



Special Instructions for Microbiology: Improving the Relevance of Microbiology Specimens

Importance of Proper Specimen Collection and Transport

The microbiology laboratory can aid in the diagnosis and management of patients with suspected or confirmed infectious diseases. However, it can do so only if the specimens provided are freshly collected, the quantity satisfactory, they have been properly obtained, are accompanied by the correct patient information, and have been transported in a prompt and satisfactory manner using the recommended collection/transport devices. The following information is given as a guideline for improving the relevance of specimens submitted to the microbiology laboratory. Refer to the individual test entries of this manual for detailed instructions on the collection, handling, and transportation of the majority of specimens encountered.

The viability of microorganisms is often limited in transport medium: specimens should therefore be transported to the laboratory without delay. For delays of 2-24 hours, hold specimens at room temperature unless otherwise specified. Unless otherwise indicated, sterile containers and syringes should contain no preservatives. Call the microbiology laboratory with specific questions not answered in this manual (800-222-5835, ext. 16141).

Guidelines for the Selection of Microbiology and Virology Tests

Specimen Type	Acid-fast Bacilli Culture	Aerobic Bacterial Culture	Anaerobic Bacterial Culture	Feces Culture	Blood Culture	Fungus Culture	Virus Culture	Chlamydia Antigen	Chlamydia Culture	Neisseria gonorrhoeae Culture	Influenza Antigen	RSV Antigen	Yersinia Culture	Ureaplasma/ Mycoplasma Culture	Legionella Culture	Bordetella Culture	Wet Prep	Rotavirus Antigen	Borrelia Culture	CMV Culture/ Antigen	Pinworm Prep	Beta Hemolytic Strep Culture	Grp A Strep Rapid Antigen	Grp B Strep Culture	Cryptosporidium/Giardia Antigens	Ova and Parasite Exam	Yeast/B V/Trichomonas Affirm™ VP111	HSV/VZV Antigen Detection	HSV Culture
Bronchoscopy Specimens	*	*				*	*								*					*									
Blood	*				*	*														*									
CSF	*	*				*	*																						
Dermis						*	*												*									*	*
Exudate (wound, abscess, drainage)	*	*	*			*	*																						
Eye/Conjunctiva		*				*	*	*	*	*																			*
Feces/Rectum ¹	*			*		*	*	*	*	*			*					*			*	*			*	*			
Fluid (not CSF)	*	*	*			*	*		*											*									
Genital Tract ([endo]cervix, vagina, urethra)						*	*	*	*	*				*		*								*			*	*	*
IV Catheter		*																											
Nasopharynx		*					*	*	*		*	*				*													
Sputum/ET	*	*				*	*								*					*									
Throat (see also Exudate/Tissue)						*	*			*												*	*						*
Tissue	*	*	*			*	*	*												*									
Urine	*	*				*								*						*									

CMV, cytomegalovirus; CSF, cerebrospinal fluid; ET, endotracheal aspirate; IV, intravenous

PCR-based tests are preferred for the following: Chlamydia/gonorrhea (urogenital), *C. difficile* (feces), enterovirus/HSV/VZV meningitis (CSF), HSV/VZV (skin), Lyme arthritis (synovial fluid), pertussis. See the reference manual entries on these tests for details.

¹ Submission of a soiled diaper for fecal testing is discouraged. If collection via diaper is necessary, line the diaper with plastic wrap before use; after defecation, remove the feces in the wrap, place both in a clean container, seal container, and promptly transport to the lab.

² Use Group A streptococcal culture for pediatric streptococcal peri-rectal pyoderma, one peri-rectal swab.

Blood Culture Guidelines

Routine blood culture media will reliably detect most common pathogenic bacteria, and many fungi, primarily yeasts. Using specialized media, blood may also be specifically cultured for yeasts and moulds, mycobacteria, and viruses. The following guideline are provided in order to maximize the detection of these microorganisms:

Routine bacterial/yeast blood culture:

- Routinely, two blood cultures (from two separate venipuncture sites) should be obtained per patient during a 24-hour period. A third culture will give an incremental increase in detection. They may be drawn at the same time, but should be collected from two different sites before the administration of antibiotics if possible. Fever of unknown origin, infective endocarditis, and concurrent antimicrobial therapy may require additional culture sets: consult with the directorial staff of the microbiology lab for specific recommendations based on the clinical situation.
- Ideally, blood should be obtained during the chill or temperature spike. Otherwise, collect blood cultures as close to the precipitating event as possible.
- Supplementary and/or special procedures may be necessary to detect unusual types of microorganisms: consult with the directorial staff of the microbiology lab for specific recommendations based on the clinical situation.
- Blood is optimally obtained by venipuncture at separate sites. Obtaining blood for culture from intravascular lines, especially if it is used to administer antimicrobial agents, is discouraged. If it is necessary to collect by IV line, the line must be flushed with an appropriate parenteral fluid by the nursing staff prior to obtaining blood for culture. Collect at least one culture by venipuncture if possible rather than two by catheter.
- Cultures are routinely held 5 days. All positive blood cultures are called to the physician.

Blood Culture Collection

- Blood should be collected in BacT Alert aerobic and anaerobic FAN bottles (bioMerieux, Durham NC), 10mL blood into each bottle, before transport to the lab. Alternatively, blood may be sent in a yellow top Vacutainer tube containing SPS. *Note that there are two yellow top Vacutainer tubes, one containing ACD, the other SPS. Only SPS is acceptable for blood culture. Specimens submitted in ACD will be rejected. Vacutainer tubes are available from Marshfield Labs.*
- Label bottles or tubes with patient identification. Avoid covering the bar code label tab on BacT Alert bottles.
- Disinfect the rubber septa of each blood culture bottle or tube with a separate alcohol wipe. Allow the rubber to air dry. Do not allow the rubber to become contaminated.

Venipuncture

Note: for collection from a venous access device:

- Refer to your institutional policy.
- Prepare the venipuncture site. Identify the vein by palpation.
 - For patients > two months of age, use a Chloraprep swab with a scrubbing action, passing multiple times over the site. Friction is an important part of the cleansing process for this product.
 - For patients \leq two months of age, use an alcohol swab and scrub the site in a circular motion. Allow the alcohol to air dry. Use a Betadine sponge and apply using a circular motion. Starting at a central point and working in a circular motion outward.
- Allow Chloraprep or Betadine to dry for 30-60 seconds. *Do not touch the vein again after this step!*
- Obtain 20mL of blood. (See Low Volume Distribution Scheme, below, if less than 20mL is obtained.)
- If using BacT/Alert bottles:
 - Adult Patients
 - Inoculate 10mL into one FAN-Anaerobic (orange top) and another 10mL into one FAN-Aerobic (green top) bottle. A minimum of 0.5mL per bottle is optimal, but any volume below 10mL will significantly decrease the chances of recovering an organism. Do not overfill bottles.
 - Pediatric Patients
 - Use this table for suggested pediatric blood draw volumes by patient weight:

Blood Volumes Suggested for Cultures from Infants and Children						
Weight of Patient		Total Blood Volume (mL)	Recommended Volume of Blood for Culture (mL)		Total Volume for Culture (mL)	% of Total Blood Volume
kg	lb		Culture Number 1	Culture Number 2		
≤ 1	≤ 2.2	50 – 99	2	–	2	4
1.1 – 2	2.2 – 4.4	100 – 200	2	2	4	4
2.1 – 12.7	4.5 – 27	>200	4	2	6	3
12.8 – 36.3	28 – 80	>800	10	10	20	2.5
>36.3	>80	>2,200	20 – 30	20 – 30	40 – 60	1.8 – 2.7

(Baron, EJ, MP Weinstein, WM Dunne, et al. 2005. Cumitech 1C, Blood Cultures IV. Pg. 4. Coordinating ed., EM Baron. ASM Press, Washington DC.)

- Low Volume Distribution Scheme (Adult and Pediatric)
 - If < 10mL of blood is obtained, divide the sample as follows:
 - >1mL sample, divide equally between both bottles.
 - ≤1mL sample, dispense into FAN aerobic (green top) bottle only.
- If using yellow top (SPS) Vacutainer bottles:
 - Collect two 8.7mL yellow top Vacutainer tubes using aseptic technique. Pediatric: if the adult volume cannot be obtained, the following collections are recommended, in this order: one 8.7mL yellow top Vacutainer tube, or two 3mL yellow top Vacutainer tubes, or one 3mL yellow top Vacutainer tube.
 - Gently tip the bottles or tubes to mix. Promptly transport to the microbiology laboratory. Maintain at room temperature: do not refrigerate or incubate.
- Do not use the Chloraprep swab:
 - On children less than two months of age due to the potential for excess skin irritation and increased risk of Chloraprep absorption.
 - On patients with known allergies to chlorhexidine gluconate or isopropyl alcohol.
 - On an open skin wound.
 - As a general skin cleaner.

Blood for Acid-fast Bacilli and Fungi

Contact the microbiology laboratory for instructions.

Cytomegalovirus antigenemia

One EDTA (lavender top) tube. Two EDTA tubes of blood must be submitted when patients are neutropenic. Store and transport to the laboratory at room temperature within 24 hours of collection. Clinic and hospital patients: arrange to draw blood so that the specimen will arrive in the lab by 1:00 pm Monday through Friday.

Antimicrobial Susceptibility Testing (AST)

AST will be performed on significant bacterial isolates with unpredictable patterns if standardized methods are available based upon Clinical Laboratory Standards Institute guidelines. Methods used include disk diffusion, minimum inhibitory concentration (MIC) by broth microdilution, or MIC by Etest[®] (AB BIODISK, Solana, Sweden.)

The microbiology laboratory uses selective reporting of susceptibility test results in order to promote rational antimicrobial usage. For more information on selective reporting, please contact the microbiology technical manager. If testing against an antimicrobial specific agent is desired contact the microbiology laboratory.

Standardized AST is not available for all bacterial species, in which case an MIC determined by a non-standardized method and without interpretation may be available upon request. Other susceptibility tests (e.g. Serum Bactericidal Test, Minimum Bactericidal Concentration [MBC], and antimicrobial synergy testing) are also available. Contact the microbiology laboratory for more information.

The microbiology laboratory publishes an annual cumulative antibiogram for commonly isolated bacteria available on this website. Printed copies are also available from the microbiology lab.

Stains and Wet Preps

Acid-fast (AFB)

Acid-fast stains are performed on lower respiratory tract specimens, skin, and other specimens to demonstrate *Mycobacterium* sp., the causative agent for tuberculosis and other mycobacterial infections. This stain is routinely performed as part of the acid-fast culture and does not have to be separately requested. If medically indicated an acid-fast stain may be requested STAT. All STAT acid-fast stains are performed on unconcentrated specimens: sensitivity may therefore be lower than a normally processed specimen. Swabs are unacceptable for AFB culture and smear.

Fungus

A fungal stain can be performed on respiratory, wound, fluid, skin, and tissues when looking for fungi. This stain is routinely performed on lower respiratory tract specimens submitted for fungal culture; for other specimen sources, order Fungus, Direct Wet Prep (KOHFS) in addition to fungal culture. **Note** Swabs are only acceptable for the identification of yeasts from the mouth, urogenital tract, and other mucosal surfaces.

Methenamine silver (MS)

MS stain may be ordered on lower respiratory specimens for the detection of *Pneumocystis jiroveci* and other fungi. (The routine Fungus smear by Calcofluor White is recommended for routine fungal microscopic exam due to its shorter turn-around time.)

The MS stain is also used by pathologists for cytological and histological studies. Contact the pathology laboratory for details. **Note:** specimens submitted for cytological studies often require the use of preservatives that are not compatible with microbiological culture studies. Submit separate specimens if both are required.

Gram

The Gram stain is used primarily to detect bacteria and yeasts in clinical specimens. A Gram stain is routinely performed on respiratory, tissue and fluid (except urine) specimens submitted for aerobic and anaerobic culture, specimen volume permitting. Gram stain may be separately ordered on other specimens. Gram stains may be ordered STAT. If swab-collected specimens are submitted for culture and Gram stain, two (2) swabs are required (one each for culture and Gram stain). Gram stains may also be performed on labeled, air-dried slides collected at bedside. The Gram stain is also used in the stool WBC test.

A definitive diagnosis of gonorrhea depends on the detection of its causative agent, *Neisseria gonorrhoeae*, by culture or PCR. However, Gram-negative diplococci contained within leukocytes in a urethral exudate from a male patient may be considered presumptive evidence of gonorrhea. Gram stain of other sources including cervical, throat, or rectal specimens should not be performed for gonorrhea since morphologically similar intracellular organisms are often found in these types of specimens. Urethral swabs from a male patient are used to prepare a smear at bedside. The slide should be labeled, allowed to air-dry and sent to the lab along with a swab specimen for gonorrhea culture. Alternately, two swabs can be submitted in bacterial transport medium and sent to the lab, where the smear will be prepared by laboratory personnel. Gram stain and gonorrhea culture should be ordered.

Vincent's Angina (Trench mouth)

A swab-collected specimen is applied to the surface of a microscope slide, allowed to air-dry, and then sent to the microbiology laboratory. A swab can also be placed into the transport medium and sent to the laboratory where the smear is then prepared by lab personnel, stained, and examined. The requisition form must specify "Rule out Vincent's Angina".