Collection of AFB (Acid Fast Bacilli) or TB Cultures

What You Will Need

- Sterile leak-proof container with biohazard bag

How to Do It

- Before collection:
  - Properly trained and qualified persons should perform the collection.
  - SWABS ARE UNACCEPTABLE FOR AFB CULTURES
  - Wash your hands carefully and put on gloves.

- Collection of AFB Cultures (refer to other collection procedures as appropriate)
  - Common specimens for AFB culture are:
    - Abscesses, wounds
    - Blood – Special tubes needed for send out to a reference lab - call Specimen Center or Microbiology Laboratory for an SPS yellow top tube. Order an “AFBL”. Draw 5 mls into special yellow top SPS tube and send to PVH Microbiology. Store and transport refrigerated.
    - Body fluids
    - Bronchial Brushes/Washes, Gastric Lavage – send to lab ASAP
    - Sputum - 5-8 cc of a 1st morning specimen is preferred. It is recommended to submit 3 specimens, 1/day or at least 8 hrs apart with at least one 1st morning specimen.
    - Tissue or Skin biopsy
    - Urine (1st morning entire specimen, with a 6 hour bladder retention)
  - Bone marrow aspirates, collected by a physician: Anticoagulants should not be used as they are toxic to microbes. Specimen must be transported immediately.
  - CSF specimens may be ordered under certain circumstances:
    1. The patient is immunocompromised
    2. The patient is not immunocompromised but, 2 or 3 of the following criteria are:
      - Greater than 5 WBCs for CSF cell count.
      - CSF Protein greater than 60ug/dl.
      - CSF Glucose less than ½ of the serum.
    3. AFB smears are not performed on CSF.
  - Stool Specimens may be ordered for AFB on patients diagnosed with human immunodeficiency virus. A screening AFB smear will be performed first, and if positive an AFB culture will follow.

More Details

- Label container with patient name, time, date, your initials, and specific source.
- Add comments for specific AFB history or organism the physician is looking for.
- Specimens should be kept at 2-8°C until and during transport, if only for AFB culture. (Routine aerobic or anaerobic bacterial cultures should not be refrigerated.

Where to Take it

Transport in a biohazard bag immediately to the University of Colorado Health Laboratories.
Collection of Anaerobic Cultures

What You Will Need

- Alcohol and betadine preps
- Sterile syringe, needle and syringe cap (for aspirates)
- Sterile containers for tissue specimens
- Culture swabs with Amies gel transport media, ESwab or special anaerobic transport containers
- Any special supplies requested by physician for special site application

How to do it

- Wash your hands and put on gloves.
- **Specimen choice: Deep Tissue or abscess aspirate, using a needle and syringe.**
  - Collect specimen from the actual site of infection.
  - Specimens should be free of "contamination" with normal flora.
  - Proper decontamination of the specimen site and aseptic technique must be followed.
  - Clinical specimens must be transported to the lab within 15-30 minutes of collection, if not in a holding medium or placed immediately into a holding medium and transported promptly to the laboratory.
  - Promptly transport to the University of Colorado Health Laboratories.
  - Anaerobic cultures will be accepted only on those specimens which have been collected and transported properly.

- **Acceptable Specimens:** (if properly collected and transported)
  - Aspirated pus
  - Tissue (biopsy, surgical, autopsy)
  - Normally sterile body fluids (CSF or shunts, pleural, pericardial, bile, joint, peritoneal, bone marrow, etc.)
  - Lung and transtracheal aspirates
  - Bronchial brushes
  - Sinus aspirates collected by needle and syringe
  - "Sulfur granules" of patients with suspect Actinomycosis, all IUDs
  - Abscesses (closed)
  - Suprapubic aspirate urine
  - Sinus aspiration of draining wounds
  - Deep wounds (pus or drainage)
  - Lesions (excluding cysts or recent burns)
  - Decubitus ulcers - if the encrusted surface is removed to exclude surface contaminants
  - Surgical specimens from sterile sites
  - Placentas (tissue collected at birth - A piece of fetal surface with attached subjacent parenchyma, and a portion of amnion if it was not already attached to the fetal surface)
  - Endometrial specimens obtained with double lumen system, uterus, culdocentesis, fallopian tubes, and ovaries
  - Bartholin’s gland
Unacceptable Specimens:
Material from superficial wound or abscess not collected properly to exclude surface contaminants and:
⇒ Culture tubes without proper transport medium
⇒ Throat/nasopharyngeal/oral cavity/external ear swabs
⇒ Sputum and Bronchoscopy specimens
⇒ Tracheostomy aspirates
⇒ Gastric contents: except in "blind loop"
⇒ Bowel contents, Feces, rectal swab
⇒ Ileostomy, colostomy effluents
⇒ Urine, voided or cath
⇒ Vaginal swabs, lochia, cervical/endocervical, perineal, or urethral swabs
⇒ Prostatic or seminal fluid

Specimen Type:
♦ For skin preparation, perform a surgical soap scrub, followed by application of 70% isopropyl alcohol and Betadine. Remove betadine with alcohol.
⇒ An alternative method is to use an alcohol scrub followed by 10% providone-iodine. Betadine is also acceptable, if allowed to remain on the skin for at least 2 minutes prior to specimen collection.

♦ Aspirates: In general, material is best obtained using a needle and syringe.
⇒ Promptly after collection expel all air from the syringe and needle. The minimum amount of specimen required is 0.5 ml.
⇒ Immediately after expulsion of air, remove the needle and place a sterile cap over the end of the syringe. NEVER TRANSPORT SYRINGES WITH NEEDLES!
⇒ Promptly transport to the lab for processing. The specimen must be processed within 15-30 minutes of collection.
⇒ If unable to transport immediately, inoculate into anaerobic transport media.

NOTE: Plastic syringes gradually admit air and therefore are acceptable only if promptly transported and processed.

♦ Tissues: The preferred specimens, should be kept moist in a sterile container and small amount of saline.
⇒ Small pieces of tissue must be transported to the laboratory within 15 to 30 minutes. NOTE: Though not recommended, if culture swab is used to transport tissue, indicate on requisition and tube, that tissue is in the culture tube so it is not overlooked.
⇒ Large pieces of tissue must be transported in a sterile container to the laboratory within 1 hour.

♦ Swabs: Swabs for collecting anaerobic cultures are not recommended because they may be more likely to be contaminated with skin flora and endogenous or colonizing anaerobes. This type of specimen is accepted only when aspiration is impossible. The E Swab or Amies swab collection system are acceptable, if the specimen is completely covered with the preservative media with no air pockets and received within 6 hours.

Where to Take it
Transport in a biohazard bag immediately to the University of Colorado Health Laboratories.
Collection of Blood Cultures - Venipuncture

What You Will Need

- BacT/Alert culture bottles: **One set =1 aerobic and 1 anaerobic bottle** drawn from the same phlebotomy procedure. **Note: Collect 2 “sets” when ever possible.**
  - Adults:
    - 1 Aerobic (green) and 1 Anaerobic (purple) for each draw site/culture
  - Children (15 years and younger):
    - 1 Pediatric (yellow) bottle if 0.5ml to 4ml of blood is collected
    - or
    - 1 Aerobic (green) bottle if 5ml to 10ml of blood is collected.
- Needles, and 20 ml or larger syringe, and/or butterfly apparatus
- Chlorahexidine, or betadine and sterile alcohol preps
- Disposable gloves
- BacT/Alert Blood Transfer Device – with Male or Female Adapters

**Always draw 2 separate blood cultures (an aerobic and anaerobic bottle - each set), from two different sites, avoiding “line” draws, when possible.**

**Note:** Do not use the blood culture bottles if they are expired, damaged or have been dropped, have cracks or a cloudy medium. The Aerobic (green) bottles and Pediatric (yellow) bottles will normally have black particles.

How to Do It

- Wash hands for at least 20 seconds or use antibacterial hand gel. Put on gloves.
- “Double Identify” the proper patient.
- Apply tourniquet to locate veins, place patient’s arm in downward position, then remove the tourniquet. **Note:** Choose a site below any existing intravenous lines.
- Place bottles on a flat surface and mark on the bottles at the broth line and 10mls above the broth line (each notch on the adult bottles is 5ml). Clean the tops of the bottles with alcohol prep and let dry.
- Apply chlorahexidine (or) in an up and down, back and forth motion. **Note:** Site must be cleaned for at least 30 seconds
- Use betadine if patient has alcohol allergy. Go in a circular motion from inside to the outside for betadine. Be careful to not go over the same area twice. Remove any extra betadine with chlorahexidine cleanser and let dry.
- **Site must dry for at least 30 seconds.** Do not blow or wave on area to accelerate drying.
- Once site has been cleaned, do not re-palpate (this may cause contamination)
**Note:** Proper skin disinfection is critical to minimize contamination of the blood culture.

**Note:** Collection of adequate volumes of blood is the most important factor in blood culture results. The Aerobic (green) bottle is the more important bottle to completely fill up to 10 mls.

- Re-apply the tourniquet and perform venipuncture:
  - If using the butterfly system, fill the Aerobic (green) bottle first, using the BacT/Alert Blood Transfer Device - with Male Adapter, with 10 mls, followed by 10 mls in the Anaerobic (purple) bottle. **Note:** Keep bottles upright. Watch 5ml marks on side of the bottles to fill with proper mount.
  
Do not force the blood into the bottles or over fill the bottles! Watch the fill carefully. The vacuum may draw more than the optimal 10 mls.

  - If using a syringe, draw 20 mls of blood. Remove needle. Attach the BacT/Alert Blood Transfer Device – with Female Adapter, add 10 mls in the Anaerobic (purple) bottle first, making sure to have 10 mls to add to the remaining Aerobic (green) bottle.

**Note:** Do not introduce any air from the syringe into the anaerobic bottle.

  - For pediatric patients, draw between 0.5 to <4 ml for the Pediatric bottle. **Note:** marks on the side of these bottles are for 4 mls.

- What to do if you draw less than 20 mls on adults:
  - If < 4 mls, fill one of the Pediatric bottles.
  - If >=4 to 10 ml, put the entire amount into the Aerobic (green) bottle
  - If 10 - 20 ml, fill the Aerobic (green) bottle with 10 mls (most important bottle) and put the remaining blood in the Anaerobic (purple) bottle.
  - **Note in specimen comments amount of blood drawn.**

<table>
<thead>
<tr>
<th>Order of Inoculation</th>
<th>Total amount of Blood Drawn with Syringe:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 cc</td>
</tr>
<tr>
<td>1. Anaerobic bottle (purple top)</td>
<td>10 cc</td>
</tr>
<tr>
<td>2. Aerobic Bottle (green top)</td>
<td>10 cc</td>
</tr>
<tr>
<td>3. Pediatric bottle (yellow top) only when &lt; 4 cc</td>
<td></td>
</tr>
</tbody>
</table>

- After the draw, apply pressure and an adhesive strip.
- Include on the label: patient name and a second identifier (date of birth or account number), time, date, your initials and draw site.
- Invert the bottle(s) to mix.
- Write on order form in the comment section and in the computer when ordering: the **draw site** (include specific IV type and site also), and **note amount of blood drawn**, if unable to get the proper amount of blood (10 mls per bottle for adult patients).
• Document any suspicion of unusual organisms in specimen comments.

• If one “set” can only be collected from a catheter line draw, which is more prone to contamination, another “set” must be collected by peripheral venipuncture.

• **Wash hands and change gloves before prepping the same or different site for the second set.**

**Where to Take It**

Take bottles to University of Colorado Health Laboratories as soon as possible. (Or within two hours of collection)

**More Details**

• **Pediatric Patients**
  Put entire sample into 1 bottle (up to 4 ml), rather than splitting a small amount into 2 bottles. You may draw as little as 0.5 ml or as much as 4 ml. If you do not have a Pediatric bottle, use an Aerobic (green) one.

• **Size of Draw**
  10 ml is needed in each of the Aerobic and Anaerobic bottles for the best results and recovery of bacteria. Correct volume directly influences recovery of significant isolates.

• **Multiple Cultures**
  Draw specimens from different sites to have the best opportunity to recover bacteria that show up intermittently or in small numbers and rule in or out contamination. Limit to 3 sets of blood cultures per 24 hours, or 4 sets in 48 hours. If more are ordered, there must be approval from a PVHS Lab Pathologist.

• **Other Body Fluids**
  If you draw peritoneal dialysates and ascites, use the same sterile technique at time of collection and place 10ml of the specimens each into the Aerobic and Anaerobic blood collection bottles in addition to sending 50 mls in a sterile container for direct plating. Write the source on the order form and on labels along with time, date and initials.

• **AFB (Acid Fast Bacillus) Blood Culture — Order “AFBL”**
  5mls of blood is drawn into one special SPS yellow top tube provided by PVHS Specimen Center or Microbiology for send out testing to a reference lab. Send back to the PVH Microbiology Lab, ASAP. Refrigerate for storage and transport.

• **Fungal Blood Culture**
  Use the same sterile technique at time of collection and place 10ml of the specimens each into the Aerobic and Anaerobic blood as for routine Blood Cultures. Mention “Looking for Yeast or Fungus” in comments.
Collection of Blood Cultures - From a line

What You Will Need

- BacT/Alert culture bottles: **2 Sets!** *(One set = 1 aerobic and 1 anaerobic bottle drawn from the same phlebotomy procedure).*

- **Always draw a second set of blood cultures (an aerobic and anaerobic bottle - each set), by venipuncture, close to the same time as the line draw.**
  - Adults:
    - 1 Aerobic (green) and 1 Anaerobic (purple) for each draw site/culture = one “set”
  - Children (15 years and younger):
    - 1 Pediatric (yellow) bottle if 0.5ml to 4ml of blood is collected
    - or
    - 1 Aerobic (green) bottle if 5ml to 10ml of blood is collected.

- BacT/Alert Blood Transfer Device – with Female Adapter
- Sterile Syringe, 20cc or larger
- Chlorahexidine and sterile alcohol preps
- Disposable gloves

**Note:** Do not use the blood bottles if they are expired, damaged or have been dropped, have cracks or a cloudy medium. The Aerobic (green) bottles and Pediatric (yellow) bottles will normally have black particles.

How to Do It

- Wash hands. Put on gloves.
- Double ID pt.
- Place bottles on a flat surface and **mark on the bottles at the broth line and 10mls above the broth line** (each notch on the adult bottles is 5ml).
- Clean the tops of the bottles with alcohol prep and let dry.

**For the most “up to date” specific instructions, call the Laboratory.**

- Apply Chlorahexidine or alcohol preps to the lumen. Allow to dry at least 30 seconds.
- Attach sterile syringe to lumen and withdraw 20mls of blood. Detach syringe from lumen.
- Aseptically attach BacT/Alert Blood Transfer Device – with Female adapter to syringe and immediately add proper amount of blood to the bottles:

**Note:** Collection of adequate volumes of blood is the most important factor in blood culture results. The Aerobic (green) bottle is the more important bottle to completely fill up to 10 mls.
Do not force blood into the bottles or over fill the bottles! Watch the fill carefully. The vacuum may draw more than the optimal 10mls.

- Fill the Anaerobic (purple) bottle first up to 10mls, making sure to have 10 ml to add to the remaining Aerobic (green) bottle. Marks on the side of the bottles are 5ml increments.

Note: Do not introduce any air from the syringe into the anaerobic bottle.

- For pediatric patients, draw between 0.5 to <4 ml for the Pediatric (yellow) bottle. Marks on the side of Pediatric bottles are in 4ml increments.

- Invert the bottle(s) to mix.

- Include on the label: patient name, a second identifier (date of birth or account number), time, date, your initials and specific IV type and draw site (ex. PICC line, RT forearm).

- Add in specimen comments when ordering blood cultures in the computer or on a requisition, the specific IV type and site.

- **What to do if you draw less than 20 ml. on adult patients:**
  - If < 4 ml, fill one of the Pediatric bottles.
  - If >= 4 to 10 ml, put the entire amount into the Aerobic (green) bottle.
  - If 10 - 20 ml, fill the Aerobic (green) bottle with 10mls and put the remaining blood in the Anaerobic (purple) bottle.

<table>
<thead>
<tr>
<th>Order of Inoculation</th>
<th>20 cc</th>
<th>11-19 cc</th>
<th>&gt;= 4-10 cc</th>
<th>&lt; 4 cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anaerobic bottle</td>
<td>10 cc</td>
<td>1-9 cc</td>
<td>10 cc</td>
<td>10 cc</td>
</tr>
<tr>
<td>2. Aerobic bottle</td>
<td>10 cc</td>
<td>10 cc</td>
<td>&gt;= 4-10 cc</td>
<td>10 cc</td>
</tr>
<tr>
<td>3. Pediatric bottle</td>
<td>------</td>
<td>--------</td>
<td>10 cc</td>
<td>10 cc</td>
</tr>
<tr>
<td>only when &lt; 4 cc</td>
<td>------</td>
<td>--------</td>
<td>Total amount</td>
<td></td>
</tr>
</tbody>
</table>

**Total Amount of Blood Drawn with Syringe:**

NOTE: Document in specimen comments the amount of blood drawn, if less than 20 ml for adult patients.

- If you suspect unusual organisms, please document in specimen comments.

- **Wash hands and change gloves before prepping for a new draw for a second set.**

**Where to Take bottles:**

Transport in a biohazard bag immediately to the University of Colorado Health Laboratories, as soon as possible. (Preferably within two hours of collection).
Bone Marrow Specimen Collection

What You Will Need

- Physician request for Bone Marrow cultures to include Routine Aerobic, AFB and Fungus Cultures. Include diagnosis in comments on requisition.
- Bone Marrow Collection “tray” from Central Supply
- Sterile syringe or sterile container for culture material

How To Do It

1. Order the Microbiology tests in the computer: Routine Aerobic, AFB and Fungus cultures.
2. Prepare puncture site as for a surgical incision.
3. With sterile technique, obtain an additional 2-10 ml of bone marrow in a syringe, after collecting aspirate smear, clot section, cytogenetics and flow cytometry specimens.
   - Remove needle, and cap tightly with sterile syringe cap OR transfer specimen to a sterile container with a tight fitting lid and no anticoagulants!
   - Label properly with at least two patient identifiers (name and date of birth or account number), specimen type, time and date drawn, and initials of person collecting.
   - Transport specimen at room temperature in a biohazard bag with requisition to University of Colorado Health Laboratories, Microbiology Department ASAP!

More Details

- Anticoagulants are inhibitory and toxic to microbes, therefore, DO NOT USE TUBES with Heparin or EDTA.
- Please put in COMMENTS any unusual organisms which the physician may be suspecting (Ex: *Brucella*, AFB, disseminated fungal infections, etc.), for special handling in Microbiology.
- Bone Marrow cultures will always get a Routine Aerobic Culture, an AFB and a Fungus Culture ordered.

Where to Take it

Transport in a biohazard bag immediately to the University of Colorado Health Laboratories.
**Collection of Chlamydia Trachomatis (with or without GC)**

<table>
<thead>
<tr>
<th><strong>SPECIMEN:</strong></th>
<th><strong>SOURCE:</strong></th>
<th><strong>AGE</strong></th>
<th><strong>METHOD:</strong></th>
<th><strong>COLLECTION DEVICE:</strong></th>
<th><strong>Mnemonic:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>conjunctiva</td>
<td>Any age</td>
<td>FLUORESCENT SMEAR (DFA) – Chlamydia only</td>
<td>Glass slide (Syva)</td>
<td>DICHF</td>
</tr>
<tr>
<td></td>
<td>nasopharyngeal swab</td>
<td>&lt; 6 months</td>
<td>Glass slide (Syva)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>nasal wash /aspirate (infant)</td>
<td>&lt; 6 months</td>
<td>Glass slide (Syva)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rectal swab</td>
<td></td>
<td>Glass slide (Syva)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cervix / urethral, adult female/male</td>
<td>&lt;12 yr. not acceptable</td>
<td>Glass slide (Syva)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GEN-PROBE (Amplified)**

- endocervix- female: Chlamydia/GC, APTIMA Gen-Probe kit, CHGCA
- urethral-male: Chlamydia/GC, APTIMA Gen-Probe kit, CHGCA
- urine-both: Chlamydia/GC, 1st 5-10ml in cup (2-8°C), CHGCA
- eye: newborn, Chlamydia only, Gen-Probe, CHA
- rectal: Chlamydia/GC, Gen-Probe, CHGCP

**CULTURE - Chlamydia**

- body fluids: Refrigerate/Sterile Container
- Swabs – all sources: Viral transport media
- Tissues: Viral transport media

**Unacceptable sources are:** vaginal and cervical - these sites do not have the columnar/cuboidal epithelial cells in which the microorganisms reside.

**What You Will Need**

- For GEN-Probe: (available from Lab Specimen Center)
  APTIMA Combo 2 Gen-Probe kit (universal swab - both male/female)
  Sterile urine container

- For Culture:
  Sterile Dacron, polyester or wire swab
  Viral transport media (refrigerated - available from Microbiology)
  Any supplies needed to collect body fluid- Sterile tube/ no media
How to do it

SPECIMEN COLLECTION:
- **Male Urethral and Endocervical:**
  - See Instruction Sheet that comes with the Aptima Combo kit or also refer to Collection of Gonorrhoeae Specimens
- **Male and Female Urine:**
  - Instruct the patient not to urinate for at least one hour prior to sampling.
  - Collect only the first 5-10 ml of urine voided into a clean urine container. Collection of larger volumes of urine may result in specimen dilution that may reduce test sensitivity. Female patients should not cleanse labial area prior to providing specimen.
  - Transport urine to the lab within 24hrs at 2-8°C for preservation.
- **Conjunctiva:**
  - Pull the lower eyelid down to expose the conjunctiva.
  - Carefully remove any exudate with a moist sterile swab.
  - Using a sterile Dacron swab (moistened) rotate firmly but gently against the conjunctival surface of the lower lid. (REFER TO THE EYE COLLECTION PROCEDURE)
- **Nasopharyngeal:**
  - Nasal washings are necessary for *Chlamydia trachomatis* on infants. (REFER TO THE NASOPHARYNGEAL WASH COLLECTION PROCEDURE)
  - Transport to the lab as soon as possible for the slide needs to be made immediately.

SPECIMEN PREPARATION:
- **For Fluorescent antibody slide:**
  - Prepare the smear according to the kit directions being careful not to make it too thick.
  - Fix it with the ampoule of methanol.
  - Transport to the lab.
- **For APTIMA Combo 2 GEN-Probe (Amplified): Genital sites**
  - Place the swab in the tube and break off at the score line.
  - Replace cap making certain swab fits into cap.
  - Screw on the cap until it snaps into place.
- **For APTIMA Combo 2 GEN-Probe (Amplified): Urine**
  - Refrigerate only first 5-10 ml of voided urine immediately at 2-8°C.
  - Transport urine on ice.
  - Transport to the laboratory as quickly as possible.
- **For culture:**
  - Break off the swab specimen into the viral transport media, and keep at 2-8°C.
  - Store body fluids in sterile containers and keep at 2-8°C.
  - Transport to the laboratory as quickly as possible.

More Details
- Use only standard Chlamydia or GC cultures for suspected cases of abuse.
- Collection is extremely important! The specimen should contain as many columnar epithelial cells as possible.

Where to Take it
Transport in a biohazard bag immediately to the University of Colorado Health Laboratories.
Collection of Clean Catch Urine Specimen

What You Will Need

- Sterile screw-cap specimen cup OR Gray Top Vacutainer C&S (Culture and Susceptibility) Collection Kits
- Antibacterial soap towelette
- Clean gauze for rinsing and drying
- Urine collection bag (U-bag) for infants

How to Do It

Note: The first morning specimen is preferred.
It is important that the patient be given clear instructions as follows:

- Instructions for females
  - Wash hands well
  - Sit on toilet and be able to swing one knee as far to the side as possible.
  - With one hand, spread the skin folds and keep them apart until urine is collected.
  - Using the soap towelette, wash the genital area from front to back.
  - Using damp gauze, wipe front to back three more times. Use each pad only once, then throw away.
  - Using dry gauze, wipe front to back two times.
  - Hold the cup with fingers on the outside; do not touch the rim.
  - Pass the first portion of urine (about 20 ml) into the toilet.
  - Without stopping the flow, catch urine in the cup. Hold the cup so it does not touch the skin.
  - Place lid on the cup carefully and tightly.
  - Write name on the label on the cup.

- Instructions for males
  - Wash hands well.
  - With one hand, pull back the foreskin on the penis.
  - Using the soap towelette, wash the end of the penis.
  - Dry the penis with the clean gauze.
  - Pass the first portion of urine (about 20 ml) into the toilet.
  - Without stopping the flow, catch urine in the cup. Hold the cup so it does not touch the skin.
  - Keep fingers away from rim and inside of the cup.
  - Place lid on cup carefully and tightly.
  - Write name on the label on the cup.
• **Instructions for babies and children**
  - The cleaning process is the same as stated above.
  - Attach the disposable collection bag (U-bag) to the child.
    - Females: make sure the sponge section is over the skin folds (labia)
    - Males: make sure the sponge section is over the penis.
  - Pull apart the plastic bag so urine will flow into it.
  - As soon as possible after collection, carefully remove bag and put into specimen cup; or follow instructions that come with the C&S Collection Kit.
  - Label container with name, time and date collected.

• **All specimens**
  - Label specimen with complete patient name, a second identifier (date of birth or account number), time, date collected.
  - Include source - clean catch or cath or suprapubic on the requisition delivered with the specimen.
  - Make sure the lid is secure and not leaking.
  - **Refrigerate immediately or within 30 minutes!**
  - If specimen is not able to be kept refrigerated or for convenience, follow the directions for preserving the sample with the Grey Top Vacutainer C&S Collection kit, which keeps the urine acceptable for culture up to 48hrs at room temperature.
  - Place in a biohazard bag and seal.
  - Refrigerate or keep cold in wet ice unpreserved urine specimens for storage and transport.

**Where to Take It**

• INPATIENT specimens: Deliver to the University of Colorado Health Laboratories without delay.
• OUTPATIENT specimens: Preserved urine in the Grey Top tube or unpreserved urine specimens (acceptable up to 24 hours, if kept at refrigerated temperatures during storage and transport) are sent to the University of Colorado Health Laboratories.

**More Details**

• Urine shall not be taken from another container such as a bedpan or urinal, nor shall it be brought from home unless proper cleansing will be followed.
• First morning specimens are more concentrated and the significant organism(s) may be in higher numbers and easier to recover.
• To avoid contaminating flora during collection it is extremely important to make sure the patient understands and follows the directions. Poor collection leads to poor results.
• The first portion of the urine flow washed most contaminants from the urethra. The midstream portion represents the bladder flora.
• Samples not collected in the Gray Top C&S Vacutainer or delayed longer than one half hour of collection without being refrigerated will give erroneous results and should not be tested.
• Obtain specimens prior to patient starting antibiotics.
Collection of Ear Cultures

What You Will Need

- Gloves
- Sterile dacron or polyester swab in a culture transport tube with media (E Swab or Amies gel)
  OR
- Sterile swab on a flexible wire shaft and culture transport tube
  OR
- Necessary supplies needed for tympanic (ear drum) puncture, as ordered by a physician.

How to Do It

- Wash your hands and put on gloves.
- Specimen choice:
  - For external ear infection, sample after cleaning the ear canal with disinfectant and rinsing with saline. Sample the canal after several minutes after cleansing by swabbing briskly over any lesions present.
  - If ear drum has ruptured and drainage is present, cleanse ear canal thoroughly with sterile moistened swab. Drainage from the ear canal should be collected only with a smaller wire culture swab, to avoid skin contaminants from the external ear canal.
  - For otitis media infections: The specimen of choice is an aspirate from a tympanic (ear drum) puncture for fluid from the middle ear. A swab is not recommended.
- Preparing the swab:
  - For a child: Bend the wire about 3/4 to 1 inch from the swab end of a wire swab.
  - If an adult: Bend the wire about 1 to 1 1/2 inches from the swab end of a wire swab.
- Insert the swab along the bottom of the ear canal, holding the shaft at the bend.
- When the fingers touch the outer ear, stop, and slightly rotate the swab. Collect fresh drainage.
- Remove swab carefully without touching other surfaces.
- Place swab in culture transport tube and push the swab as far down into the transport media.
- Label culture container with patient name, time, date, your initials and detailed source.

Where to Take It

Take specimens to the University of Colorado Health Laboratories as soon as possible.

More Details

- An external ear culture will not determine the cause of otitis media (middle ear infection) unless there has been a recent rupture of the ear drum.
- If a child has his or her ear tubes removed, fluid retrieved from the drainage tubing can also be collected in a sterile vial or syringe for transport to the laboratory.
Collection of Eye Specimens (Conjunctiva)

What You Will Need

- Sterile saline
- Sterile Dacron or polyester swab in a culture transport tube with appropriate transport medium.
  AND/OR a Sterile swab on a flexible wire shaft.
- OR, Needle and syringe, or other special supplies and proper culture plates needed for aspirates or corneal scrapings collected at the bedside by the physician. **NOTE:** Corneal scrapings submitted for Fungus Cultures, must be collected by a physician and directly inoculated to special media. Call the Microbiology Lab for the special media to be available prior to patient’s appointment. Also refer to Collection of Fungus Cultures within this manual.

How to Do It

Depending on the site of the eye infection, culture both eyes (for comparison) with separate swabs, unless the same infection is the same in both eyes. Specify detailed source: left or right conjunctiva, lid margin, or cornea sample. Obtain specimen before topical medications are applied.

- Wash your hands and follow appropriate barrier precautions.
- Using the sterile swab, obtain any exudate or drainage from the conjunctiva by touching the lower lid and around the lacrimal duct area in the inside corner of the eye. If no exudate is present, pre-moisten the swab with sterile saline, and roll the swab over the conjunctiva and/or lid margins.
- Place swab in culture transport tube and push the swab as far down as possible into the transport media.
- Label specimen completely with specific source information.
- Transport immediately to the laboratory.

**NOTE:** More serious infections, wounds or corneal scrapings for culture or inclusion bodies must be collected by a physician. Refer to specific collection procedures for Wounds, Viral or Chlamydia cultures.

Where to Take It

Transport immediately to the University of Colorado Health Laboratories.
Collection of Fungus Cultures, Fungal Stains and Hair, Skin, and Nail Cultures

What You Will Need

- Sterile saline
- Sterile cotton balls or gauze
- 70% alcohol (isopropyl "rubbing" alcohol)
- Sterile tongue depressor or scalpel blade
- Sterile, leak proof container.

How to Do It

- Before collection
  ♦ Properly trained and qualified persons should do collection of hair, skin and nail cultures!
  ♦ Collect as much specimen as possible.
  ♦ Swabs are discouraged and should not be used if tissues, aspirates, biopsies, skin scrapings, hair, and nails can be obtained. ESwabs or other swabs with Amies media are acceptable for Yeast cultures. **NOTE: SWABS ARE UNACCEPTIBLE FOR AFB CULTURES, or Fungal stains, if also ordered.**
  ♦ Wash your hands carefully and put on gloves.

- Site preparation and collection
  ♦ **Skin scrapings** – Wash affected area with 70% isopropyl alcohol to remove dirt, medication, and contaminating surface bacteria. Using a scalpel blade or tongue depressor, scrape areas at active margin of the lesion, not white inner area. Do not draw blood. Place the scales in a sterile container.

  ♦ **Hair** – A woods lamp may be used to select hairs for culture. Fluorescent, distorted, or fractured hairs should be removed with tweezers. Pluck 10-12 hairs with tweezers, removing the basal portion of the hair or the hair stubs. Infected hairs can be easily removed, but normal hairs are more difficult to dislodge. A comb or brush may be used to collect loose hair and skin squames. On the scalp, the edges of the white patches are likely to contain the best material. Place hairs in a sterile container.

  ♦ **Nails** – Nails should be cleansed with 70% isopropyl alcohol, and the outermost layer should be removed by scraping with a scalpel. Deeper scrapings, debris from under the edges of infected nails and nail clippings from infected areas are also suitable for culture. Collect clippings of nails, especially near the bed of the nail. Place nails in a sterile container.

Routine Fungus Cultures
• Specimens for fungal culture are collected as described for bacterial culture within this manual for:
  Abscesses, wounds
  Blood – use same procedure as for regular bacterial cultures. Mention for yeast or fungus in comments.
  Body fluids
  Eye culture
  Genital specimens
  Skin biopsy
  Sputum - 1st morning specimen preferred
  Tissue
  Urine (1st morning entire specimen with a 6 hour bladder retention)
• A physician collects bone marrow aspirates. Anticoagulants should not be used. Specimen must be transported immediately.
• A physician must collect corneal cultures. It is usually collected at the office using direct inoculation to Blood agar, Chocolate agar, and Sabourauds agar.
• Specimens often submitted for yeast cultures and should be ordered as “CUYE”:
  Mouth or Tongue
  Stool
  Throat
  Vaginal
  • Label container with patient name, time, date, your initials, and specific source.
  • Special stains for fungus
  • Fungal smears are performed by pathology on specimens other than swabs. Submit a pathology requisition.
  • KOH smears are performed in Microbiology, primarily on esophageal, hair, skin, nail, and vaginal specimens.

Where to Take It

Transport in a biohazard bag immediately to the University of Colorado Health Laboratories.

More Details

• Fungal specimens submitted for Histoplasma capsulatitum and Blastomyces dermatitidis survive for only short periods and must be set up immediately.
• Include comments or warnings if suspecting a certain organism, especially Coccidioides immitis!
• Fresh specimens from tissue, aspirates or bone marrow should be received in the laboratory immediately.
• All specimens should be received within 20 hours of collection.
• Hair, skin and nail specimens must be transported at room temperature.
• Sterile body sites and swabs should be stored and transported at room temperature.
• Sites which have the possibility of bacterial contamination should be kept refrigerated.
• Add comments for pertinent history, patient diagnosis or suspected organism.
Collection of Gonorrhoeae Specimens for Culture

What You Will Need

- Sterile cotton balls or Dacron or polyester swabs (for cleaning surface of cervix or urethra)
- E swab or Sterile Dacron or polyester swab with Amies transport culture swab. OR
- Sterile special smaller swab on flexible plastic or wire shaft (for eye and urethra) to transport in an E swab tube or Amies gel media.

How to Do It

Endocervix:
- Wash your hands and follow appropriate barrier precautions.
- The patient is placed in the lithotomy position.
- A speculum is inserted using only water for a lubricant and the cervical os is visualized.
- Remove excess mucus with a cotton ball or Dacron swabs.
- For gonococcal and cervical cultures collected for other procedures, a Dacron or polyester swab is inserted in the distal portion of the endocervical canal, rotated gently and allowed to remain for 10-30 seconds.
- Insert the collection swab into the culture tube and immerse in the transport media.
- Transport the culture swab to the lab within 6 hours.

Urethral:
- Wash your hands and follow appropriate barrier precautions.
- Insert a sterile wire swab into the urethra about 2 cm then gently scrape the mucosa by rotating the swab as it is withdrawn.
- If purulent material is present within the urethra or if other lesions are present, culture as the second site, in addition to the endocervix (in females).
- Insert the special smaller collection swab (flexible plastic or a wire swab) into the culture tube and immerse in the transport media.
- Transport the culture swab in E swab media or Amies gel media to the lab within 6 hours.

Miscellaneous:
- Rectal swab: Can be obtained without an anoscope by inserting a Dacron swab approximately one inch into the anal canal. Move the swab from side to side to sample the crypts and left for 10-30 seconds to allow absorption of the organisms onto the swab. If feces are present on the swab, discard it and try again. Transport the swab the same as described above for endocervix and urethra.
- Urine: Refer to COLLECTION OF CHLAMYDIA TRACHOMATIS PROCEDURE and order as a Chlamydia/GC amplified Probe test.
- Ocular specimen (newborns): Refer to EYE COLLECTION PROCEDURE, note in comments as a special request to look for G.C. Order as a routine culture (CURO)
Where to Take It

Transport to the University of Colorado Health Laboratories ASAP or same day of collection.

More Details

- The preferred sites for culturing *Neisseria gonorrhoeae* are the endocervix in females and the urethral meatus in males. G.C. screens may be performed on specimens from the eye, throat or rectum, when specifically requested.

- Joint Fluid cultures are held 3 days to include recovery of G.C., but please note in comments if there is a suspicion of G.C.

- Collect only exudate and scrapings of material and cells from the above mentioned selected sites. VAGINAL SPECIMENS ARE NOT ACCEPTABLE!

- **NOTE: It is important to obtain cells from the proper infected areas.**

- G.C. culture is still the Gold Standard for legal purposes (assault or abuse cases).

- If DNA probe testing is requested, refer to the Chlamydia/GC Collection section of this manual.

- Urine specimens are only recommended for the Amplified Probe testing and not for culture.

- Appropriate culture swabs are available from the University of Colorado Health Laboratories.
Collection of Herpes Simplex (HSV) Culture Specimens

What You Will Need

- **Universal Viral Transport Media**, M4, M5 or any other liquid Viral Transport Medium – Conical tube with pink fluid (tissue culture based intended to stabilize viruses during transport, controlling mycotic and bacterial overgrowth). Available from the laboratory. Store at 2-25 °C  **Note: Do not use if cloudy, yellow or expired.**
- Sterile Dacron swab.  **Do not use calcium alginate wire swabs or wooden shafted swabs.**
- Sterile syringe/needle, if aspirate is to be obtained.
- Biohazard bag
- Refrigerator or wet ice

How to Do It

- **Special specimen collection requirements:**
  Proper collection and handling of patient specimens are the most important factors in successful HSV detection.
  - Personnel should be trained in collection and culture techniques and follow appropriate barrier precautions.
  - Avoid the production of aerosols during collection.
  - Collect the specimen during peak infectivity, ie. acute phase.
  - Collect in a manner which ensures greatest amount of virus.
  - Avoid the collection of patient blood, (blood can inhibit the growth of the virus and the cell culture), and contamination from adjacent areas.
  - Avoid creams, lotions, ointments, alcohol, Betadine, or a recent sitz bath which may reduce viable virus yield significantly, prior to collection, or report to the physician when the site is sampled.
  - Label specimens properly with the patient’s name and a second identifier (date of birth or acct.#), time, date and detailed source information.
  - Complete the requisition to include detailed source and proper test request.
  - **Store all specimens for viral culture in a biohazard bag refrigerated at 2-8° C!**

- **Procedure for Specific Specimen Sites:**
  - Lesions and/or vesicle fluid:
    - If possible, aspirate vesicle fluid with a syringe with a 26-27 gauge needle then expel into transport media. For ulcerated lesions, use a sterile cotton or Dacron swab to remove and discard pus without disrupting the lesion base, Use a fresh sterile collection swab dipped in viral transport medium to vigorously swab the lesion base to obtain cells. Break off swab into transport media. Crusted lesions should have the crust removed and discarded by lifting the crust from the lesion with a sterile needle, then collected as an ulcerated lesion.
  - Amniotic fluid/Body fluid:
    - Submit the entire specimen in sterile tube(s) for optimal concentration.
  - CSF : **Order HSV PCR instead!**
    - (In the case of encephalitis when a biopsy is not available) collect 2 ml from adults and 1 ml of CSF from infants and children, if possible. Submit CSF specimen ASAP.
◆ **Eye exudates:**
  ⇒ Rub palpebral conjunctiva with sterile cotton or Dacron swab and immediately break off into viral transport medium. Refer to Eye Specimen Collection Procedure.

◆ **Tissue/biopsy specimens:**
  ⇒ Submit entire specimen in sterile container to the lab as soon as possible.

◆ **Newborn cultures (asymptomatic children/mothers with lesions):**
  ⇒ Collection must be done as soon after birth as possible before bathing. The specimens may be pooled to save cost if necessary using pre-moistened cotton or Dacron swabs on the following acceptable sites:
  - Conjunctiva
  - Mouth
  - Nasal
  - Throat or NP
  - Rectal
  - Deep surface (ear or skin fold) - last choice

◆ **Sputum:**
  ⇒ Mix well and pipet ~5 drops of a representative portion of the specimen, or use a swab to inoculate the tube of transport medium.

◆ **Blood:** (not recommended)
  ⇒ Use heparinized tubes for collection of blood. Keep tubes cold (2-8°C) and transport within one hour of collection. (tubes will be centrifuged and the buffy coat will be used to inoculate the culture).

- **Important notes about the Transport medium:**
  ⇒ The transport medium must be red to pink. If yellow or expired, DO NOT USE!! Obtain a new collection kit from Microbiology.
  ⇒ To preserve infectivity of virus, place swabs and small amounts of aspirate (not CSF) **immediately** into the Universal Viral Transport Medium provided by the University of Colorado Health Laboratories.
  ⇒ **Store at 2-8°C** for best recovery within 48 hrs., or at or below -70°C for longer storage.
  ⇒ The Universal Viral Transport Media is also acceptable for PCR testing.

**Where to Take It**

**Refrigerate or keep on wet ice at 2-8°C** and transport to University of Colorado Health Laboratories.
Collection of Nasal Screening for MRSA (Methicillin Resistant Staph aureus)

What You Will Need
- Orders for MRSA nasal surveillance screening
- Copan Transystem Transport swabs (2), red cap – for Rapid PCR testing
  Or
- E swab or BBL culturette containing dual swabs in Amies gel media, blue cap – for culture

How to Do It

Note: Verify the orders for MRSA screening only - If a culture is wanted to recover other organisms also, order a routine aerobic culture (CURO).

- On inpatients only - Order STAT, “MRSAPCRSC” for the rapid PCR method.
- On outpatients - Order “MRSASC” for the screen by culture method.

NOTE: This is not the same as an NP collection. Do not go up into the sinus cavity, just collect from the anterior nares.

- Wash hands. Put on gloves and follow other appropriate barrier precautions.
- Remove excess drainage from nose with a tissue. Ask the patient to tilt his/her head back.
- Insert dry swabs approximately 1-2 cm into the nostril.
- Rotate the swabs against the inside of the nostril for 3 seconds. Apply slight pressure with a finger on the outside of the nose to help assure good contact between the swab and the inside of the nose.
- Using the same swab, repeat for the second nostril.
- Remove the plastic transport tube. Twist off the tube cap and discard it. Place the swabs into the plastic tube. The swabs should go all the way into the tube until they rest on the top of the sponge at the bottom of the tube.
- Make sure the red cap is on tightly.
- Label the plastic transport tube with two forms of identification (full patient name and either a birthdate or account number), and the time/ date of collection and your initials.

Where to Take It
- Store and transport the swab at room temperature (specimen is good for 24 hours), or store swab at 2-8°C for up to 5 days.
- University of Colorado Health Laboratories.

More Details
- The PCR surveillance test is to determine carrier states of patients at high risk for MRSA infection.
- The PCR test is for patients who need to be screened when entering the hospital and a STAT result is needed, for weekly testing in ICU or before being transferred into another facility.
- Refer to the Poster from Cepheid for how to collect the nose PCR test on the next page.
Xpert™ MRSA Specimen Collection Protocol

1. A Cosm Vesturi Transyster double-swab must be used to collect the specimen.
   Cepheid Collection Device 8060-0070

2. Insert the dry swabs 3-2 cm into the nostril and rotate swabs against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nose.

3. Repeat Step 2 in second nostril with the same swabs.

4. Place the swabs back into the tube.

5. Specimens that can be tested within 24 hours can be kept at room temperature. If not, it is recommended that they be refrigerated. Specimens stored at 2-8°C are stable for up to 4 days.

For Technical Assistance please contact: Cepheid Technical Support 888-838-3222, Option 2
Collection of Nasopharyngeal Swabs

What You Will Need

- For Influenza A/B, RSV or other respiratory virus testing – (Note: Do not use calcium alginate swabs.)
  - Rapid Influenza or RSV Immunoassay (RIA): Nylon fiber flocked NP sterile swab with a flexible plastic shaft with 1ml of Universal (viral) transport media (UTM)
  - Influenza PCR testing (FLUPCR): Flocked NP sterile swab with 3ml of UTM
  - Biohazard bag, refrigerator and/or ice for storage and transport.
  - Gloves, gown, and mask for specimen collection

- For routine NP cultures -
  - Nylon fiber flocked NP sterile swab or Dacron or rayon-tipped NP wire swab preserved in E Swab culture media, Amies or Stuarts media.

Note: Specimens for some respiratory viral cultures, and Pertussis require nasal washings. Washings are collected by trained personnel at University of Colorado Health Laboratories.

How to Do It (Refer to separate How to…poster for Nasopharyngeal Swab)

- This procedure must be done ONLY by trained personnel.
- Wash hands. Put on gloves, gown, and mask.
  - Remove excess drainage from the nose with a tissue.
  - Have another person hold the patient’s head securely, if necessary. Insert the swab on the inside edge of the nostril until it stops. (The swab is at the back of nasal cavity.) Do not touch the swab below the perforated breaking point.
  - Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx. (Refer to separate poster for picture of Nasopharyngeal Swab)
  - Rotate the swab several times and remove from nostril.
  - Label container with patient’s name and birthdate, source, date/time and initials.

- For Influenzae A/B, RSV or other respiratory viruses:
  - Insert the swab into proper UTM for tests ordered (For RIA only: dry swabs in paper wrapper, culture tube or in saline are also acceptable if performed within 8 hrs)
  - REFRIGERATE (2-8°C) immediately and during storage and transport.
  - Transport specimens ASAP to the Lab in a biohazard bag.
  - Influenza testing can be performed up to 48 hrs for RIA or 72 hrs for PCR if NP swab is stored in UTM at 2-8°C.

- For routine NP cultures:
  - Place swab in culture media to keep moist. Transport at room temperature in a biohazard bag to the Microbiology Lab within 6 hrs.

Where to Take It
University of Colorado Health Laboratories.
Collection of Nasal Wash/Aspirate for Viral Tests and Viral Cultures

What You Will Need (for those collections performed at University of Colorado Health Laboratories.)

- K-66 tubing for machine
- #8 or #5/6 suction catheter
- Luki tube
- Suction machine (table top or wall mounted)
- gloves
- goggles
- Mask
- sterile saline vial

How to Do It

- This procedure must only be performed by trained personnel
- Double identify
- Explain procedure to patient or family
- Wash hands
- Put on gloves, goggles, gown, and mask
- Position patient lying down- Have someone help restrain patient if necessary by holding his/her arms up next to his/her head
- Assemble equipment
- Connect to suction machine:
  - K-66 tubing
  - Luki tube
  - #8 or #5/6 suction catheter

  Calibrate suction machine based on age: NOTE It is VERY important to have your thumb over the catheter hole while calibrating to ensure that the suction is not set too high!

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Table top Calibration</th>
<th>Wall Mount Calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>Birth to 44 weeks</td>
<td>6-8 cmHg</td>
<td>60-80 mmHg</td>
</tr>
<tr>
<td>Pediatric</td>
<td>45 weeks to 12 years</td>
<td>8-10 cmHg</td>
<td>80-100 mmHg</td>
</tr>
<tr>
<td>Adult</td>
<td>13 years and older</td>
<td>10-12 cmHg</td>
<td>100-120 mmHg</td>
</tr>
</tbody>
</table>

- Instill 3-6 drops of saline into both nostrils
- With your thumb off of the catheter opening, thread the catheter into the nostril (until resistance is met), plug the suction opening with your thumb and rotate in a circular motion, letting your thumb on and off of the suction
- Take your thumb off the suction opening and then remove the catheter out of the nostril
- Repeat step above in other nostril until 0.5mLs of aspirate are obtained for each test ordered
- If needed, rinse suction tubing with a small amount of saline being careful not to make specimen too dilute. A small amount of blood in the specimen presents no problem
- Separate the Luki tube from the catheter and connecting tube. Close its connecting end pieces together. Use Parafilm, if needed, to keep tubing connections closed
- Label specimen
- Dispose of used equipment in a red isolation bag for proper disposal

Where to Take It
Take it to the University of Colorado Health Laboratories
Collection of Perinatal Strep Cultures

**Note:** CDC 2010 Guidelines recommend universal prenatal screening of vaginal / rectal specimens for Group B Strep colonization of all pregnant women at 35-37 weeks gestation.

**What You Will Need**

- Sterile Dacron, polyester swabs or ESwab with transport media.

**How to Do It**

- Wash your hands and follow appropriate barrier precautions.
- Place the patient in the lithotomy position.
- **NOTE:** A speculum should not be used, as it may push organisms up into the vagina.
- Swab the lower vagina (vaginal introitus), followed by the rectum (insert swab through the anal sphincter) using the same swab or two different swabs. Cervical cultures are not appropriate.
- Place swab(s) into culture tube moistened with transport media. Vaginal and rectal swabs, if collected separately, should be placed in the same transport medium and handled as one culture.
- Label specimen clearly for a Perinatal Strep Screen (PSS) for Group B Strep.
- Fill out a Requisition properly to accompany specimen.

**Note:** There is a line on the PVHS Microbiology requisition to check if the patient is penicillin allergic and the physician needs the lab to perform special susceptibility testing (for erythromycin and clindamycin) on positive cultures. OR - Please add in comments requests to perform susceptibilities, if culture is positive.

- Transport to the lab on same day of collection.

**Where to Take It**
Take it to the University of Colorado Health Laboratories.
Collection of Sputum

What You Will Need
- Sterile specimen cup, wide mouth
- OR sterile suction trap with tubing (if suctioning a sample)

Note: Blood cultures, not sputum cultures are the best for diagnosing bacterial pneumonia.

How to Do It
- Give clear instructions to the patient.
- The patient must rinse out his/her mouth vigorously with water. NO MOUTHWASH.
- Remove dentures.
- The patient must collect the sputum from a deep cough in the sterile cup. The first morning specimen is the best.
  - If necessary, explain the difference between “spit” (saliva) and sputum.
  - If the sputum is suctioned, follow the nursing policy for collection.
  - Once sputum is collected, screw on lid tightly and label with patient name and a second identifier (birth date or acct.#), time, date, your initials and source.

Where to Take It
Take specimen to the University of Colorado Health Laboratories within two hours.
Note: Some significant bacteria may die within minutes.

More Details
- Specimen evaluation
  ⇒ A Gram Stain is done on all sputa to determine if the samples are acceptable for culture.

- Repeat Collections
  ⇒ If a sample is unacceptable with oral contamination (saliva), the lab will call the patient provider or nursing floor to request a repeat specimen.
  ⇒ If a repeat collection is not good, a sample may need to be collected by Respiratory Therapy.
  ⇒ If no acceptable samples can be obtained after 3 tries, the doctor should be notified by nursing staff for further instructions.

- Patient History/Diagnosis
  ⇒ Comment on the order if a patient has chronic bronchitis or has a suppressed immune system (e.g. AIDS, cancer), otherwise samples may appear as saliva on a Gram Stain and be rejected for culture. Make note of Cystic Fibrosis patients as special cultures are required for these patients.

- Acid Fast Cultures (Tuberculosis)
  For Acid-Fast (tuberculosis) cultures three or more consecutive samples are recommended, collected at least 8 hours apart, with at least one being a 1st morning specimen.
Collection of a Stool Sample

Before Collection

Some medicines affect test results. Consult with the physician and before collecting an unacceptable sample, wait 10 days before collecting a stool specimen after cessation of the following:

- Antibiotics
- Antidiarrheals (Ex. Kapectate, Peptobismol, etc.)
- Antacids (Ex. Rolaids, Maalox, etc.)
- Oily laxatives
- Suppositories
- Recent barium X-ray test

**NO “ROCK” HARD STOOL SPECIMENS WILL BE ACCEPTED for O&P or Stool Cultures. NO FORMED STOOL SPECIMENS WILL BE ACCEPTED FOR Clostridium difficile testing.**

INPATIENTS: If patient has been in the hospital longer than three days and develops diarrhea, request a *Clostridium difficile* test, not a culture or O&P. Only one *C. difficile* PCR test is needed to assess for *C. difficile* infection.

NOTE: No more than one same test order per day (~24 hrs part) will be accepted on stool specimens.
Up to three samples collected over a 10 day period is recommended for multiple O&P orders.

What You Will Need

- **Patient instructions (Stool Collection Instructions), special collection devices, containers and preservative kits are available at all Poudre Valley Health System Laboratories.**
- **For Culture, *C. difficile*, Ova and Parasites, Giardia/Cryptosporidium:**

  - **OUTPATIENTS:**
    - Check orders to include the proper collection containers.
    - Occult Blood testing requires the patient to use the Hemoccult cards (follow special patient instructions that come with the cards)
    - *C. difficile* testing must be collected as unpreserved, fresh, **unformed (must conform to the shape of the container)** stool only. Store and transport at 2-8°C.
    - Preservative collection kits are greatly encouraged for convenience and better recovery when testing for Culture or Ova and Parasites.
      - Orange top C&S vial: Used for Bacterial Culture.
      - PARAPAK Pink top Formalin and Grey top ZN-PVA vials: Used for Ova and Parasites, and just the Pink top Formalin vial for the Giardia/Cryptosporidium Ag.

  **NOTE:** Approximately one tablespoon of stool is needed to fill each vial up to the red line.

  - **INPATIENTS:** (or if no preservative kits are available)
    - Transport fresh stool specimens for Culture and O&P to the Lab within 30 minutes!
    - Culture and O&P tests should not be performed on patients in the hospital >72hrs. Consider one *C. difficile* PCR test instead – submit fresh, **unformed** stool, order as **Urgent**, for the purpose of printing.
• For other stool tests:
  ♦ INPATIENTS and OUTPATIENTS:
    ⇒ A fresh sample is needed for the following tests. Collect the sample in a clean, dry container and bring to the lab within 30 minutes or keep refrigerated. Approximately one tablespoon of stool for is needed for each test: 
    Rotavirus, Qualitative Fat or Reducing Substances.

How to Do It (Collect and Preserve Stool specimens in Preservative kits)

• Collect stool in a clean, dry container, or on plastic wrap stretched across the toilet.
• Make sure the sample does not mix with water or urine.
• Fresh specimens require 1-2 tablespoons of stool for each test ordered.
• Fill each collection tube (if used) so the sample raises the level of the liquid to the red line.
• When using the kits, include bloody or liquid portions of stool sample.
• Mix the collection tubes well (separate bowel movements may be combined to fill to line).
• Mark the box on the collection tube which best describes the sample appearance or shape.
• Make sure all containers are labeled properly with patient name, birth date, date and time of collection.

Where to Take It

Transport to University of Colorado Health Laboratories within 30 minutes if fresh, or within 72 hours if in special collection kits (refer to special specimen requirements for C. difficile testing).

More Details

• Diapers are not accepted! If diapers are used to collect a stool specimen: Line diaper with plastic wrap so stool does not soak into or contact the diaper and transfer the specimen to clean dry well sealed container.
  OR
  Use a urine collection bag to collect the stool sample (this will also keep out urine).
• If there are multiple orders for the same test(s), wait 24 to 48 hours between stool sample collections for results of the first samples as well as to allow for better recovery of pathogens from different samples.
• Clostridium difficile testing must be performed on fresh, liquid or soft stool specimens only. Specimens are acceptable up to 5 days from the time of collection, if kept refrigerated. (Specimens may also be frozen if longer storage/transport time is needed).
  Only one test for C. difficile by PCR is needed. If new diarrheal illness occurs more than 7 days after a prior negative test a single repeat specimen may be indicated. Testing again within a few weeks after a positive result should not be used as “Test of Cure”.

Patient History/Diagnosis

• If there is a recent history of travel to coastal areas or a foreign country, include this information in comments on the requisition.
• Specify antibiotics and/or chemotherapy treatments.
• Note in comments, if patient has had bloody diarrhea in past few days.
Write special requests for specific pathogens such as Yersinia, Vibrio, Cryptosporidium, and other unusual parasites on the requisition.
Collection of Throat Swabs

What you will need

- Double swabs in **Red-capped** Liquid Stuarts media culture tube for a **Rapid Strep with Strep Culture backup**. (Dry swabs are also accepted.)
- OR - 1 or 2 swabs or ESwar with or without culture medium if for **Strep culture only**
  - Sterile tongue depressor
  - Surgical Mask
  - Special cultures require special media and handling: Viral cultures, *Neisseria gonorrhoeae*, Diphtheria, and Pertussis (see Special Cultures). Refer to separate collection procedures and/or call the University of Colorado Health Laboratories for instructions.

How to do it

- Instruct the patient to tilt the head back, take a deep breath and say “AH” to visualize the back of the throat. **Note:** Put on the surgical mask at this time for protection from aerosols.
- Look for any areas of redness, swelling, pus pockets, or membrane formation and make sure to include those areas when swabbing.
- Place gentle pressure on the tongue with a tongue depressor so the swab will not touch the tongue. (Tongue bacteria may interfere with testing.)
- Have the patient say “AH” again, which helps lift the uvula and prevents gagging.
- Guide the swab(s) to the back of the throat (posterior pharynx) while not touching the tongue, uvula or other parts of the inside of the mouth.
- Swab in a gentle figure-eight sweeping motion, touching all areas of the mucosa behind the uvula and between the tonsillar pillars and any inflamed or exudative areas.
- Carefully remove the swab, avoiding contact with the tongue or other parts of the mouth.
- Place the swab(s) into the sterile swab container appropriate for the tests ordered.
- Label culture tube with patient name, date, time, your initials and source.

Where to Take It

Send specimens to the University of Colorado Health Laboratories, soon as possible.
More Details

- If a Rapid strep test is negative, a reflex Strep Throat Culture, looking for Group A Beta Strep is standard practice and will be ordered automatically unless requested otherwise.
- **Routine throat cultures are not accepted.** Order only a Rapid Strep Screen (RSS) or Strep Culture (CUSS) for patients with pharyngitis and looking for Group A Beta strep.

Special cultures

- For viral cultures, refer to Collection of Viral Cultures Procedure.
- Diphtheria and *N. gonorrhoeae* (GC, or gonorrhea) are collected as above but with different swabs with transport media. Contact PVH Microbiology for special instructions and swabs.
- Diphtheria requests should also include a nasal culture and are a send-out test.
- Whooping cough (*Bordetella pertussis*) requires a nasal wash and can be collected at University of Colorado Health Laboratories during normal business hours.
- Order a Yeast Culture (CUYE) if looking for Candida, also called (Thrush or Monilia).
Collection of Vaginal Specimens for KOH, *Trichomonas* and Bacterial vaginosis

What You Will Need
- Speculum without lubricant
- Blue capped Culturette swabs with Amies gel media, or
- Sterile saline (~1ml) in a tube and plastic pipette to collect secretions

How to Do It
- Wash your hands and follow appropriate barrier precautions.
- Place the patient in the lithotomy position and use a speculum to open the vaginal canal.
- Collect secretions with a pipette or swab of the mucosa high in the vaginal canal.
- Place swab into culture tube moistened with transport media, or add secretions collected with a pipette into a tube with a small amount of saline.
- Label specimen clearly with all patient information (at least 2 identifiers), the time and date of collection and your initials.
- Fill out a Requisition or order in the computer properly with tests to be ordered and to accompany specimen. Order "KOH" for yeast, "TRICH" for a *Trichomonas* prep, and/or a Gram stain, “SMGR” for Bacterial vaginosis for each individually, OR “WET PREP” for all three tests.
- Transport to the lab immediately for best recovery of motile *Trichomonas* parasites.

Where to Take It
Take it to the University of Colorado Health Laboratories.

More Details
- Follow separate procedure specifically for Gonorrhoeae Specimens for Culture or *Chlamydia Trachomatis*.
- Vaginal cultures are difficult to interpret, of little value and will not be accepted.
- A yeast culture maybe be ordered as “CUYE”
- Wounds or abscesses should be ordered as such with detailed specimen descriptions and will be processed as routine aerobic cultures.

Specimen evaluation
- Specimens are examined for motile *Trichomonas* by wet prep, yeast by KOH prep, and characteristic clue cells and bacteria of Bacterial vaginosis by performing a Gram stain.
- Yeast cultures are examined and held three days.
Collection of Viral Cultures

What You Will Need

- Sterile, screw-top container OR Universal Viral Transport Media
- Needle and Syringe
- Dacron swab (if used) DO NOT USE calcium alginate wire or wooden shaft swabs.
- Biohazard bag
- Refrigerator or wet ice
- Orders and/or requisition with source and what type of virus(s) to be tested for.

NOTE: Viral Specimens should be stored at refrigerator temperature (2-8°C) or on wet ice within 1 hour of collection and during transport. Do Not Freeze!

How to Do It

- **Biopsy Specimen (brain, liver, lung):**
  - Fixed-tissue or specimens in Formalin are not acceptable.
  - Collect fresh tissue from appropriate site using a separate sterile instrument to cut or remove each sample. Each specimen need not be more than 1-2 cm in diameter.
  - Place each sample into an individual sterile, screw-top container, with a very small amount of sterile saline or tissue on moist gauze (so that specimens will not dry out, if bacterial cultures are also ordered*. OR-
  - If orders are just for Viral cultures, Place in Universal Viral Transport Media.

- **Blood**
  - Draw 10 ml of whole blood (minimum 3 ml) in EDTA (purple) tube, for CMV only.
  - Draw 10 ml of non-anticoagulated blood for most other viruses, as serum is preferred.

  Note: If for PCR testing, serum or plasma (heparin or EDTA) is needed, ½ cc per virus. Except, 2cc for Enterovirus is needed.

- **Cerebrospinal fluid or Body fluids:**
  - Aseptically collect 2 ml (minimum 0.5 ml) in a sterile tube.

  NOTE: IT IS RECOMMENDED THAT THIS BE A TUBE SEPARATE FROM THOSE USED FOR OTHER LABORATORY TESTS, (so that it can be kept refrigerated).

- **Cutaneous/Vesicular lesion (most common for Herpes Simplex Virus):**
  - If present, wash vesicle with sterile saline and aspirate fluid with a tuberculin syringe.
  - Place fluid in sterile, screw-topped container.
  - If a swab must be used for collection, place in Universal Viral Transport Media.
  - If bacterial cultures are also ordered, a separate swab must be collected with appropriate preservation.*

- **Respiratory Aspirate: Refer to Collection of Nasal wash/aspirate procedure, if needed.**
  - Collect aspirates from the nose, nasopharynx and oropharynx.
- Send in a sterile, leak proof screw-top container, kept refrigerated for transport.

- **Sputum: Refer to Collection of Sputum, if needed.**
  - Obtain specimen in a sterile leak proof container.

- **Stool/Rectal Swab:**
  - Insert Dacron swab(s) at least three (3) centimeters into anal opening; rotate to obtain feces on swab.
  - Place swab(s) in **Universal Viral Transport Media**.

- **Throat swab: Refer to Throat Swab Collection, if needed.**
  - Using a Dacron or rayon swab, rotate the swab on the tonsil crypts and on the back of the throat (5 seconds for each area is ideal). See also Collection of Throat Swabs.
  - Place swab in **Universal Viral Transport Media**.

- **Urethral swab:** (Refer to Chlamydia or Gonorrhea Collection, if needed.)
  - Insert the swab at least 2 cm into the urethral opening and rotate gently to obtain epithelial cells.
  - Place swab in Universal Viral Transport Media.

- **Urine:** Refer to Clean-Catch Urine Collection, if needed.
  - Obtain 2 ml (minimum 0.5 ml) fresh, clean-catch specimen in a sterile container.

**Label specimens with patient name, date, time, your initials, and source.**

**Keep at refrigerated temperatures for storage and during transport.**

**Where to Take It**

Transport in a biohazard bag immediately to the University of Colorado Health Laboratories.

**More Details**

- Make sure screw-tops are on tight and correctly so that there is no leakage.
- All specimens must be stored in individual biohazard bags.
- Virus samples should always be kept at **2-8°C**, for best recovery.
- Viral cultures must be clearly identified so they are not mixed in with other specimens.
- The laboratory cannot process specimens which are not collected, stored, and transported properly.

*For Bacterial culture requirements or other special tests, refer to other sections in Microbiology Specimen Collection.*
Collection of Wounds and Abscesses

Background
- The use of aseptic technique in specimen collection and meticulous decontamination of skin are critical for accurate results.
- The culture will include a gram stain, to access the quality of the specimen.
- **Recommended: Collect as much fluid, pus, or tissue as possible.**
- Swabs SHOULD NOT be used if fluid, aspirates or tissue can be obtained
- Refer also to the “Collection of Anaerobic Cultures” in this manual for more details.

What You Will Need
- **Skin cleanser:**
  - Isopropyl alcohol and providone-iodine (betadine) swabs or
  - Chlorhexidine gluconate swabs or
  - Where mucus membranes are involved, a mild, approved antiseptic

- **Collection Container:**
  - Sterile needle and syringe with cap (fluids) or
  - Sterile container, gauze and normal saline (tissue specimens) or
  - If not possible to collect fluid or tissue, use: **E Swab**, flocked swab with Amies liquid – for aerobes, anaerobes and fastidious organisms.  
    Or the BBL CultureSwab Plus double swabs with Amies gel medium is also acceptable
  - **Sterile gloves**

How to Do It

- **Site Preparation and specimen collection:**
  - Wash your hands carefully and put on gloves.

**Intact skin over undrained abscess**
- Decontaminate the skin starting at the collection point, moving in a spiral outward.
  - If using betadine and alcohol, apply betadine first then remove with alcohol.
  - Allow each cleanser to dry before proceeding.
- After collecting sample, remove needle, purge air and cap syringe.

**Wound beds or sinus tract**
- Remove bio-burden and surface slough to expose tissues more likely to contain active pathogens and less likely to contain normal flora.
- Rinse collection area with sterile normal saline and sharply remove specimen.
- For tracts, sample with curette as close to the base of the tract as possible.
- Place specimens on sterile saline moistened gauze to keep them from drying and seal.
- If a swab must be used, the specimen should be taken from the advancing margin of the lesion, and **not** just pus or exudate. Sample the lesion margin and abscess walls **firmly** with the swab avoiding **any** contact with intact skin.
What to Document

- Label container with:
  - Patient name and a second identifier (birth date or acct#)
  - Time, date, your initials and the specific anatomic site.
- On the requisition:
  - Specify either “surface” or “deep” wound, and be as specific as possible about the specimen. Include the diagnosis, and information such as post-surgical, past infections or looking for specific pathogens.
  - Specify if the patient has an important history of exposure to animals or other sources of disease.
  - Note if patient is on antibiotics, and which one(s).

Where to Take It

- Transport specimens to the University of Colorado Health Laboratories immediately.
- Call for STAT pick-ups for unpreserved specimens (not in any holding media), especially if anaerobes are ordered.

Refer also to

- Collection of Anaerobic Cultures
- Microbiology Specimen Collection
- Microbiology Specimen Time Limits