

## Microbiology

### General Guidelines for Proper Specimen Collection (See also: Collection of Specimens for Viral Testing)

- ◆ Collect specimen before administering antibiotics or antivirals when possible.
- ◆ Collect specimen with as little skin contamination as possible to ensure that the sample collected represents the infected site.
- ◆ Utilize appropriate collection devices. Use sterile equipment and aseptic technique to collect specimens to prevent introduction of microorganisms during invasive procedures.
- ◆ Clearly label the specimen container with patient's name, hospital number or other identifying number (i.e. birth date, requisition number), date, and time of collection.
- ◆ Collect an adequate amount of specimen. Inadequate amounts of specimen may yield false-negative results.
- ◆ Develop an understanding of the microbiology laboratory's source identification schemes. Know when to add "rule-out" requests. Our laboratory routinely screens for Shigella, Salmonella, E. Coli 0157, and Campylobacter species in stool specimens, but not for Yersinia, Vibrio, Aeromonas, or Plesiomonas.
- ◆ If a specimen is collected through intact skin, cleanse the skin first. For example, use 70% alcohol followed by iodine solution (1 to 2% tincture of iodine or 10% solution of providence iodine). Prevent burns caused by the tincture of iodine by removing excess iodine after the specimen has been collected.
- ◆ Before collecting the specimen, consider the risk/benefit ratio of the collection procedure to the patient.
- ◆ Collect fluid specimens in sturdy, sterile, screw cap, leak proof containers with lids that do not create an aerosol when opened.
- ◆ Specimens obtained by a physician using needle aspiration should be transferred to a sterile tube or anaerobic transport vial prior to transport of the specimen to the laboratory. If there is little material in the syringe, the physician should draw a small amount of sterile nonbacteriostatic 0.85% NaCl through the syringe and then transfer the specimen to a sterile tube. Alternatively, and **ONLY** if the specimen will be compromised by transferring it from the syringe, a small amount of sterile 0.85% NaCl may be drawn into the syringe prior to removal of the needle. DO NOT TRANSPORT SYRINGES WITH NEEDLES ATTACHED AND/OR RECAPPED. Attach syringe cap ONLY if necessary. The physician should use a protective device while removing the needle to avoid injury and should cap the syringe with a sterile cap prior to transporting it to the laboratory.
- ◆ Any Microbiology and Virology specimens collected in formalin are **UNACCEPTABLE** for culture!

## General Guidelines for Transport of Microbiology Specimens

(See also: Collection of Specimens for Viral Testing)

- ◆ Transport all specimens to the laboratory promptly.
  1. To ensure the survival and isolation of fastidious organisms and to prevent overgrowth by more hardy bacteria.
  2. To shorten the duration of specimen contact with some local anesthetics used in the collection procedure that may have antibacterial activity.
  3. To provide a more accurate diagnosis of the infectious disease process.
  4. All collections must be labeled with correct date and time of collection and the specific site.
- ◆ Alternatives to prompt delivery
  1. Refrigerate most specimens as 2° – 8° C. The following are exceptions:
    - a. Blood cultured in Bactec bottles must be kept at room temperature or incubated at 35° - 37° no longer than 20 hours. An ISOLATOR tube is held at room temperature. Blood for viral culture MUST be held at room temperature and transported within 6 hours of collection.
    - b. Specimens may harbor temperature sensitive organisms and should be left at room temperature.  
Exception: urines in non-preserved transport containers should be refrigerated.
    - c. An anaerobic transport system MUST be used for anaerobic culture requests.
    - d. Stool specimens should be collected in STOOL transport vials. See following tables. Diapers and swabs are NOT ACCEPTABLE for parasite determinations.
    - e. In general, spinal fluid (CSF) specimens are held at room temperature. Alternatively, incubate at 35 - 37 C. CSF for viral culture should be refrigerated.
  2. Use of specimen transport systems:
    - a. Anaerobic transport systems are used to ensure the viability of anaerobic organisms in transit to the laboratory. See Table 1.
    - b. Aerobic transport methods are listed in Table 2. Although sterile swabs can be used for collection and transport of specimens, the test request should be considered prior to specimen collection. Certain types of swabs should be used for collection and transport of certain cultures, as described in Table 2.
    - c. Specific transport containers for specimens are listed in Table 3.

**TABLE 1. ANAEROBIC TRANSPORT SYSTEMS**

<b>System</b>	<b>Description</b>
<b>Port-a-Cult Tube</b>	Tube is used for transporting swabs. Contains a semi-solid medium with reducing agent and redox indicator. Any purple discoloration of medium indicates exposure to air. Viability of anaerobes, facultative and aerobic organisms are maintained for up to 72 hours at 20-25° C. This tube should not be used to transport small volumes of body fluids, aspirates or tissues.
<b>Syringe/Needle Aspirates</b>	Express air from syringe, remove needle, and recap with syringe cap. Small amounts of fluid may be transferred to a vacutainer red top tube for transportation. If fairly large volume (5 ml or more), anaerobic bacteria will survive for 24 hours at room temperature. See specimen collection guidelines for physician collection of syringe aspirates.
<b>Bactec Blood Bottles</b>	Fluids collected in large quantities >10 mL may be directly inoculated into Bactec blood culture vials; 8-10 ml in each bottle (aerobic and anaerobic). Transport immediately at room temperature. Alternatively, incubate at 35-37. If Bactec bottles are used, 1 mL of fluid in a red top vacutainer should also be sent.
<b>Sterile Screw Cap Containers</b>	These are best for transporting tissue and biopsy specimens. If specimen is small, place on sterile gauze moistened with sterile non-bacteriostatic 0.85% NaCl. Do not wrap in gauze. <b>DO NOT USE FORMALIN</b>

**TABLE 2. AEROBIC TRANSPORT SYSTEMS**

<b>System</b>	<b>Description/Comments</b>
<b>Culturette Swab (Stuart's Media)</b>	Sterile, disposable culture collection and transport system consisting of a plastic tube containing a rayon-tipped swab and transport medium to prevent drying of bacteria and maintain pH
<b>Cotton Swabs (Cotton Swabs are NOT recommended for use)</b>	Residual fatty acids may inhibit <u>MRSA</u> , Group B Beta Strep and <u>Chlamydia</u> sp. If cotton is glued or spun to wooden applicator stick, wooden stick may inactivate viruses and interfere with some <u>Ureaplasma</u> identification tests. Therefore; wooden shafted swabs are UNACCEPTABLE FOR <u>MRSA</u> , <u>Group BBS</u> , Chlamydia, Ureaplasma and VIRAL CULTURE. Calgiswab® also unacceptable for viral culture.
<b>Nasopharyngeal/ Mini-tip Culturettes</b>	Flexible wire shafts with small rayon tips provide for easier urethrogenital, and nasopharyngeal specimens collection or culture of <u>B. pertussis</u> , <u>N. gonorrhoeae</u> and respiration virus in children.
<b>Sterile Screw-cap Containers</b>	Useful for collection of urine, sputum, stool, Bronchoalveolar Lavage, catheter tips and specimens for routine and viral cultures. For small specimen place on sterile gauze moistened with sterile nonbacteriostatic 0.85 % NaCl in a cup NEVER place biopsy specimen in formalin or wrap in gauze.
<b>Sterile Petri Dishes</b>	Useful for hair or skin-scraping specimens. Tape petri dish securely prior to transport.
<b>Sterile Tubes: Screw-cap Glass or Plastic tubes, Sterile Vacutainer Tubes without additives</b>	Useful for collection of sterile fluids, drainage, or bronchial brush specimens for routine bacterial, viral, or chlamydial culture. These tubes are <u>NOT</u> to be used for transport of anaerobic specimens.
<b>B-D Urine Tubes</b>	Vacutainer tube containing 0.5 ml freeze-dried boric acid- sodium formate maintenance formula. Maintenance formula holds bacterial population in urine specimen for 48 hours at room temperature comparable to those in urine specimens without additives, but held under refrigeration for the same period. Preservative may be toxic to some uropathogens. UNACCEPTABLE for Virus culture, Legionella antigen or Histoplasmosis antigen testing.
<b>Ova &amp; Parasite Vials</b>	Two (3) container package: one PVA, one formalin to preserve feces for parasite detection, and one has Carey Blair media for stool culture. Preservative containers are unacceptable for other stool tests. ALWAYS inoculate all three vials.
<b>M4 RT Tube (red capped)</b>	Sterile swabs and transport tubes containing Hanks BSS, antibiotics and fungizone. Suitable for Chlamydia Trachomatis PCR, GC PCR, C. Trachomatis and Chlamydia Respiratory Culture. Once inoculated, store <u>refrigerated</u> until it is to be transported.
<b>M4 Tube (blue capped)</b>	Use sterile transport tubes containing Hanks BSS, antibiotics and fungizone. For collection and transport of Mycoplasma and Ureaplasma cultures only. <u>Once inoculated, store refrigerated</u> until it is to be transported.
<b>GenProbe Aptima Tubes</b>	Use with the Aptima Systems ONLY. Good for detecting C. Trachomatis and Gonorrhoeae by PCR. UNACCEPTABLE for other testing including C. Trachomatis and N. Gonorrhoeae culture.
<b>Viral Transport Medium</b>	*See also Collection of Specimens for Viral Testing
<b>Blood</b>	Bactec system for routine blood culture and isolator system if systemic yeast and mycobacteria are suspected. Yellow (ACD) or Green (Lithium or Sodium Heparin) top tube for virus isolation.

<b>TABLE 3. SPECIMEN TRANSPORT GUIDE</b>	
<b>Source and type of specimen</b>	<b>Transport Method</b>
<b>CNS</b> CSF Brain abscess CNS Biopsy	Sterile screw-cap tube Anaerobic transport system If swab submitted, send in anaerobic transport system. If specimen is tissue, send in sterile cup with small amount of nonbacteriostatic 0.85% NaCl, ( <b>NEVER</b> place in formalin.)
<b>Gastrointestinal system</b> Stool Rectal Swab Gastric lavage/washings Duodenal aspirate Rectal biopsy  Sigmoidoscopy specimen	Sterile screw-cap cup and/or Ova & Parasite Vials. Culturette (for pinworm, use pinworm collection kit.) Sterile screw-cap cup or sputum trap Sterile screw-cap cup or sputum trap Sterile screw-cap cup. If specimen is small, send in sterile cup with small amount of nonbacteriostatic 0.85% NaCl. ( <b>NEVER</b> place in formalin.) Sterile screw-cap cup. If specimen is small, send in sterile cup with small amount of nonbacteriostatic 0.85% NaCl. ( <b>NEVER</b> place in formalin.)
<b>Eye</b> Conjunctiva scrapings Corneal scrapings Intraocular fluid	Culturette Send prepared smears and/or directly plated media. Send prepared smears and/or directly plated media. Send prepared smears and/or directly plated media, capped syringe <u>without</u> needle with air expelled or sterile tube.
<b>Genital Tract, Female</b> Amniotic fluid  Fallopian tube Bartholin fluid Endometrial Vulval  Cervical  Urethral Vaginal	1 - 2 ml of sample in sterile screw-cap tube. Amounts greater than 2 ml may be directly inoculated into blood culture vials. Do not refrigerate. 1 - 2 ml of sample in sterile screw-cap tube or anaerobic transport system. 1 - 2 ml of sample in sterile screw-cap tube or anaerobic transport system. Sterile screw-cap cup or tube or anaerobic transport system. Capped syringe without needle, culturette, viral culturette, or M4RT tube for Chlamydial transport. Culturette, viral culturette, M4RT tube, female GenProbe specimen collection kit, M4 tube. Culturette, viral culturette, M4RT tube, M4 tube. Culturette, viral culturette, M4RT tube, M4 tube.
<b>Genital tract, Male</b> Anal swab  Urethral  Epididymis Prostatic massage Semen Penile lesion	Culturette, viral culturette, M4RT tube. Mini-tip culturette, viral culturette, M4RT tube, male GenProbe specimen collection kit, M4 tube. Sterile screw-cap cup or anaerobic transport system. Sterile screw-cap tube, or cup or culturette. Sterile screw-cap tube, or cup or culturette, M4 tube Capped syringe without needle, culturette, viral culturette, M4RT tube.

**TABLE 3. SPECIMEN TRANSPORT GUIDE – con't**

<b>Source and type of specimen</b>	<b>Transport Method</b>
<b>Lower respiratory tract</b>	
Lung biopsy	Sterile screw-cap cup; if specimen is small, place it in a small amount of nonbacteriostatic 0.85 % NaCl. (NEVER place in formalin.)
Induced sputum/Expectorated Sputum	Sterile screw-cap cup.
Tracheal/endotracheal aspirate	Sterile screw-cap cup or sputum trap
Bronchoalveolar Lavage	Sputum trap or sterile screw-cap cup
Bronchial washings	Sputum trap or sterile screw-cap cup
Transbronchial biopsy	Sterile screw-cap cup with 1 - 2 ml nonbacteriostatic 0.85 % NaCl.
Bronchial brush	Sterile screw-cap tube.
Transtracheal aspirate	Sterile screw-cap cup or tube, or anaerobic transport system.
Lung aspirate	Sterile screw-cap cup or tube, or anaerobic transport system.
<b>Upper respiratory tract</b>	
Throat swab	Culturette or viral transport system.
Nasal swab	Culturette or viral transport system.
Oral culture	Culturette or viral transport system (HSV).
Nasopharyngeal swab	Mini-tip culturette, viral transport system or Starswab II/Amies charcoal media
Tympanocentesis fluid	Anaerobic transport system or capped syringe without needle.
Sinus aspirate	Anaerobic transport system or capped syringe without needle.
Nasopharyngeal suction	Sterile screw-cap cup or viral transport system.
Nasal washings	Sterile screw-cap cup or viral transport system.
<b>Sterile body fluids:</b> (excluding: CSF, urine, blood)	Sterile screw-cap container, capped syringe without needle, anaerobic transport system, red top vacutainer tube. Volumes greater than 10 ml may be inoculated directly into blood culture vials along with specimen in red top vacutainer tube.
Pleural, peritoneal, ascites, joint and synovial fluid.	
<b>Subcutaneous tissue and skin</b>	
Ulcers or nodules, superficial wounds, Exudates	Capped syringe without needle.
Biopsy	Sterile screw-cap cup; if specimen is small, add a small amount of sterile nonbacteriostatic 0.85 % NaCl to prevent drying.
Burn specimens	Sterile screw-cap container.
Superficial fungal lesion	Sterile screw-cap container
<b>Deep wounds, aspirates, tissues</b>	
Site wounds, swabs	Anaerobic transport system.
Deep wounds, abscesses	Sterile screw-cap container or anaerobic transport system.
Soft tissue aspirates	Capped syringe without needle.
Bone	Sterile screw-cap container; if specimen is small, add a small amount of sterile nonbacteriostatic 0.85 % NaCl to prevent drying.
Punch skin biopsy, tissue	Sterile screw-cap container; if specimen is small, add a small amount of sterile nonbacteriostatic 0.85 % NaCl to prevent drying.
<b>Urine</b>	
Clean catch (CCMS)	B-D vacutainer or sterile screw-cap container.
Ileal loop	B-D vacutainer or sterile screw-cap container.
Straight catheter	B-D vacutainer or sterile screw-cap container.
Suprapubic aspirate	Capped syringe without needle or anaerobic transport system.
Bladder washout	Sterile screw-cap container. <b>Label with correct times and sites</b>
Bilateral urethral catheterization	Sterile screw-cap container. <b>Label with correct times and sites.</b>

## General Guidelines for Blood Culture Collection

**TABLE 4: Recommendations for Blood Culture Collection**

<b>Clinical Condition</b>	<b>Collection Protocol</b>	<b>Comments</b>
<b>For Adults/Adolescents</b>		
Severe Septicemia	(2) Cultures prior to therapy	One (1) 10-15 ML sample from each arm
Acute Endocarditis	(2) Cultures w/in 1-2 hr. of evaluation and prior to therapy	Space each collection at least 1 hr. apart with two (2) separate venipunctures
Subacute endocarditis	(2) Cultures w/in 24 hr. on day (1). If negative 24 hr. later obtain (2) more	Space each collection at least 1 hr. apart. Patients receiving antibiotics prior to admission, obtain two (2)separate cultures on each of three (3) successive days. Cultures should be incubated at least (14) days. <b>*NOTE:</b> contact the microbiology lab when cultures need to be held longer than (5) days.
Low grade intravascular infection	(2) Cultures w/in 24 hr.	Specimens should be collected at Least 1 hr. apart at first sign of febrile episodes.
Bacteremia of unknown origin Patient on therapy	(4) Cultures w/in 48 hr.	Take specimen immediately prior to next dose of antibiotic. Cultures should be incubated for at least (14) days *NOTE: contact the microbiology lab when cultures need to be held longer than (5) days
Febrile Episodes	No more than two (2) total cultures	Bacteremia may precede episodes of fever and chills by about one (1) hr.
<b>Small Children (&lt; 4 years)</b>	1-2 ML. samples	(2) Cultures usually suffice for diagnosing bacteremia in the newborn. Less than 1 mL of blood will detect Bacteremia when concentration of organism is sufficiently high. If so, inoculate (1)Peds Plus bottle.

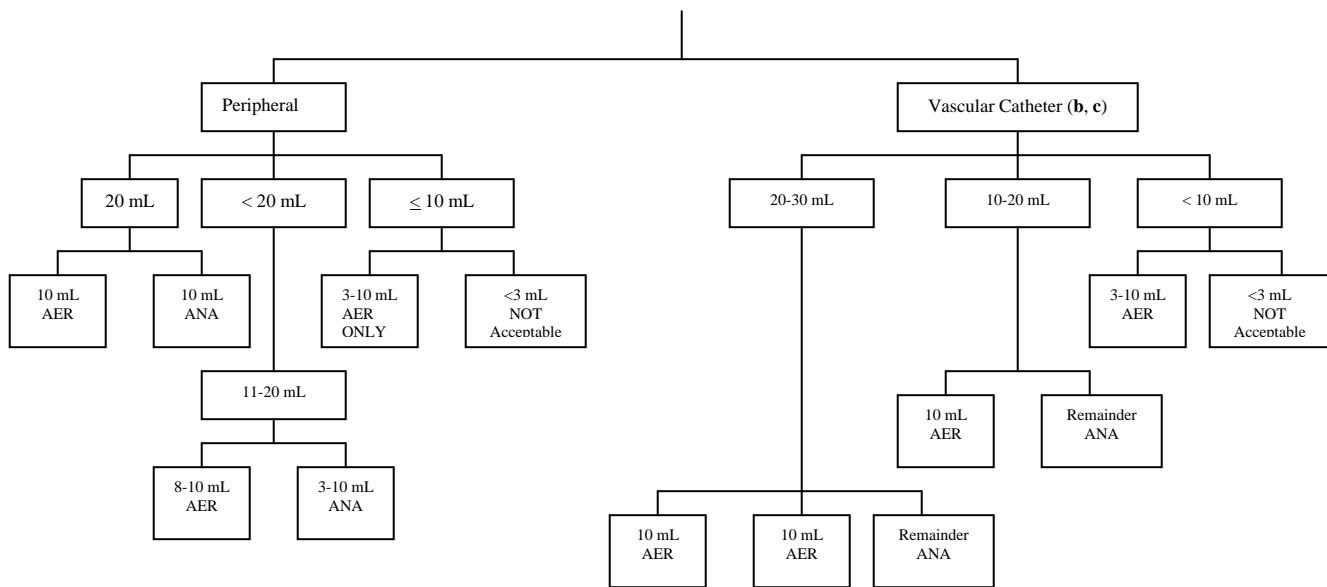
- ◆ The volume of blood for culture is **critical!** Concentration of organisms in most cases of Bacteremia is low, especially if the patient is on antimicrobial therapy.
- ◆ Most cases of Bacteremia are detected using 2 sets of separately collected blood cultures. More than 2 sets yield little additional information.
- ◆ In children less than four, the concentration of organisms during bacteremia is higher than those four years and older; therefore, less blood is acceptable for culture utilizing the Peds Plus bottles.
- ◆ The yield of blood cultures in adults increases approximately 3% per ml of blood cultured. Culture bottles with inadequate blood volumes may demonstrate no growth when in fact microorganisms are present in the blood. To accurately ascertain bacteremic episodes and obtain the best sensitivity, the maximum amounts of blood (specified on the collection bottles) should be obtained for culture.
- ◆ One blood culture “set” is defined as a sample of blood drawn at a single time at a single site, regardless of how many bottles are injected into. These “sets” typically should be collected ten minutes apart unless ordered otherwise. For blood cultures obtained from an intravascular line it is permissible to obtain both “sets” with one large volume draw.
- ◆ Ideally blood cultures are obtained from peripheral venipuncture (i.e. - two sets from two separate venipunctures).
- ◆ Draw blood cultures prior to initiating or changing antimicrobial therapy if at all possible.
- ◆ Aseptic technique is critical to obtaining accurate results. When obtaining specimens via skin puncture, a second person may be needed to assist in extremity stabilization and to place blood into bottles while pressure is held over puncture site.

### Inpatient Collection Policy

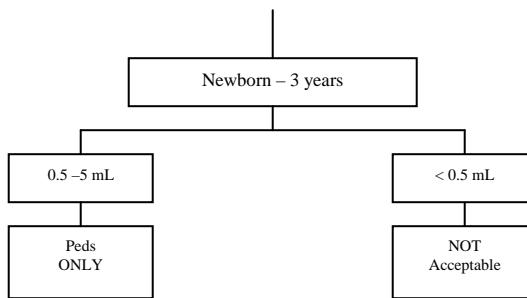
- ◆ RN's, LPN's, AST's, house staff or physicians who have received competency validation in blood culture specimen collection may draw Blood cultures.
- ◆ A physician's order is required to obtain blood cultures from an intravascular line. It is critical to label the specimen with the specific line it is drawn from (eg - arterial, PICC, etc.).
- ◆ Blood can be collected from veins, arteries, or intravascular catheters

**Collection Guideline****Volume of Blood for Collection**

**“ADULT BLOOD/PER STICK”(a, b)**  
**(≥ 4 years old)**



**PEDIATRICS and NEWBORN BLOOD per STICK (b)**



**KEY:**

- a) Collect 2 sets (needle sticks) within 24 hours. AER: Bactec 26 PLUS Aerobic (blue foil cap/silver label)
- b) Maximum draw preferred. ANA: Bactec Lytic Anaerobic/F (purple foil cap/purple label)
- c) Should not replace venipuncture if possible. PED: Bactec Peds Plus (silver foil cap/pink label)

**TABLE 5. Media for isolation of organisms from Blood Culture Media.**

CULTURE	MEDIA TYPE	AMOUNT OF BLOOD	
Bacteria/Yeast	Bactec 26 Plus (Silver Label) or Bactec Peds Plus (Pink Label)	8 – 10 ML	Adults
		0.5 – 5 ML	Peds
Anaerobes	Bactec Lytic (Purple Label)	3 – 10 ML	
Systemic Fungus (Histoplasma Capsulatum or Cryptococcus Neoformans)	Adult Isolator Pediatric Isolator	9 – 10 ML	Adults
		0.5 – 1.5 ML	Peds
Mycobacteria	Adult Isolator	9 – 10 ML	
CMV/Adenovirus/HSV	ACD (Yellow Top), EDTA (Purple) Heparin (Green Top: sodium or lithium))	8 – 10 ML	Adults
		3 ML	Peds

- ◆ Blood for CMV/Adenovirus/Herpes (HSV) cultures:
  1. Blood for viral culture is ONLY processed Monday - Friday, 7 AM to 1 PM, Saturday if received in lab by 8 AM.
  2. Blood MUST BE in the laboratory within 6 Hours of collection
  3. DO NOT REFRIGERATE! STORE AT ROOM TEMPERATURE.
- ◆ If viremia, fungemia, brucellosis, tularemia, bartonella, or an infection with a cell-wall deficient or fastidious bacterium is suspected, the microbiology laboratory should be contacted for special instructions.

## Equipment

Alcohol Swabs	Appropriate Blood Culture Bottles	10 - 30 ml syringe	Exam Gloves
Tincture of Iodine Sepp	Needle/Butterfly for Venipuncture	NICU: Providone-iodine	

## Procedure (Observe Universal {Standard} Precautions):

- ◆ Put on gloves (clean exam gloves are acceptable).
- ◆ Disinfect the septa of the blood culture bottles utilizing 70% isopropyl alcohol. NOTE: Do not use iodine/providence-iodine to prevent septa from deteriorating.
- ◆ Identify site for specimen collection.
- ◆ **Peripheral Venipuncture/Arterial Puncture:**
  1. Vigorously cleanse site by rubbing in concentric circles with 70% isopropyl alcohol to disinfect the skin.
  2. Vigorously disinfect the site by starting at the center and rub in concentric circles utilizing tincture of iodine solution for one-two minute duration. NOTE: NICU utilize betadine. For iodine sensitive patients, utilize 70% isopropyl alcohol for one-two minute duration.
  3. Allow the disinfectant to dry (iodine and alcohol function by drying). NOTE: Do not palpate over the site the needle will enter once the site is disinfected (to decrease contamination risk to the specimen).
  4. Draw blood via a syringe and needle/butterfly.
  5. Remove needle/butterfly and apply pressure to site until bleeding stops.
  6. Place blood into appropriate collection bottles/tubes. Inoculate aerobic bottle first. Refer to collection diagram. It is not necessary to change needles prior to inoculating bottles/tubes.
  7. If septum becomes contaminated with blood, clean with 70% isopropyl alcohol.
  8. Once hemostasis is obtained at puncture site, cleanse residual iodine/betadine from site with alcohol to prevent skin irritation.
- ◆ **Line Draws**
  1. If more than one intravascular catheter is available to obtain blood then select line least likely to be colonized/contaminated with microorganisms. Most typically it should be the newest line in; however, consider factors such as breaks into the line for blood draws, tubing changes or catheter manipulations.
  2. Cleanse line adapter, as needed, if one is present, vigorously with 70% isopropyl alcohol prior to obtaining specimen.
  3. Follow “Collection Diagram” for placing blood specimen into bottles. Inoculate aerobic bottle first.
- ◆ **Specimen Labeling**
  1. Label each bottle with patient label containing name and hospital number. Include date, time, specific line site if line draw, Accudata/I-Stat code or name of person collecting specimen.
  2. **DO NOT COVER BAR CODE OF BOTTLE WITH LABEL!!!**

## General Guidelines for Sterile Body Fluids Collection (excluding CSF, urine, and blood)

- ◆ Clean the needle puncture site with alcohol, and disinfect it with an iodine solution to prevent introduction of infection. (If tincture of iodine is used, remove with 70 % isopropyl alcohol after the procedure to avoid burns.)
- ◆ Expel any air bubbles from the syringe, and immediately inject the specimen into a sterile screw-cap container. If sufficient fluid (10 - 20 ml), inject fluid into blood BACTEC bottles; also send fluid in a red vacutainer tube for stains and direct culture. The specimen may be sent in the syringe with the needle removed.

<b>TABLE 6. Collection considerations for Sterile Body Fluids</b>		
<b>Culture</b>	<b>Volume (ml) *</b>	<b>Comments</b>
Bacteria	1 - 10	If gonococcal arthritis is suspected, indicate on order so Laboratory can add Thayer-Martin plate to set up.
Fungi	>10	Fluid for <u>Histoplasma capsulatum</u> (AIDS), <u>Cryptococcus</u> sp., <u>Candida albicans</u> , and <u>Candida tropicalis</u>
Anaerobes	1 - 10	Transport anaerobically
Mycobacteria	>10	
Virus	1 - 3	Transport in sterile tube on ICE.

\*Amounts are guidelines. Greater volumes will increase the chance of organism recovery

### Catheter Cultures

#### General Considerations

- ◆ Transport to laboratory **IMMEDIATELY** at room temperature.
- ◆ Catheter cultures are examined and reported in a semi-quantitative manner.
- ◆ Specimens **MUST** be received in the laboratory within 2 hours of collection to minimize overgrowth and/or death of organisms.
- ◆ FOLEY CATHETER TIPS ARE UNACCEPTABLE FOR CULTURE.
- ◆ ONLY cut segments should be sent. DO NOT PLACE segments in anaerobic transport system.
- ◆ Catheter tip cultures are examined ONLY for growth of aerobic organisms and yeast; they are not appropriate for anaerobic culture.
- ◆ Greater than 15 colonies are suggestive of colonization.

#### Short catheters (2 - 3 inches)

- ◆ Aseptically remove the catheter. Cut the catheter at the skin interface point, using sterile technique.
- ◆ Place the catheter segment in a sterile wide-mouth screw-cap container. Appropriately label the specimen container according to type and location.
- ◆ Transport to the laboratory **IMMEDIATELY**.

#### Long catheters (8 - 24 inches)

- ◆ Aseptically remove the catheter. Submit 2 segments for analysis.
  1. Cut a 2-inch segment of the catheter, which was within the blood vessel, using sterile technique. Place the segment in a wide-mouth screw-cap container. Appropriately label the specimen according to type and location.
  2. Cut a second 2-inch segment of the catheter from the skin interface. Place the segment in a separate wide-mouth screw-cap container. Appropriately label the specimen container according to type and location.
- ◆ Transport to the laboratory **IMMEDIATELY**.

## General Guidelines for Central Nervous System (CNS) Specimen Collection

### **Spinal fluid (CSF)**

Suggested volumes are: for routine (1ml), fungal (2ml), mycobacterial (2ml), and viral (2ml) cultures respectively.

- ◆ **Lumbar puncture**

A total of three tubes are generally required for microbiology, hematology, and chemistry testing. The second tube drawn will generally go to microbiology, and the last tube drawn will generally go to hematology. (In traumatic taps, the CSF will often clear as the later tubes are collected.) **NOTE:** **ALWAYS** send the **MOST TURBID** tube to microbiology.

### **Other CNS specimens**

- ◆ **Brain abscess**

1. Ninety percent of brain abscesses will grow anaerobic bacteria. See Table 1 for anaerobic transport systems. In the event that an anaerobic transport system is unavailable, transport the specimen without delay to microbiology for immediate processing.
2. See Table 1 for transport of specimens in a syringe.

- ◆ **CNS biopsy specimens**

1. Obtain biopsy sample from the lesion at surgery and send to the microbiology laboratory in an anaerobic transport system.
2. **DO NOT ADD FORMALIN!**

**TABLE 7. Collection considerations for CNS Specimens**

<b>Culture</b>	<b>Volume (ml) *</b>	<b>Comments</b>
Bacteria	1	Send cloudiest CSF specimen to microbiology immediately; tube #2 is preferred
Fungi	2	Rule out <u>Cryptococcus</u> sp.
Mycobacteria	2	<u>M. tuberculosis</u> , <u>M. avium</u> , <u>M. intracellulare</u>
Anaerobes	NA	Brain abscess or CNS biopsy specimens
Parasites	NA	Brain abscess or CNS biopsy specimens
Virus	1 - 2	Send to virology on ice

\*-Amounts are guidelines. Greater volumes increase the chance of organism recovery. NA-not applicable

## General Guidelines for Gastrointestinal Tract Specimen Collection

The gastrointestinal tract includes the esophagus, stomach, duodenum, small intestine and colon.

### **Fecal specimens**

1. Submitted primarily for the detection of Campylobacter, Shigella, and Salmonella sp. E. Coli 0157:H7 and Clostridium difficile toxin. In certain cases, detection of Yersinia, Vibrio, Aeromonas, and Plesiomonas sp. must be specifically requested
2. General considerations:
  - a. Keep stool specimens cool; do not incubate them.
  - b. DO NOT USE TOILET PAPER to collect stool. Toilet paper may be impregnated with barium salts, which are inhibitory for some fecal pathogens.
  - c. Diaper specimens are unacceptable.
3. Have patient obtain stool specimen by one of the following methods:
  - a. Pass stool directly into a wide-mouth, leak proof container with a tight fitting lid.
  - b. Pass stool into a clean, dry bedpan, and transfer stool into a sterile leak proof container with a tight fitting lid
4. Stool for ova and parasites MUST be placed in proper preservative containers. DIAPERS AND SWABS ARE NOT ACCEPTABLE.
  - a. **Routine O&P:** Giardia and Cryptosporidium screen by immunofluorescence on a single stool specimen.

- b. **Special Studies O&P:** Concentration/trichrome stain by special request only when clinical suspicion combined with patient history and 3 specimens collected.
- c. No more than 1 specimen/24 hours.
- d. **Special Stool Cultures and O&P:** Stool cultures and O&P exams will be performed by special request only on immunocompromised patients, transplant patients, and examination for potential nosocomial transmission.

### Rectal swabs

1. Submitted primarily for the detection of N. Gonorrhoeae, Shigella sp., and Enteroviruses.
2. Swabs of small lesions are appropriate for detection of herpes simplex virus (HSV). See Collection of Specimens for Viral Testing

### Gastric aspirates

The patient should fast prior to each of the following procedures.

1. **Gastric Lavage**  
Submitted primarily for the detection of M. tuberculosis in patients (most frequently children) unable to produce quality sputum. Lavage should be performed after the patient wakes in the morning so that the sputum swallowed during sleep is still in the stomach.
2. **Duodenal aspiration**  
Submitted primarily for detection of Giardia sp. and larvae of Strongyloides stercoralis and Ascaris lumbricoides.

**TABLE 8. Collection considerations for Gastrointestinal Specimens**

Culture	Comments
Bacteria	* Stools: 2 stools recommended * Gastric biopsy: rule out <u>Helicobacter pylori</u> * Rectal swab: rule out enteric pathogens (especially <u>Shigella</u> sp. and/or <u>N. Gonorrhoeae</u> )
Fungi	Gastric aspirate, gastric biopsy, esophageal brush, esophageal biopsy
Pinworm	For pinworm collection kit and instructions, contact microbiology at 491-6000. Collected when patient gets up in the morning before patient bathes or defecates.
Mycobacteria	Gastric aspirate or gastric biopsy; feces.
Parasites	Place stool specimen in preservative. Three daily stool specimens are recommended for special studies. One stool specimen recommended for routine O&P *Duodenal aspirates are useful for detecting <u>Giardia</u> sp. and larvae of <u>S. stercoralis</u> and <u>A. lumbricoides</u> *Use rectal biopsy specimens for <u>E. histolytica</u> and <u>B. coli</u> *Use small bowel biopsy specimens for <u>Giardia</u> sp., <u>Cryptosporidium</u> , <u>Microsporidium</u> sp., and CMV
Virus	Place stool in a sterile screw capped container and rectal swab in viral transport medium Place esophageal and rectal biopsies in viral transport medium. Do not freeze.

## General Guidelines for Genital Tract Specimen Collection

### General considerations

- ◆ Genital tract specimens are submitted primarily for the detection of sexually transmitted pathogens such as N. Gonorrhoeae, Chlamydia Trachomatis, lymphogranuloma venereum (LGV), HSV, human papilloma virus (HPV-with histological confirmation), trichomonads, Haemophilus Ducreyi, Group B Streptococci, Mycoplasma Hominis, Ureaplasma Urealyticum and Candida infections.
- ◆ Become familiar with the various tests, the appropriate situation, sources and collection devices for each sexually transmitted pathogen. For example, N. Gonorrhoeae, and C. Trachomatis are available as Polymerase Chain Reaction (PCR) and culture. PCR is recommended for certain sources and screening. Culture is recommended for test of cure after treatment is completed, alleged sexual assault cases and sources that are not approved for PCR testing. Refer to Tables 2,3 and 9 for appropriate information.
- ◆ If infection is not caused by any of these pathogens, anaerobic bacteria may be involved. If an anaerobic infection from an appropriate source (Table 9) is suspected; transport the specimen in an anaerobic transport system (Table 1).

**TABLE 9. Collection considerations for Genital Tract Specimens**

<b>Culture</b>	<b>Recommended Specimens</b>
Bacteria Culture	Prostatic fluid, cervical, vaginal
Fungus Culture	Anal, vaginal, cervical
Anaerobe Culture	Epididymis aspirate, amniotic fluid, abscess fluid
<u>T</u> richomonas Vaginalis	Vaginal, prostatic fluid
<u>N</u> . Gonorrhoeae (PCR)	Cervical, urethral (male)
<u>N</u> . Gonorrhoeae (Culture)	Cervical, urethral, anal or vaginal
<u>C</u> . Trachomatis (PCR)	Cervical, urethral (male), conjunctival
<u>C</u> . Trachomatis (Culture)	Urethral, vulval, cervical, rectal, throat
HSV	Genital or perianal lesion
LGV	Rectal, cervical, urethral, bulbo or ulcer material
<u>H</u> . ducreyi	Material from ulcers of genitalia and perianal areas and from inguinal nodes
<u>M</u> ycoplasma Hominis & <u>U</u> . Urealyticum	Urethral, epididymis, prostatic fluid, semen, cervix or urine

### **General Guidelines for Ocular Specimen Collection**

#### **General considerations**

1. Obtain viral and chlamydial samples before topical anesthetics are instilled.
2. Obtain samples for chlamydia cultures or chlamydia PCR with Chlamydia transport system and viral cultures in viral culturette or in viral transport medium.
3. Send prepared slides and inoculated media to the laboratory immediately.

**TABLE 10. Collection considerations for Ocular Specimens**

<b>Culture</b>	<b>Comments</b>
Bacteria	Inoculate media directly with ocular scrapings. (If <u>N. gonorrhoeae</u> is suspected, inoculate a Thayer-Martin plate.)
Fungi	Inoculate media directly with ocular scrapings.
Anaerobes	Use anaerobic transport system, or inoculate media directly. If inflammation occurs after extra capsular cataract extraction, rule out <u>Propionibacterium</u> sp.
Parasites	Use to detect <u>Acanthamoeba</u> sp. Send specimen in sterile container.
Chlamydia	Use chlamydia transport system. PCR testing recommended for conjunctiva.
Virus	Use viral culturette or viral transport medium.
Mycobacteria	Inoculate medium directly with ocular scrapings.

## General Guidelines for Respiratory Specimen Collection

### **General considerations**

1. Twenty-four hour sputum collections are NOT acceptable for culture.
2. If *Corynebacterium diphtheriae*, *Arcanobacterium haemolyticum*, *Bordetella pertussis*, *Chlamydiae* or *Mycoplasma*, are suspected, the laboratory MUST be informed prior to collection because special techniques and/or media are required for isolation of these agents.

**TABLE 11. Collection considerations for Respiratory Specimens**

<b>Culture</b>	<b>Volume (ml) @</b>	<b>Comments</b>
Bacteria	NA	Saliva is unacceptable. One specimen per day is appropriate for routine culture. Refer to guidelines for proper sources.
Fungi	3 - 5	Collect early-morning specimens resulting from deep cough or Sputum induction. Lung biopsy specimens or lung aspirates are also appropriate.
Anaerobes	1	Sinus aspirate, tympanocentesis fluid, transtrachael aspirate, and lung aspirates or biopsy specimens are appropriate.
Mycobacteria	5 - 10	Collect 3 early-morning fresh specimens (one per three days) resulting from deep cough or sputum induction. Lung biopsy specimens or lung aspirates are also appropriate
<u>Pneumocystis</u> sp.	2	Use induced sputum, BAL, or lung biopsy specimen.
Parasites	3 - 5	Can be examined for amoebae, helminth eggs ( <u>Paragonimus westermani</u> ), hooklets of <u>Echinococcus</u> sp., larvae of hookworm, and <u>Ascaris</u> and <u>Strongyloides</u> sp.
<u>Viral</u>	NA	Place sputum or respiratory swab in viral transport medium.

@ - Amounts are guidelines. NA = not applicable.

## General Guidelines for Subcutaneous Tissue and Skin Specimen Collection

**TABLE 12. Collection considerations for Subcutaneous Tissue and Skin Specimens**

<b>Culture</b>	<b>Comments</b>
Bacteria	Syringe aspirates or biopsy specimens are preferable to swab specimens.
Anaerobes	Uncommon in burn, ulcer, nodules, or superficial skin infections; useful following bites or trauma
Fungi	Useful in diagnosing dermatophytes, yeast, filamentous fungi, and dimorphic fungi
Mycobacteria	Useful in diagnosing <u>M. marinum</u> , <u>M. fortuitum</u> , and <u>M. chelonei</u>
Virus@	Useful in diagnosing HSV and varicella-zoster virus

@-Rate of recovery of virus is higher from the youngest lesions (vesicles), than from pustules, ulcers and crusted lesions.

## **General Guidelines for Urine Specimen Collection**

### **General considerations**

- ◆ NEVER COLLECT a urine specimen from a bedpan or urinal.
- ◆ Thoroughly clean the urethral opening (and vaginal vestibule in females) prior to collection procedures to ensure that the specimen obtained is not contaminated with colonizing microorganisms in this area.
- ◆ Soap, rather than disinfectants, is recommended for cleaning the urethral area. If disinfectants are introduced into the urine during collection, they may be inhibitory to the growth of microorganisms.
- ◆ Transport specimens to the laboratory within 2 hours of collection if not collected in a preservative tube. Refrigeration is required prior to transport for urines sent in non-preserved containers. Bacterial counts remain stable in non-preserved urine for at least 24 hours at 4C. DO NOT FREEZE.
- ◆ Use sterile cups or tubes to transport urine. Urine transport kits are available through SPD.
- ◆ Transport suprapubic bladder aspirate (SPA) specimens for anaerobic culture in a sterile tube. Transport to laboratory immediately after collection.
- ◆ Always transport urine for viral cultures in a sterile container. PRESERVATIVE TUBES ARE NOT ACCEPTABLE.
- ◆ Any urine collection procedure involving catheterization must be done with scrupulous aseptic technique to avoid introducing microorganisms.
- ◆ Send the first morning voided urine. Three consecutive first morning urine specimens are recommended for mycobacterial culture.
- ◆ Twenty-four hour urine specimens are NOT ACCEPTABLE FOR CULTURE.
- ◆ Bag urines ARE NOT ACCEPTABLE FOR CULTURE.
- ◆ Preservative tubes are not acceptable for Histoplasmosis or Legionella Antigen testing. Use sterile cups or tubes

### **Collection techniques**

#### **Clean-catch urine (CCMS) specimens (female)**

1. The person obtaining the urine specimen should wash hands with soap and water, rinse, and dry. If the patient is collecting the specimen, she should be given detailed instructions, including diagrams or a pictorial display.
2. Cleanse urethral opening and vaginal vestibule area with soapy water or clean gauze pads soaked with liquid soap
3. Rinse the area well with water or wet gauze wipes.
4. Hold labia apart during voiding.
5. Allow a few milliliters of urine to pass. (Do not stop the flow of urine.)
6. Collect the midstream portion of urine in a sterile container.

#### **Clean-catch urine (CCMS) specimens (male)**

1. The person obtaining the urine should wash hands with soap and water, rinse, and dry. If the patient is collecting the specimen, he should be given detailed instructions, including diagrams or a pictorial display.
2. Cleanse the penis, retract the foreskin (if not circumcised), and wash with soapy water.
3. Rinse the area well with sterile water.
4. Keeping the foreskin retracted (to minimize contamination with skin flora) allow a few milliliters of urine to pass. (Do not stop the flow of urine.)
5. Collect the midstream portion of urine in a sterile container.

**TABLE 13. Collection considerations for Urine Specimens**

Culture	Volume (ml) @	Comments
Bacteria	0.5 - 10	Do not collect 24-hour specimen. After proper cleansing of patient, use morning midstream void. Volumes greater than 3 ml place in PRESERVATIVE TUBE FOR ROUTINE URINE CULTURE.
Fungi	>20	Do not collect 24-hour specimen. First morning void is recommended.
Mycobacteria	>20	Do not collect 24-hour specimen. Three first morning consecutive voided urine specimens are recommended.
Anaerobes	1	Use suprapubic aspirate. Send in anaerobic transport system.
Virus	10 - 50	Do not collect 24-hour specimen. First morning void is recommended. Transport to the laboratory immediately. DO NOT SEND IN PRESERVATIVE TUBE
Parasites	24 – hour collection	Use for detecting <u>Schistosoma haematobium</u> eggs, <u>Trichomonas vaginalis</u> trophozoites in males, and <u>Onchocerca volvulus</u> microfilariae.
@-Amounts are guidelines. In general, greater volumes increase the chance of organism recovery.		

### **General Guidelines for Deep Wound, Aspirates, and Tissue Specimens**

**TABLE 14. Considerations for Deep Wound, Aspirate and Tissue Specimens**

Culture	Comments
Bacteria	Biopsy specimens or aspirates are better than swab specimens.
Anaerobes	Useful in diagnosing actinomycosis; (must include special request for actinomyces on patient orders). Send in anaerobic transport.
Fungi	Useful in diagnosing <u>Pseudallescheria boydii</u> , <u>Bipolaris</u> sp., <u>Exophiala</u> sp., and <u>Fusarium</u> sp.
Mycobacteria	Useful in diagnosing <u>M. tuberculosis</u> , <u>M. bovis</u> , and <u>M. kansasii</u> .

### **General Guidelines for Viral Specimen Collection**

#### **Collect Specimens listed below for Syndromes shown**

- Upper respiratory infection: throat, nasal or NP swab, stool or rectal swab
- Lower respiratory infection: throat, nasal or NP swab, BAL
- Cutaneous lesions and vesicles: vesicle fluid or lesion scrapings
- Mucous membrane disease: throat or nasal swab, whole anticoagulated blood
- Central Nervous System infection: throat or nasal swab, spinal fluid
- Undiagnosed febrile illness: whole anticoagulated blood
- Congenital anomalies: throat or nasal swab, urine, whole anticoagulated blood
- Diarrhea: stool, throat or nasal swab

#### **Samples for both/either virus isolation or viral antigen detection are collected according to guidelines below:**

1. Specimens should be collected as soon as possible after the onset of illness.
2. Aliquots of viral medium (Hank's balanced salt solution with gentamicin and amphotericin B) are available in the Virology Laboratory at 491-6999 so that specimens may be inoculated into M4 or universal transport medium (when required) before being transported to the laboratory.
3. Do not use calcium alginate (Calgiswab®); use sterile cotton or Dacron swab or CoPan flocked swab.

4. Prompt transport and handling of viral culture specimens is imperative for virus isolation.

**Storage Instructions:** Transport to the laboratory immediately. Do not freeze. Leave blood at room temp; refrigerate all other specimens.

**Causes for rejection:** Improper specimen type, delayed transport to the laboratory, specimen fixed in formalin. If an unacceptable specimen is received, the appropriate physician or nursing station will be notified and another specimen will be requested before disposal of the original specimen.

**Specimens received in the laboratory by 1 pm on Monday - Friday and by 8:00 am on Saturday will be processed the same day.**

## General Guidelines for Antimicrobial Susceptibility Testing

### **General**

1. Antimicrobial susceptibility testing is performed on all potential pathogens recovered in any amount from normally sterile body sites or present in significant numbers from sites, which have indigenous flora.
2. Representative isolates from all positive cultured specimens are saved for one week.
3. All blood and spinal fluid isolates are saved indefinitely.
4. Tests are performed daily, and results usually available 24 - 48 hours after isolation of microorganisms.

### **Minimum Inhibitory Concentration (MIC)**

1. The MIC is the lowest concentration of an antimicrobial, which inhibits growth of the microorganism tested.
2. It is achieved using an automated (Vitek), manual broth micro dilution test systems, or E-Test Strips. .
3. The test method is effective for rapidly growing, aerobic, non-fastidious bacteria.
4. Selection of antimicrobials on each test panel is determined based on organism's type (gram positive or gram negative), body site, (urine or nonprime), and spectrum of activity.
5. MIC values (susceptible, intermediate, resistant) are reported in terms of achievable levels of antimicrobial agent in serum and urine.

### **Agar Disk Diffusion (Kirby-Bauer)**

1. The Agar Disk Diffusion test uses a single-potency disk for semi quantitative interpretation (susceptible, intermediate, resistant).
2. It is occasionally used for fastidious organisms or newly introduced antibiotics.

### **Agar Screen Test**

1. The Agar Screen Test uses a single breakpoint concentration of antimicrobial for qualitative interpretation (susceptible and resistant).
2. It is used for Staph aureus (Oxacillin-Methacillin), and Enterococci (Vancomycin).

### **E-Test**

1. The E-Test uses a strip coated with a logarithmic gradient of an antimicrobial agent applied to an inoculated plate.
2. After incubation, an ellipse of inhibition is formed.
3. At the intersection of the ellipse with the strip, the MIC is read from the interpretative scale.
4. It is used for Streptococcus Pneumoniae and other fastidious organisms.

### **Beta Lactamase Test**

1. Beta-lactamases are degradative enzymes capable of hydrolyzing beta-lactam antimicrobials (penicillins, cephalosporins).
2. Routine testing for beta-lactamase production is performed on all *Bacteroides* spp., *Haemophilus* spp., *Moraxella catarrhalis*, and *Neisseria gonorrhoeae* when susceptibility testing is indicated.

### **Selective Testing**

1. **Anaerobes**
  - a. A reference lab on request, performs susceptibility testing of anaerobes only.
  - b. The beta-, test is routinely used on *Bacteroides* spp and other anaerobic gram-positive rods.

- c. A positive test predicts resistance to penicillin and the first generation-cephalosporins.
2. **Enterococci:**
- a. Non-urine isolates of enterococci are tested against ampicillin, vancomycin, and high concentrations of gentamicin and streptomycin.
  - b. All urine isolates are screened for vancomycin resistance (VRE).
  - c. For treatment of serious infections, high doses of ampicillin or penicillin are required; for endocarditis and other life-threatening infections, an aminoglycoside (i.e. gentamicin) must be combined with penicillin to achieve bactericidal effect.
  - d. Occasional isolates of enterococci (*E. faecium*) will be resistant to the penicillins. In this case, vancomycin should be considered for therapy.
  - e. In the treatment of endocarditis, vancomycin alone will not kill enterococci, and the addition of an amino glycoside is required.
  - f. Amino glycoside susceptibility screening is performed to detect "high level resistance".
  - g. Amino glycosides alone are ineffective against enterococci, however, in combination with a penicillin or vancomycin, low concentrations of gentamicin or streptomycin will produce bactericidal synergy.
  - h. In order to predict this synergistic effect, the isolate is tested against very high concentrations of amino glycosides.
3. **Haemophilus Influenzae:**
- a. Isolates of *H. influenzae* are tested with the rapid beta-lactamase test for ampicillin and penicillin resistance.
4. **Mycobacterium Tuberculosis**
- a. All isolates of *M. tuberculosis* are tested for multiple drug resistance using radiometric susceptibility test system.
  - b. The drugs used for testing include; isoniazid, streptomycin, rifampin, and ethambutol.
  - c. Results are available 4 to 7 days after inoculation and interpreted as susceptible or resistant. Mycobacteria other than tuberculosis are sent to a reference laboratory for testing upon request.
5. **Methicillin Resistant Staphylococci**
- a. MICS are routinely performed on all clinically significant isolates of *Staph aureus* and coagulase negative Staphylococci, which includes testing for oxacillin (methicillin) resistance
  - b. Requests for MRSA only are tested by the oxacillin agar screen method.
  - c. When *in vitro* tests show staphylococcal isolates to be resistant to oxacillin or methicillin, none of the other penicillinase-resistant penicillins, cephalosporins, or beta-lactam/beta-lactamase inhibitor combinations have been found effective for treating infection.
6. **Streptococcus Pneumoniae**
- a. All isolates of *S. pneumoniae* are screened for penicillin, ceftriaxone & levofloxacin resistance by the E-test.
    - i. E-test MIC's are interpreted as sensitive (<0.06 meg/ml)
    - ii. relative resistance (0.12 - 1.0 meg/ml),
    - iii. and absolute resistant (>2.0 meg/ml).
  - b. Penicillin- resistant *S. pneumoniae* are also resistant to other antimicrobial agents, including chloramphenicol, tetracycline and SXT.
  - c. These strains also show a decreased susceptibility to other beta-lactam antimicrobials including extended spectrum cephalosporins.
  - d. Blood and spinal fluid isolates are also tested against Ceftriaxone (E-test MIC); and by Kirby Bauer method- Chloramphenicol, Erythromycin, SXT, and Vancomycin.

## Antibiograms

1. Antibiograms representing cumulative antimicrobial susceptibility statistics from general patient and critical care units are prepared yearly. These are available by request from the Microbiology Laboratory.
2. They provide clinician guidance on the choices of empiric therapy, measure trends in antibiotic resistance patterns, and allow pattern recognition that complements epidemiological investigations, so unusual resistant trends can be traced.