CRITERIA FOR UNACCEPTABLE SPECIMENS

The following is a list of those clinical specimens or situations that under normal circumstances will be considered unacceptable for processing. The respective nursing station and/or physician must be notified regarding the unacceptability of a specimen. Specimens unacceptable per the criteria noted below, but which are not readily re-obtainable (tissue, bronchial washings, CSF, etc.) should not be discarded prior to discussion with the senior microbiology staff. On those clinical specimens found to be unsatisfactory, an entry must be logged into the Laboratory Information System (LIS). Inform patient area regarding the unacceptability of the specimen and document the telephone call in the LIS, include date, time, person spoken to and reason for specimen being unacceptable.

1. Specimen in non-sterile container.

2. Specimens in leaking containers.

3. Specimen in unlabeled containers.

4. Specimens in which the patient name on the specimen and the lab slip do not correspond; specimens in which the nature of the specimen as indicated on the lab slip does not correspond with whatever specimen identification might be present on the specimen label.

5. Specimens where there is a prolonged delay between collection and receipt by the laboratory.

6. Unauthorized specimens (employees, etc.) without prior approval.

7. Specimens other than clinical material without prior approval.

8. Processing of specimen would produce information of questionable medical value (e.g. Foley catheter tip, bacterial antigen testing on specimens other than CSF, etc.).

9. The quantity of the specimen is insufficient for testing (the specimen is considered QNS).

10. The specimen has been submitted for anaerobic culture from a site known to have anaerobes as part of the normal flora.
PROCEDURE REGARDING COLLECTION AND TRANSPORT OF MICROBIOLOGIC SPECIMENS

All specimens, once obtained, will be sent to the Central Accessioning Area. They will be held there in the appropriate storage temperature. A messenger will then deliver the specimens to the Microbiology Laboratory at an interval of every 2 hours.

All hospital patient areas are supplied with culturette transport collection kits. This transport media (AMIES liquid, Stuart liquid and/or Cary-Blair) is only to be used for bacteriologic specimens collected on cotton-tipped swabs.

Nose, Throat, Wounds, Rectal etc.:

Collect specimen on cotton-tipped swab. The cotton swab tip is inserted into the transport medium, which is then capped and sent to the laboratory. At least two (2) swabs are required to have adequate material for a Gram Stain and Culture. Transport media is used for SWABS ONLY.

Culture of Catheters, Tubing, C.S.F., Sputum, Stool, Urine Cultures, and all other Fluid Specimens (Pleural, Peritoneal, Joint, etc.):

Collect with aseptic technique and send in a plain sterile container.

Blood Cultures:

A Blood Culture set consists of two (2) bottles of blood culture medium. Aseptically inoculate 3-4 ml, aliquots of patient blood into each of the two (2) 50 ml blood culture bottles provided.

Tuberculosis (A.F.B.) Cultures: (Sent to Kings County Hospital)

Sputum - collect early morning specimens on each of three (3) consecutive days and send each in a plain sterile container. It is not necessary to submit a 24-hour collection of sputum.

Urine - collect early morning specimen in special collection and transport system. It is not necessary to submit a 24-hour collection of urine.

Gastric Lavage - a gastric lavage should be collected only when patient can produce little or no sputum. Collect specimen in a plain sterile container and
send as soon as possible since gastric fluid is toxic for A.F.B.
Bronchial Washings, Joint Fluid, Pus, CS.F., Tissue, etc. – collect specimen in a plain sterile container.

Stool – the laboratory will not culture a stool specimen for A.F.B. culture unless the smear demonstrates AFB.

Fungal Cultures:

Specimens for fungal culture can be collected from any anatomical site, however, since there is no one medium that will support the growth of all fungi, it is essential that the physician indicate the specific fungus culture he desires. If this is not done, isolation of HISTOPLASMA CAPSULATUM, NORCARDIA ASTEROIDES, other species of Nocardia, and all species of ACTINOMYCES may be missed. The physician should call the lab before sending any specimens to the laboratory when ACTINOMYCOSIS is suspected, since the latter requires special procedures for isolation.

Skin Scrapings – collect with aseptic technique and send in plain sterile container.

All Other Specimens – please follow the procedures noted above for Tuberculosis (A.F.B.) cultures with the exception that it is not necessary to routinely submit three (3) sputum specimens for respiratory fungus isolation.

Ova and Parasite Examinations: (Sent to Kings County Hospital)

Stool – collect a freshly passed specimen in a plain container or Para Pak collection kit and send to the laboratory. If a delay is unavoidable, place the specimen in a refrigerator.

Malaria – send slides to Hematology laboratory.

Requisition Forms:

All specimens should be properly labeled and accompanied by a properly completed computer generated label. Please contact the lab regarding requests for any special studies or the isolation of a particular pathogen.

BLOOD CULTURE COLLECTION
SITE SELECTION
Select a different body site for each culture drawn.

Avoid drawing blood through indwelling intravascular catheters unless blood cannot be obtained by venipuncture. Blood collected from intravascular catheters should be done with the knowledge that contamination may be an issue.

SITE PREPARATION (ChloraPrep Single Swabstick Applicator)

DO NOT USE:
On children less than 2 months of age because of the potential for excessive skin irritation and increased drug absorption.
On patients with known allergies to chlorohexidine gluconate or isopropal alcohol.

Tear pouch at side notch to reveal applicator handle. Do not touch foam applicator tip. Place foam flat side down on treatment area.

Use repeated back and forth strokes of the swab stick for approximately 30 seconds. Completely wet the treatment area with antiseptic. Allow the area to air dry for approximately 30 seconds. Do not blot or wipe.

DO NOT touch or palpate the area after cleaning.

DISINFECTING BLOOD CULTURE VIALS-
Remove the flip-off caps from BacT/ALERT culture vials.
Wipe top of each vial with a separate 70% isopropyl alcohol pad and allow to dry.
Do not use iodine to disinfect tops of vials.

VENIPUNCTURE
Avoid touching the venipuncture site. If it is necessary to touch the site after it has been cleaned, wipe your fingers with 70% alcohol before touching the site.

When using the Blood Collection Set (“butterfly”) the phlebotomist MUST carefully monitor the volume collected by using the 5 ml graduation marks on the vial label. If the volume is not monitored, the stated maximum amount collected may be exceeded. This condition may adversely create a ‘false’ positive result, due to high blood background.
If using a needle and syringe, typically a 20 ml syringe is used for adults. Draw 16 to 20 ml of blood for one blood culture set (aerobic and anaerobic). Aseptically inject 8 to 10 ml of specimen into each vial.

For pediatric patient:
1) newborns (nursery) – draw 1 to 3 ml of blood or as much as possible and transfer the entire amount into BacT/ALERT Pediatric FAN only;
2) children – draw 1 to 8 ml per venipuncture and split equally between aerobic and anaerobic vials. If less than 3 ml is collected, transfer the entire amount into an aerobic vial only; and
3) if BacT/ALERT Pediatric FAN vial is used, draw 1 to 3 ml of blood and transfer the entire amount into a Pediatric FAN vial.

After all specimens have been collected from the individual, care for the venipuncture site following guidelines recommended by your institution.

The inoculated BacT/ALERT vials should be transported as quickly as possible to the laboratory.

Volume
The volume of blood cultured is critical because the number of organisms per ml of blood in most cases of bacteremia is low, especially if the patient is on antimicrobial therapy. In infants and children, the number of organisms per ml of during bacteremia is higher than adults, so less blood is required for culture.

SPECIMEN LABELING

Each vial should be labeled with the appropriate patient information:

Patient’s name
Hospital number (Patient ID)
Patient’s location (room and bed #)
Date and time of collection
Collector’s initials
Site of venipuncture
Or other information as per facility

Each request slip should also have all the information above.

NUMBER AND TIMING

Most cases of bacteremia are detected using two to three sets of separately collected blood cultures. More than three sets of blood cultures yield little additional information. Conversely, a single blood culture may miss intermittently
occurring bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms.

**COLLECTION OF AUTOPSY SPECIMENS FOR BACTERIOLOGIC EXAMINATION**

The proper collection methods for various autopsy specimens for bacteriologic examination are indicated below. Please note that all specimens must be appropriately labeled and must be accompanied by a properly completed laboratory requisition. Requests for any special studies or for the isolation of a particular pathogen must be so noted on the form. Please call the laboratory when in doubt concerning the procedure to be followed regarding collection of specimens for the isolation of specific pathogens.

<table>
<thead>
<tr>
<th>SPECIMENS</th>
<th>METHOD OF COLLECTION</th>
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<tbody>
<tr>
<td>Blood</td>
<td>Inoculate 5 ml of blood into each of two Blood Culture bottles</td>
</tr>
<tr>
<td>Swab</td>
<td>Insert into Transport Medium</td>
</tr>
<tr>
<td>Tissue</td>
<td>Place in sterile screw-capped container</td>
</tr>
<tr>
<td>Fluids and Exudates</td>
<td>Collect in sterile tube using sterile syringe.</td>
</tr>
</tbody>
</table>

**SPECIAL INSTRUCTIONS**

Please note the following when obtaining autopsy material for bacteriology cultures.

Obtain all cultures as soon as possible after entering the body and before any vessels are ligated.

Sear an area on the surface of the organ to be cultured with a hot thin steel spatula.

Elevate the organ to be cultured preventing contaminated fluids or secretions from flowing across the seared area.

Either lance a small area within the sterile field and pierce the organ with a sterile swab, or remove a 1 cubic centimeter block of tissue, using a separate set of sterile instruments for each biopsy (the exercising of care to prevent contamination while obtaining autopsy cultures is more important a consideration than which of the above two sampling techniques is chosen: swabs or tissue).
Unless otherwise indicated, susceptibility studies need not be performed on bacterial isolates recovered from autopsy specimens. Exception to this policy might include the following: recovery of an unusual isolation or a special request.

When it is determined that a patient has expired after the specimen was submitted for microbiologic examination the laboratory workup of specimen (including indicated susceptibility studies) is to continue as per established laboratory protocol. While it is obviously not necessary to call in the susceptibility results, all laboratory results must be reported.

COLLECTION TECHNIQUES FOR OPTIMAL RECOVERY OF ANAEROBES

Introduction

The role that anaerobes play in human infection has recently become more recognized with the introduction of advanced and improved bacteriologic recovery and characterization techniques. Many of these anaerobic organisms occur as part of the normal flora of the skin, pharynx, intestinal, and genital tracts. Anaerobes demonstrate a relatively low order of virulence and seldom invade healthy tissue; however, these organisms may produce clinical infection in debilitated, traumatized, or immunologically suppressed patients. The anaerobic bacteria most often associated with human infection include the following genera:

- BACTEROIDES AND FUSOBACTERIUM – Gram negative, non-sporulating rods.
- CLOSTRIIDIUM – Gram-positive, sporulating rods.
- PEPTOSTREPTOCOCCUS – Gram-positive cocci in chains.
- PEPTOCOCCUS – Gram-positive cocci in clusters.

Members of the genus Bacteroides are the most frequently involved in anaerobic infections. In addition, it is common to recover multiple species of anaerobes either alone or mixed with aerobic organisms from infectious processes.

Acceptability of Specimens

Acceptable specimens for meaningful anaerobic culture should be devoid of contamination by the normal anaerobic flora of mucocutaneous surfaces. Culture of the latter would be expected to yield normal flora, and thus constitute a wasted effort, and moreover, may present the physician with meaningless and often misleading results. The following table lists those specimens considered to be suitable and unsuitable for anaerobic culture.

<table>
<thead>
<tr>
<th>GENERAL ANAEROBIC CLASS</th>
<th>SUITABLE ANAEROBIC CULTURE</th>
<th>UNSUITABLE CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body fluids</td>
<td>blood, ascitic, bile, bone marrow,</td>
<td></td>
</tr>
</tbody>
</table>
synovial, seminal, prostatic, pericardial, pleural, cerebrospinal

Exudates aspirated pus from deep wound or abscess, pus from superficial wounds or abscesses or “sulfur granules” if present

Genital specimens, placenta, Bartholin’s gland, culdocentesis, vaginal, cervical female endometrial, fallopian tube, septic abortion urethral

Genital specimens, prostatic or seminal fluid urethral

Male Lesions deep wounds and abscesses burns, cysts, and ulcers

Surgical specimens all tissue: appendix, gallbladder, etc.

Respiratory transtracheal aspirate throat, tonsil, nose, nasopharyngeal, expectorated sputum, ear, sinus, tracheal, bronchial

Gastro- Intestinal stool, colostomy ileostomy

Urine suprapubic aspirate voided or catheterized urine

The Bacteriology laboratory cultures and reports anaerobes routinely, new methodologies are being instituted to enhance the recovery of anaerobic organisms. In order to make optimum use of these techniques it is necessary to know the nature of the specimen submitted with respect to anatomic location, and diagnosis if known.

Collection of Specimens
The transport of clinical specimens from the bedside to the laboratory is a critical step in the laboratory’s ability to culture anaerobes. The following collection
techniques are recommended in order to insure optimum recovery of these organisms. 

Body fluids – collect in sterile tube for transport to laboratory.

Pus – pus aspirated from a deep abscess or other aspirated material is best left in the syringe with the needle removed and send to the laboratory immediately. Exudates – collect on cotton swabs and promptly place in transport medium. Please submit two swabs so that adequate material is available for a direct Gram-stained smear. Blood – inoculate 3-5 ml aliquots into each of two blood culture bottles.

ALL SPECIMENS SHOULD BE SENT TO THE LABORATORY AS SOON AS POSSIBLE AFTER COLLECTION.