to the laboratory.</td>
</tr>
<tr>
<td>Culturette</td>
<td>A transport medium which maintains viability of microorganisms in specimen to be transported to the laboratory.</td>
</tr>
<tr>
<td>Viral Culturette</td>
<td>A transport medium for maintaining the viability of viruses to the laboratory.</td>
</tr>
</tbody>
</table>
**Chlamydia Transport Medium**  
A special sucrose phosphate medium for transporting.

**Transgrow**  
Modified Thayer-Martin medium with 5% CO₂ designed to maintain viability of *Neisseria gonorrhoeae*.

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

## SUPPLEMENTAL PRIMARY CULTURE MEDIA FOR RECOVERY OF SPECIAL FASTIDIOUS ORGANISMS

<table>
<thead>
<tr>
<th>MEDIUM</th>
<th>PURPOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordet-Gengou Agar</td>
<td>Selective medium for recovery of <em>Bordetella pertussis</em>. Inoculation of medium with a nasopharyngeal swab is recommended instead of the use of a cough plate.</td>
</tr>
<tr>
<td>Brucella Broth</td>
<td>Enriched peptic digest case in medium designed for recovery of Brucella species from clinical specimens and other infected material.</td>
</tr>
<tr>
<td>Fletcher’s Semisolid Medium</td>
<td>Basal medium used with serum enrichments for the recovery of <em>Leptospira</em> species.</td>
</tr>
<tr>
<td>Loeffler Medium and Tellurite Medium</td>
<td>Used for inoculation of nasopharyngeal cultures. Also used to prepare methylene blue stains of suspicious colonies.</td>
</tr>
<tr>
<td>Thiosulfate Citrate Blue</td>
<td>Primary recovery of <em>Vibrio</em> species from fecal or other contaminated material.</td>
</tr>
<tr>
<td>Campy BAP</td>
<td>Special media with a variety of antibiotics which are designed to inhibit normal fecal flora but not...</td>
</tr>
</tbody>
</table>
effect growth of *Campylobacter fetus* ssp., *jejuni* when incubated at 42°C. All stools are routinely screened for *C. fetus* ssp. *jejuni*.

**Yersinia Selective Medium (CIN)**
Selective medium containing cefsulodin, irgasan and novobiocin for isolating Yersinia species.

**V Agar**
Special media containing human blood to detect hemolysis of *Gardnerella vaginalis*.

**Glucose-Cysteine Agar**
Special media with thiamine for isolating *Francisella tularensis*.

---

### SPECIMEN PROCESSING IN BACTERIOLOGY

<table>
<thead>
<tr>
<th>Type of Specimen</th>
<th>Media and Conditions</th>
<th>Normal Flora</th>
<th>Common Pathogen</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat</td>
<td>Blood agar</td>
<td>Alpha and gamma strep</td>
<td>Group A, beta –hemolytic streptococci</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chocolate agar (for pediatric if requested)</td>
<td>Commensal <em>Neisseria</em></td>
<td>(Haemophilus influenzae)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MacConkey (aerobic) (all above CO₂, 35°C)</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>(Corynebacterium diphtheriae)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MacConkey Aerobic (All above CO₂, 35°C)</td>
<td>Diphtheroids</td>
<td>(Bordetella pertussis)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic media</td>
<td><em>Streptococcus pneumoniae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>N. Meningitidis</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory</th>
<th>Blood Agar</th>
<th>Larynx, trachea, sinus, sputue:</th>
<th>Group A,beta streptococci</th>
<th>Gram Stain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chocolate Agar</td>
<td><em>S.epidermidis</em></td>
<td><em>H. influenzae</em></td>
<td>Sputum</td>
</tr>
<tr>
<td></td>
<td>MacConkey Aerobic (All above CO₂, 35°C)</td>
<td>Non-beta streptococci</td>
<td><em>S. aureus</em></td>
<td>All aspirates</td>
</tr>
<tr>
<td></td>
<td>Anaerobic media</td>
<td>Comensal <em>Nisseria</em></td>
<td><em>Enterobacteriaceae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilus</td>
<td><em>Pseudomonas sp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. pneumoniae</em></td>
<td></td>
</tr>
</tbody>
</table>
## Transtracheal: none

<table>
<thead>
<tr>
<th>Type of Specimen</th>
<th>Media and Conditions</th>
<th>Normal Flora</th>
<th>Common Pathogens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Blood agar +</td>
<td>None</td>
<td><em>Escherichia coli</em> and other enterics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MacConkey</td>
<td></td>
<td>Enterococci</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(All 35°C, CO₂)</td>
<td></td>
<td><em>Pseudomonas sp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Staphylococci</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001 mml loop used for clean catch specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01 ml loop used for catheterized specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>MacConkey</td>
<td>Enterobacteriaceae</td>
<td><em>Salmonella sp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Xylose-Lysine-desoxy-cholate</td>
<td>Enterococci</td>
<td><em>Shigella sp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selenite F</td>
<td></td>
<td><em>Arizona sp.</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selective medium for</td>
<td></td>
<td><em>Yersinia enterocolitica</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Campylobacter</td>
<td></td>
<td><em>Campylobacter fetus ssp. jejuni</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>42°C, 5%, O₂</td>
<td></td>
<td>Aeromonas sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood, CAN</td>
<td></td>
<td>Pleisomonas sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cinn/22°C, Aerobic, TCBS</td>
<td></td>
<td>Edwardsiella</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(All 34°C, aerobic, unless otherwise specified)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Genital for GC only**

- Modified Thayer-Martin (MYM) (35°C, CO₂)
- Lactobacilli
- Diptheroids
- Alpha streptococci (inhibited on MTM)
- *Neisseria gonorrhoeae*
- Gram Stain
<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Media/Agar</th>
<th>Isolates</th>
<th>Stain Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine genital</td>
<td>MTM MacConkey-aerobic</td>
<td>Group A and B streptococci</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V agar (all 35°C, CO₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genital – surgical or aspirates</td>
<td>Blood agar</td>
<td>None</td>
<td>Same as above plus</td>
</tr>
<tr>
<td></td>
<td>Chocolate agar MTM MacConkey – aerobic Anaerobic blood agar (all 35°C, CO₂)</td>
<td>S. pneumoniae, N. meningitidis, H. influenzae</td>
<td>Gram Stain anaerobes</td>
</tr>
<tr>
<td>Sterile body fluids, CSF, joint fluid, pleural fluid, peritoneal</td>
<td>Blood agar Chocolate agar Fildes (35C, CO₂)</td>
<td>Identify all isolates</td>
<td>Gram Stain</td>
</tr>
<tr>
<td>Blood</td>
<td>Submitted in two bottles: one 35C aerobic and one 35C anaerobic</td>
<td>None</td>
<td>Any isolate potentially significant</td>
</tr>
<tr>
<td>Wound (superficial includes eye and ear)</td>
<td>MacConkey agar 35C, aerobic Blood Agar Chocolate (eye, head and chest) (All 35C, CO₂)</td>
<td>S.epidermidis, S.aureus, Beta-hemolytic streptococci, P. aeruginosa</td>
<td>Gram Stain</td>
</tr>
<tr>
<td></td>
<td>S.epidermidis, Diphtheroids Consensual Neisseria anaerobes Other skin flora</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H. influenzae Biotype III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound (surgical or aspirate Tissue specimens)</td>
<td>Same as above plus Anaerobic blood agar K-V blood agar*</td>
<td>None</td>
<td>Same as above plus Anaerobes Potentially any isolate</td>
</tr>
</tbody>
</table>
- Kanamycin-Vancomycin
ALTERNATIVE AVENUES TO CONSIDER IN LABORATORY DIAGNOSIS

When should Mycobacterium sp. be considered?

1. Most all sputum specimens.
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.

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

7. The specimen is a skin biopsy of a granulomatous lesion.
# A DICTIONARY OF CLINICAL SPECIMENS

## BLOOD

## BODY FLUIDS

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Description</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>Ascitic fluid</td>
<td>Serous fluid aspirated from the abdominal cavity (the peritoneum).</td>
</tr>
<tr>
<td>Bile</td>
<td>A 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>Pericardial fluid</td>
<td>Fluid contained within the membranous sac that encases the heart.</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>Same as ascitic fluid.</td>
</tr>
<tr>
<td>Pleural fluid</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>Spinal fluid</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>Transudate</td>
<td>Fluid which has passed through a membrane or extruded from a tissue and characterized by its low viscosity, lack of protein, and cells or cellular debris, and by having a specific gravity under 1.013.</td>
</tr>
</tbody>
</table>

## EYE
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>The mucous membrane covering the anterior surface of the eyeball and the 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>
<tr>
<td><strong>GENITAL (FEMALE)</strong></td>
<td></td>
</tr>
<tr>
<td>Bartholin’s gland</td>
<td>One of two, small, mucous scripting glands on either side of the vaginal 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 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 vaginal/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>
<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>(See placenta) A result of a cesarean section</td>
</tr>
</tbody>
</table>
Urethral  From the membraneous canal conveying urine from the bladder to the exterior of the body
Uterus  Hollow, muscular organ in the female in which the fetus develops
Vaginal  From the canal that extends from the vulva to the cervix
Vulva  The region of the external female genitals

**GENITAL (MALE)**
Lesion  A more or less circumscribed pathologic or traumatic injury to tissue
Penile exudate  Exudate expressed through the urethra
Prostate  A gland which, in the male, surrounds the neck of the bladder and the urethra
Urethral  See above

**LOWER RESPIRATORY TRACT**
Bronchial  Referring to the large air passages which dichotomously branch within the lungs.
Bronchial aspirate  Material collected from the bronchi by means of instrumentation
Fiberoptic  Collection of material with an instrument designed for visualization of the lower respiratory area and for specimen collection
Sputum  Matter ejected from the lungs, bronchi, and trachea through the mouth
Tracheal  The tube from the larynx to the bronchi
Transtracheal aspirate  Material obtained by surgical passage of a catheter through the tracheal wall and into the lower respiratory area.
UPPER RESPIRATORY TRACT

Ear
Unless specified, refers to the external ear

Mouth and dental
Gums, gingivae, teeth, root canals, tongue, etc.

Nasopharynx
That part of the pharynx above the soft palate

Nose (nasal)
In microbiology the term usually refers to culture obtained from about 1-2 cm deep within the nostril

Sinus
Any body cavity, hollow space, or open channel

Throat
That area within the deep oral cavity between and including the tonsillar pillars

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

SURFACE SPECIMENS

Burn
A traumatic lesion caused by contact of tissue with heat

Cyst
Any liquid or exudate containing sac

Decubitus
Ulceration due to prolonged pressure of lying down or sitting

Exudate
Fluid-containing protein, cells or solid material escaped from blood vessels as a reaction to injury or inflammation

Laceration
A cult

Lesion
See Genital (male)

Paronychia
Inflammation around the folds of skin around the fingernails
Skin  External body covering

Stoma  Any small opening or orifice on a free surface, i.e., the opening from a colostomy or ileostomy site

Suture  A surgical “stitch”

Ulcer  A loss of integrity of a cutaneous or mucous surface lining resulting from the sudden or gradual sloughing of necrotic tissue.

Vesicle  A small blister containing a serous liquid

**SURGICAL SPECIMENS**

**Abscess**  Localized collection of pus in a cavity formed by disintegration of tissue

**Aspirate**  Removal of fluids from a cavity by suction

**Biopsy**  Surgical removal of small portions of tissue from a living body for the purpose of establishing a precise diagnosis

**Bone**  Mineralized connective tissue that comprises the skeleton of vertebrates

**Clot**  A semisolid mass, usually of blood or lymph

**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**  See **SURFACE SPECIMENS**

**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  See lesion, GENITAL (MALE)

URINE

Catheterized  Urine aspirated from a urinary catheter
Midstream  Urine collected in a container after the first few milliliters of urine has been passed
Suprapubic  Urine surgically aspirated with syringe and needle by direct puncture into the bladder

REFERENCES

1. Colorado Association for Continuing Medical Laboratory Education. 1979. CACMILE Telediaglogs in Microbiology. Developed under CDC Contract #200-78-0833. Elmer Koneman, M.D., Project Director.


SUPPLEMENTAL READING


URINE

1. Cleansing Procedures

   **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.

   **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

   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-trachael 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

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.

FECES FOR PARASITOLOGY AND ROUTINE CULTURE
Fecal specimens should be collected in a clean, wax-lined carton and delivered to the laboratory immediately. The specimen must still be warm by the time it reaches the laboratory. Specimens **MUST** be collected prior to barium enemas. The time of collection and the diagnosis must be on the requisition sheet.

Rectal swabs will be accepted from the VA Hospital in Port-A-Cul swab systems for *C. difficile* and *Campylobacter* culture, as long as the medium is not oxidized.

**FLUIDS, SPINAL**

All spinal fluids should be collected in sterile tubes and brought to the laboratory **IMMEDIATELY** and handed to the secretary at the reception desk. Time of collection must be marked on the requisition sheet, with a diagnosis.

**FLUIDS, OTHER THAN SPINAL**

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**

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.

**URETHRAL AND VAGINAL OR CERVICAL DISCHARGES**

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.

1. Remove cap of bottle only when ready to inoculate medium.
2. 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.

3. Tighten the cap immediately to prevent loss of CO$_2$.

**CAUTION**: Keep neck of the bottle in an upright position to prevent CO$_2$ loss.

Desiccation or a change in temperature can easily kill the fastidious Gonococcus.

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

**THROAT CULTURES**

Sterile swabs are used for collecting these specimens. The tonsillar area should be swabbed with at least two (2) swabs and moist swabs must be delivered to the laboratory immediately. Dry swabs are of no benefit to the patient. The diagnosis must be on the requisition sheet as well as time of collection.

**SKIN OR NAIL SCRAPINGS**

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. Viable 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**

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**
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.

Each time that 10 ml of blood is drawn for culture, it is to be placed into each of two blood culture bottles (Bactec, Aerobic and Anaerobic). The patient’s arm should be cleansed with green soap, followed by disinfection with 70% isopropyl alcohol and last, 2% iodine applied in concentric fashion to the venipuncture site. The iodine should remain intact on the skin for at least one minute. The intended venipuncture site should not be touched unless palpation is performed with a sterile glove, or previously cleansed finger.

In order to reduce the introduction of contaminants into the bottles, it is necessary to disinfect the rubber diaphragm on the bottle with 70% isopropyl alcohol. A new needle should be used for introducing the blood into the bottle. Never use the same needle that was used on the patient.

It is recommended that the volume of blood added to the bottle be 1/10 the volume of broth present in the bottle. Therefore, 5 ml of blood should be added to 50 ml bottles and 10 ml to 100 ml bottles. For special culturing procedures, refer to the BLOOD CULTURE section of the Procedures Manual.

**COLLECTION OF URINE FROM 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 Phenyl Ethyl Alcohol (PEA) Agar Plate and McConkey Agar plates are streaked for isolation. A Fildes broth is inoculated on all patients if over a month old. A Thio with sucrose is included on all patients on antibiotics.

INTERPRETATION

Any growth is considered significant and is identified and antibiotic susceptibility testing is performed.
REFERENCES


COLLECTION OF THROAT SPECIMEN

PURPOSE

The purpose of collecting a throat specimen is usually to recover Group Streptococcus or other potential pathogens are requested.

MATERIALS

Sterile Swab, Sterile Tube or Transport Container, 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. 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 *Bordetella pertussis*, *Corynbacterium diphtheriae*, *Neisseria gonorrhoeae* or any other fastidious organisms. Smears and anaerobe cultivation are not performed on throat specimens.

**INTERPRETATION**

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

**REFERENCES**


NASOPHARYNGEAL SPECIMEN COLLECTION

Specimen collection procedures appropriate for use with BD Directigen™ RSV, BD Directigen™ Flu A and BD Directigen™ Flu A+B rapid EIA tests.

<table>
<thead>
<tr>
<th>Product</th>
<th>Transport Medium</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directigen™ RSV (40 tests)</td>
<td>Liquid Amies, Reg. Alum. Wire</td>
<td>253040</td>
</tr>
<tr>
<td>(20 tests)</td>
<td>Liquid Amies, Flex. Alum. Wire</td>
<td>253020</td>
</tr>
<tr>
<td>Directigen™ Flu A (20 tests)</td>
<td>Liquid Stuart, Reg. Alum. Wire</td>
<td>256020</td>
</tr>
<tr>
<td>Directigen™ Flu A+B (20 tests)</td>
<td>Liquid Stuart, Soft Alum. Wire</td>
<td>256010</td>
</tr>
<tr>
<td>BBL™ CultureSwab™</td>
<td>Liquid Amies, Soft Alum. Wire</td>
<td>220129</td>
</tr>
<tr>
<td>BBL™ CultureSwab™</td>
<td>Liquid Amies, Flex. Alum. Wire</td>
<td>220130</td>
</tr>
<tr>
<td>BBL™ CultureSwab™</td>
<td>Liquid Stuart, Reg. Alum. Wire</td>
<td>220131</td>
</tr>
<tr>
<td>BBL™ CultureSwab™</td>
<td>Liquid Stuart, Soft Alum. Wire</td>
<td>220132</td>
</tr>
<tr>
<td>BBL™ CultureSwab™</td>
<td>Liquid Stuart, Flex. Alum. Wire</td>
<td>220133</td>
</tr>
<tr>
<td>BBL™ CultureSwab™ Plus</td>
<td>Amies Gel w/Charcoal, Reg. Alum. Wire</td>
<td>220134</td>
</tr>
<tr>
<td>BBL™ CultureSwab™ Plus</td>
<td>Amies Gel w/Charcoal, Soft Alum. Wire</td>
<td>220123</td>
</tr>
<tr>
<td>BBL™ CultureSwab™ Plus</td>
<td>Amies Gel w/Charcoal, Flex. Twisted Wire</td>
<td>220124</td>
</tr>
<tr>
<td>Viral Cultrette™</td>
<td>Hanks’ Balanced Salt Solution</td>
<td>261514</td>
</tr>
</tbody>
</table>

Patient’s head should be inclined from vertical as shown for proper specimen recovery.
Nasopharyngeal Wash: Bulb Method

Materials: Saline
1-2 oz. tapered rubber bulb*
Viral Transport Medium (VTM)
Specimen container

1. Suction 3-5 ml saline into a new sterile bulb.
2. Insert bulb into one nostril until nostril is occluded.
3. Instill saline into nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
4. Empty bulb into suitable dry, sterile specimen container or one containing VTM, according to virology laboratory requirements.

* Length and diameter of bulb as appropriate for infant, child or adult.
Nasopharyngeal Wash: Syringe Method

**Materials:**
- Saline
- 3-5 ml syringe*
- 2" 18-20 gauge tubing*
- Viral Transport Medium (VTM)
- Specimen container

1. Fill syringe with saline; attach tubing to syringe tip.
2. Quickly instill saline into nostril.
3a. Aspirate the recoverable nasal specimen. Recovery must occur immediately, as the instilled fluid will rapidly drain.
3b. *(Alternate)* In appropriate cases, patients may tilt head forward to allow specimen to drain into suitable sterile container.
4. *(If aspirated)* Inject aspirated specimen from syringe into suitable dry, sterile specimen container or one containing VTM, according to virology laboratory requirements.

* Length and diameter of syringe and tubing as appropriate for infant, child or adult.
Nasopharyngeal Wash: Syringe Method

Materials: Saline
3-5 ml syringe*
2" 18-20 gauge tubing*
Viral Transport Medium (VTM)
Specimen container

1. Fill syringe with saline; attach tubing to syringe tip.
2. Quickly instill saline into nostril.
3a. Aspirate the recoverable nasal specimen. Recovery must occur immediately, as the instilled fluid will rapidly drain.
3b. (Alternate) In appropriate cases, patients may tilt head forward to allow specimen to drain into suitable sterile container.
4. (If aspirated) Inject aspirated specimen from syringe into suitable dry, sterile specimen container or one containing VTM, according to virology laboratory requirements.

* Length and diameter of syringe and tubing as appropriate for infant, child or adult.
Vacuum-assisted Nasopharyngeal Aspirate Method

Materials: Portable suction pump
Sterile suction catheter
Mucus trap (i.e., Luken’s tube)
Viral Transport Medium (VTM)

1. Attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter; turn on suction and adjust to suggested pressure.
2. Without applying suction, insert catheter into the nose, directed posteriorly and toward the opening of the external ear. NOTE: Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior naris and external opening of the ear.
3. Apply suction. Using a rotating movement, slowly withdraw catheter. NOTE: Catheter should remain in nasopharynx no longer than 10 seconds.
4. Hold trap upright to prevent secretions from going into pump.
5. Rinse catheter (if necessary) with approximately 2.0 ml VTM; disconnect suction; connect tubing to arm of mucus trap to seal.

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Catheter Size (French)**</th>
<th>Suction Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature infant</td>
<td>6</td>
<td>80-100 mmHg</td>
</tr>
<tr>
<td>Infant</td>
<td>8</td>
<td>80-100 mmHg</td>
</tr>
<tr>
<td>Toddler / Preschooler</td>
<td>10</td>
<td>100-120 mmHg</td>
</tr>
<tr>
<td>School age</td>
<td>12</td>
<td>100-120 mmHg</td>
</tr>
<tr>
<td>Adolescent / Adult</td>
<td>14</td>
<td>120-150 mmHg</td>
</tr>
</tbody>
</table>

** To determine length of catheter tubing, measure distance from tip of nose to external opening of ear.
**Nasopharyngeal Swab Method**

*Materials:* BD BBL CultureSwab flexible, soft, or regular aluminum wire products *or* Nasopharyngeal swab with synthetic fiber tip 1-2 ml Viral Transport Medium (VTM) Specimen container

1. Insert swab into one nostril.
2. Rotate swab over surface of posterior nasopharynx.
3. Withdraw swab from collection site; insert into transport tube or container with VTM.
SECTION V

VIRAL SPECIMEN COLLECTION
VIRAL SPECIMEN COLLECTION

I. 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.

II. PATIENT INFORMATION REQUESTED

A. 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 birthdate)

III. COLLECTION OF SPECIMENS/BASIC INFORMATION

A. Collect-specimens promptly, preferably within 3 days and generally as soon as possible after the onset of symptoms.
B. Collect postmortem specimens aseptically, as soon as possible after death.

C. Immediately transport all specimens on wet ice to the laboratory.
   1. 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.)
D. Do not use any preservatives or fixatives. For certain types of specimens, the laboratory will supply viral transport media.

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

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

A. Nasal Excretions

1. Collect with a sterile cotton swab and place in Virocult Transport Tube supplied by the laboratory.

2. Nasal washings can be obtained by instilling 4-5 ml or sterile saline into each nostril with the head titled back slightly; the head is then brought forward and the saline is allowed to flow into a small container held beneath the nose.

3. Transport to the laboratory immediately on wet ice.

B. Throat Washings

1. The patient should gargle with approximately 10 ml of saline solution and expectorate into a sterile container.

2. Transport to the laboratory immediately on wet ice.

C. Throat Swabs

1. Rub a sterile, dry cotton swab from a Virocult Transport tube on the tonsils and the back of the pharynx. Insert into the Virocult supplied by the laboratory. **DO NOT USE CALGISWABS.**

2. Transport to the laboratory immediately on ice.

D. Vesicular Fluids or Skin Scrapings

1. 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.
2. Vesicles are first washed gently with ordinary soap and water and rinsed. The cleansed vesicles may be opened and the exude absorbed onto a dry, sterile viral culturette.
3. Transport to the laboratory immediately on ice.

E. Spinal Fluids

1. Collect 3-5 ml in a sterile screw-capped viral or tube and transport on ice to the laboratory immediately.
   
   a. 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.

F. Body Fluids

1. “Sterile” fluids: pleural, peritoneal, pericardial, joint, collect as above for spinal fluids.
2. Transport to the laboratory immediately on ice.

V. CHLAMYDIA ISOLATION

A. Specimen Collection and Transport

   A good specimen should ideally contain host cell scrapings. Therefore the infected area should be vigorously swabbed. Use calgiswabs (calcium alginate) and NOT cotton swabs for optional isolation of chlamydia.

B. 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.

C. Sputums or throat washings are suitable for Isolations.

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

IT MUST BE SPECIFIED ON THE REQUISITION SLIP IF THE SPECIMEN IS TO BE SENT TO THE REFERENCE LAB.

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

REFERENCES


SPECIMEN HANDLING FOR VIROLOGY

I. GENERAL COMMENTS
   A. Accession number range is 3750-4199.
   B. All paperwork must match specimen label, and complete information must be given. Initial each requisition.
   C. Make sure requisition is clocked in.
   D. Note if specimen is NOT received on ice.
   E. ONLY VIROcults should be used for specimen submitted via swab. Make sure ampule has been crushed.
   F. Other specimen types should be submitted in sterile container, except stool which can be submitted in cardboard box.
   G. When multiple requests are on the same specimen, a minimum of 0.5 cc per request is needed, except stools for CDIT – need 1 cc minimally. Otherwise, allocate as much as you can.
   H. Tissue can be homogenized and split for Virology, if multiple requests are ordered. If only for Virology, leave intact.
   I. Only FRHD and CHM are frozen. Serum submitted for FRHD must be separated from red cells and put into a labeled tube before freezing.

II. SPECIMEN TO BE FROZEN AT –70°C: CHM, FRHD
   A. CHLAMYDIA (CHM)
      1. Place computer label on tube, after matching paperwork.
2. **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.

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

4. There is generally a 48-hour turn around time once the culture is set up.

B. Freeze and Hold Specimen (FRHD)

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

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

3. For special studies, such as “xoma”, retain paperwork at primary plating in folder.

### III. RESPIRATORY SYNCYTICAL VIRUS (Specimen should be an NP suction)

A. In House Specimens

1. Must be received by 10:00 a.m.

2. Both cultures (RSV) and RSVE.A or fluorescent antibody (RSVFA) must be requested. If not, have the Control Area add the tests on.

3. Call/bring to Virology immediately.

B. Outside Specimens

1. Request should be for only RSVE.A or RSVFA, due to the fastidious nature of the virus.

2. If received by 10:00 a.m., call/bring to Virology. Otherwise, place in refrigerator.
IV. **OTHER VIRUSES (TO BE REFRIGERATED)**

A. **Cytomegalovirus (CV)**

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

B. **Adenovirus (ADV), Enterovirus (EV), Herpes Simplex Virus (HC)**

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

C. **Influenza ((INF)**

Specimen type should be NP suction or throat swabs. Notify the Virology laboratory during working hours when received. Refrigerate specimen.

D. **Unknown (W) Viral Requests**

Any specimen type except whole blood may be submitted for culture. Refrigerate specimen.

E. **Respiratory Battery (NF Suction)**

1. Must be received by 10:00 a.m. for FA to be done that day.

2. Call/bring to Virology immediately.

V. **CLOSTRIDIUM DIFFICILE TOXIN TEST (CDIT)**

A. Test is performed on stools only.

B. Minimum of 1 cc/1 gram of stool is needed.
C. The test is set up daily, turn around time is 48 hours.

D. Turn around time for EIA is within 24 hours.

VIRAL SPECIMEN COLLECTION

I. 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.

II. Patient Information Requested
A. 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.

S.

Relevant Immunization

6. Brief Clinical History

7.

Identifying Patient Number (i.e. Hospital number, Social Security or Birthday)

III. Collection of Specimens/Basic Information

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

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

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

1. With a few exceptions, specimens to be tested within 24 hours may be held at 4C 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.)

D. Do not use any preservatives or fixatives. For certain types of specimens, viral transport media will be supplied by the laboratory.

E. Label each specimen with the patient's name, type of specimen, and date of collection.

-53

REV0504A\42
IV. Types of Specimens and Methods of Collection for All Virus Isolation Except Chlamydia

A. Nasal Excretions

1. Collect with a sterile cotton swab and place in Virocult Transport Tube supplied by the laboratory.

2. Nasal washings can be obtained by instilling 4-5 ml or sterile saline into each nostril with the head tilted back and the saline is allowed to flow into a small container held beneath the nose.

3. Transport to the laboratory immediately on wet ice.

B. Throat Washings

1. The patient should gargle with approximately 10 ml of saline solution and expectorate into a sterile container.

2. Transport to the laboratory immediately on wet ice.

Throat Swabs

1. Rub a sterile, dry cotton swab from a Virocult Transport tube on the tonsils and the back of the pharynx. Insert into the Virocult supplied by the laboratory. DO NOT USE CALGISWABS.

Transport to the laboratory immediately on ice.

D. Vesicular Fluids or Skin Scrapings.

1. 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.

2. 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 viral culturette.

3.

E. Spinal Fluids

Transport to the laboratory immediately on ice.

1. Collect 3-5 ml in a sterile screw-capped vial or tube and transport on ice to the laboratory immediately.

a.
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.

F. Spinal Fluids

1. Collect 3-5 ml in a sterile screw-capped vial or tube and transport on ice to the laboratory immediately.
   a. 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.

G. Body Fluids

1. "Sterile" fluids: pleural, peritoneal, pericardial, joint, collect as above for spinal fluids.
   2. Transport to the laboratory immediately on ice.

V. Chlamydia Isolation

A. Specimen Collection and Transport

A good specimen should ideally contain host cell scrapings. Therefore the infected area should be vigorously swabbed. Use calcium alginate (calcium alginate) and NOT cotton swabs for isolation of chlamydia.

B. 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.

C.

Sputums or throat washings are suitable for isolations.

D. Eye cultures should be swabs of the conjunctival surfaces NOT the purulent discharge.

Specimens must be promptly transported on ice in special transport media (available from the lab) to the laboratory. Transport media in the refrigerator but for prolonged storage freezing in transport media at -70°C is recommended.

VI. Criteria for Rejection

A. Specimens must be brought to the laboratory on wet ice as soon as possible after collection. Time of collection specimens which have been sitting at room temperature are not suitable for viral isolation and will not be processed.
B. Sputa and broncial washings are not considered ideal specimens due to their toxicity on tissue culture, throat or nasal washings or swabs are preferred.

IT MUST BE SPECIFIED ON THE REQUISITION SLIP IF THE SPECIMEN IS TO BE SENT TO THE REFERENCE LAB.

REV0504A\43

-55

If you have questions regarding specimen handling, call for a supervisor, telephone 233-5411.

**Questions related to appropriateness of specimen or clinically related questions** should be directed to Dr. David Wilson, telephone 233-6323.

References


I. GENERAL COMMENTS

A. Accession number range is 3750-4199.

B. All paperwork must match specimen label, and complete information must be given. Initial each requisition. Make sure requisition is clocked in.

D. Note if specimen is NOT received on ice.

E. Only VIROcults should be used for specimen submitted via swab. Make sure ampule has been crushed.

F. Other specimen types should be submitted in sterile container, except stool which can be submitted in cardboard box.

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

G.

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

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

II. SPECIMEN TO BE FROZEN AT -70°C: CHM, FRHD

A. Chlamydia (CHM)

Place computer label on tube, after matching paperwork.

2. buy 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.

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

4. There is generally a 48 hour turn around time once the culture is set up.

B. Freeze and Hold Specimen (FRHD)

1. Red-stoppered tubes--after clotting, centrifuge, remove serum and place into a correctly labeled plastic tube. Other body fluids may be frozen in the tube received or transferred to a sterile plastic tube.

For special studies, such as "xoma", retain paperwork at primary plating in folder so marked.

III. RESPIRATORY SYNCYTICAL VIRUS (Specimen should be an NP suction)
A. In House Specimens

1. Must be received by 10:00 a.m.
Both culture (RSV) and fluorescent antibody (RSVFA) must be requested. If not, have the Control Area add the tests on.
Call/bring to Virology immediately.

B. Outside Specimens

1. Request should be for only RSVFA, due to the fastidious nature of the virus.
2. If received by 10:00 a.m., call/bring to Virology. Otherwise, place in refrigerator.

IV. OTHER VIRUSES (to be refrigerated)

Cytomegalovirus (CV)

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

B. Adenovirus (ADV), Enterovirus (EV), Herpes Simplex Virus (HV)

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

Influenza (INF)

Specimen type should be NP suction or throat swabs. Notify the Virology laboratory during working hours when received.

D. Unknown (W) Viral Requests

Any specimen type except whole blood may be submitted for culture. Refrigerate specimen.
E. Respiratory Battery (NF Suction)

1. Must be received by 10:00 a.m. for FA to be done that day.

2. Call/bring to Virology immediately.

**CLOSTRIDIUM DIFFICILE TOXIN TEST (CDIT)**

A. Test is performed on stools only.
B. Minimum of 1 cc/1 gram of stool is needed.
C. The test is set up daily, turn around time is 48 hours.