Stanford HEALTH CARE
STANFORD MEDICINE

PATHOLOGY & LABORATORY MEDICINE

Specimen Collection

Home
Test Directory
Test Requisitions
Specimen Collection
Critical Values
Administration
Clinical Departments
Pathology Departments
Esoteric Departments
Featured Services

Specimen Collection > Microbiology

ANTIBIOGRAM CHARTS

LPCH

SUH

Specimen Submission

Request Forms

Use individual requisition forms available from the laboratory. All test requests require a physician's written order to process a specimen. Follow the collection instructions for each type of specimen.

Patient Identification

All patients from whom clinical specimens are obtained must be positively identified, utilizing at least two unique identifiers prior to specimen collection. Positive identification is the responsibility of the person collecting the sample.

Required Information

All specimens must be labeled.

Specimen Labeling: The following information must be legibly recorded on a label affixed in an irreversible fashion to the specimen container:

- Patient's full name (not a nickname)
- Medical Record Number or other unique identifier (ID)
- Date and, if appropriate, time when specimen was obtained
- Specimen source
- Signature/ initials of collector
- The label should be affixed directly to the specimen container and not the bag

Bar-coded pre-printed labels with accession numbers generated by an information system may be used.

- Place the labeled specimen in the provided leak proof sealed plastic biohazard bag
- Place the matching requisition in the outside pouch of the bag

Transport specimens promptly: See specific test for temperature requirements
The date and signature/ initials of the collector must be recorded after the specimen has been collected and after verifying that the patient name and ID on the label agrees with that on the test requisition. This is the single most important factor in preventing errors in patient specimen identification.

Use of a request form wrapped around the container is not acceptable as a specimen label.

Specimens will not be accepted if the information on the specimen label does not match the information on the accompanying requisition.

Required Information on the Requisition Form

On all requests forms, the following information is required

- Patient's name & address
- Patient's gender
- Date of birth
- The last six digits of the patient's social security number or other unique identifier (ID#)
- Date and if appropriate, time of collection
- Test requested
- Type or source of the specimen
- Requesting physician/ or Client Number
- Clinical information if requested
- All applicable medical necessity codes (ICD)
- Complete billing and insurance information

Providing additional relevant information may be important in alerting the laboratory of the need for special handling or specimen work-up.

Tests sent to reference laboratories must have patient history information. The need for such information is indicated on the test request form.

Back To Top

Reliability and Value of Test Results

The reliability and value of test results depends on numerous factors. Improper collection, transport, or processing of a specimen can decrease the quality of patient care or result in unnecessary additional testing or treatment.

Please refer to the Test Directory/Laboratory Guide or call Customer Service at 1- (877) 717-3733 for instructions.

Laboratory personnel cannot label specimens nor complete requisition forms (source, time of collection, patient's name), which they have not collected. Mislabeled (specimen label does not match requisition) or unlabeled specimens will be rejected.

Back To Top

Microbiology General Information and Specimen Collection Instructions

If infection with any of the following organisms in Table 1 is suspected, please alert the Laboratory. Call Customer Services and request the Microbiology Department. Write a warning note on the requisition.

Specimen Rejection Policy

The physician will be notified of the reason for the specimen rejection. The specimen will be held in the Clinical Microbiology Laboratory, for 24 hours before being discarded (unless it has leaked). Action must be taken by the physician's office staff or nursing staff after notification to properly resolve the rejection problem.

Table 1.
Alert Laboratory of the Following Suspected Infections

Bacterial Agents	Fungal Agents
Bacillus anthracis	
Bartonella	Coccidioides immitis
Brucella	
Francisella tularensis	
Pseudomonas pseudomallei	
Yersinia pestis	

Back To Top

Table 2.
Examples of Common Specimen Types - How to Transport

Specimen Type	Transport Device
Aspirate	Anaerobic transport tube
Biopsies	

	For anaerobic culture, place in the anaerobic transport tube. Recommend at least 1 cm3 for complete cultures. If anaerobic culture is not requested, the sample may be placed in a sterile tube with a few drops of sterile non-bacteriostatic saline to keep it moist.
Cervix	Appropriate for GC culture only. Use a Culturette®. Swab. Transport immediately.
Cerebrospinal fluid	Collect in special CSF sterile plastic tube provided in kit. If additional tubes are required, use sterile screw-capped plastic tubes. Anaerobic transport is required if anaerobic culture is required.
Drainages	Use anaerobic transport tube. If anaerobes are not ordered, use sterile tube or Culturette® swab.
Exudates	If swab, use Culturette®. If liquid and anaerobes requested, use anaerobic transport tube.
Fluids	If possible, inject maximum of 10 mL in equal amounts into an aerobic and anaerobic blood culture bottles. Transport additional fluid (at least 1 mL) in a sterile tube for Gram stain and plated media and in an anaerobic transport tube if anaerobic culture is requested. Blood culture bottle is recommended for joint fluids.
Fungus	Aspirate, biopsy, blood, body fluid, bronchoalveolar lavage, hair, nails, sinus, skin, sputum stool, urine or genital. See Fungus Collection Instructions. Collect 2mL or 1cc tissue in a sterile container; 50 mL body fluid, 5 mL aspirates. See Special Blood Culture Collection Section
Pus	If anaerobic culture is requested, inject aspirated pus into anaerobic transport tube. If swab, use Culturette® (aerobic culture only).
Skin scrapings	Place in sterile container.
Sputum, Routine	Obtain the specimen when the cough is productive, early morning is best. Collect in a sterile plastic container. Refrigeration is not recommended.
Sputum for AFB	Submit three (3) first morning expectorate sputum samples, collected on three (3) different days. Refrigerate the samples. At least 3 mL sputum is required for AFB cultures.
Tracheal aspirate (endotracheal aspirate)	Submit specimen in a sterile container with a screw cap lid.
Urethral swab	Culturette® (charcoal preferred)
Urine	

	Collect as clean catch in a sterile plastic container and aliquot into special Vacutainer Urine Culture transport tube with growth inhibitor (gray top). Refrigerate specimen if urine culture transport tube is not used. For urinalysis aliquot into a yellow top urine preservative transport tube.
Vaginal swab	For yeast culture. Collect in a Culturette® and send to the laboratory immediately. If bacterial vaginosis studies are requested, use a second swab to prepare slide or send second swab to laboratory. For group B strep screen, collect both vaginal and anal swab(s). Vaginal swabs are not appropriate for GC culture, only cervical swabs are acceptable for GC culture.
Wound	Collect on sterile aerobic swab using a culturette and transport promptly. If requesting a gram stain collect 2 Culturettes®. Only tissue or aspirated material is acceptable for anaerobic cultures. For anaerobic cultures, use an anaerobic transport device. See Microbiology Brochure for compete information.

Back To Top

Turnaround Time for Results - General

Stat Requests

Gram Stain, KOH, India Ink Preps, and Cryptococcal Latex tests are the only tests routinely available on a stat basis.

Results will be available within 2 hours of receipt in the laboratory depending on the specimen type and preparation time. Results will be called to the ordering physician. Please note on the requisition the phone number to call.

Note: Other tests not performed in the Microbiology section of the laboratory may be ordered STAT, such as Malaria, Rapid Antigen for flu A and B, RSV, and Group A Strep.

Routine Cultures

Preliminary results are usually available 1 day after receipt. Susceptibilities are usually available 2 days after receipt of the specimen. Negative cultures are held 2-14 days depending on specimen source.

Anaerobic Cultures

Tissues and fluids, sent in an anaerobic transport tube, are acceptable for anaerobic culture. Preliminary results are usually available 2 days after receipt. Negative results are routinely held 5 days. Swabs are not acceptable for anaerobic culture. (See Microbiology Brochure)

Fungus Cultures

Preliminary results are available after 2-4 days (for rapid growers). Negative cultures are held 3 weeks. (Surveillance cultures 2 weeks.) Swabs are not acceptable for fungus cultures.

AFB Cultures

Usual time to detect positives is 10 days to 3 weeks. Positives are called. Negative cultures are routinely held 6 weeks. Swabs are not acceptable for AFB cultures, except for skin lesions from which Mycobacterium marinum or M. haemophilum are sought.

Legionella Cultures
Usual time for detection is 4-7 days. Negative cultures are routinely held 7 days.

Parasitology
Results on specimens received Monday-Thursday are usually available within 24 hours, (not performed on weekends).

Serology
Turnaround varies. See specific test.

Specimens sent out to the State Laboratory require a convalescent serum taken 3-4 weeks after the acute serum before testing will proceed. Please label specimens as acute or convalescent.

Specimen Rejection

Inappropriate storage or excessive delays in transport of the specimen may result in the specimen being rejected for culture
Inadequate sample (QNS)
Specimen container contaminated or leaking
Poor quality sample: Sputum samples are screened for evidence of oropharyngeal contamination and will be rejected if failing to pass the screen.

Poor quality sample: Sputums are screened for evidence of oropharyngeal contamination and will be rejected if failing to pass the screen.

Back To Top

Blood Culture Recommendations and Rationale Summary

A series of two to three blood cultures is usually recommended to rule out bacteremia. The following are guidelines approved by the American College of Physicians.

One blood culture is rarely, if ever, sufficient (sensitivity is approximately 90%)
Two blood cultures are necessary and sufficient to rule out or establish a diagnosis of bacteremia when the anticipated pathogen bacteremia is low to moderate for example, in a patient with pneumonia, gastrointestinal sepsis, or fever and neutropenia.
Three blood culture sets should be obtained to rule out bacteremia when the probability of bacteremia is high or when continuous bacteremia is the diagnosis being pursued
Three or at most four blood sets should be obtained to rule out bacteremia when the probability of bacteremia is high and either the anticipated pathogens are also common contaminants as in prosthetic valve endocarditis, unusual organisms, or the patient with suspected endocarditis has received antimicrobials within the prior 2 weeks

More than a series consisting of two to four blood cultures is indicated in some circumstances such as to evaluate clinical importance of low virulence organisms or common culture contaminants, to follow-up patients who have fever lasting more than 3 days after initiation of antibiotic therapy with a second culture series, to obtain fungal cultures when the probability of fungemia is high, or to evaluate bacteriologic cure in patients with serious infections requiring prolonged treatment.
More than three to four cultures/day or more than six/week are usually excessive. For pediatric patients, obtain as much blood as practical (1-4 mL minimum) and use pediatric culture system.

Draw cultures prior to initiating antibiotics

In vivo suppression of organisms may not be reversed in culture
Drawing cultures 2-3 days or up to 2 weeks after antibiotics have been discontinued may be necessary to detect bacteremia with partially treated infections such as endocarditis

Back To Top

Collection of Blood Cultures

Materials Needed

Blood Culture Kit (with SafSite Transfer Set)
Guaze/Coverlett Adhesive Dressing/Sterile Band-Aid/1 inch Paper tape
70% alcohol pledgets or ampules
ChloraPrep One Step (preferred) or 2% tincture of iodine
(1) - 20 mL syringe for each set of blood cultures
Sterile needle or winged infusion set (butterfly)
Gloves
Tourniquet appropriate blood culture bottles or tubes

It is important to take the time to properly disinfect the draw site because contaminated cultures may provoke unnecessary treatment or procedures for some patients. Drawing blood for culture from catheters is not an outpatient procedure and may be performed only by authorized staff. For Hospital in-patients refer to unit specific protocols for drawing blood for culture from catheters.

Blood Culture Collection Procedure:

Identify the venipuncture by palpating the vein
Prepare the site by either of the two following methods
- Preferred method: Using Chlorhexidine (ChloraPrep One Step) by vigorously rubbing the site in a side to side motion
- Tincture of iodine is acceptable but the site must be first gently scrubbed with 70% alcohol followed by cleansing with 2% tincture of iodine vigorously rubbing the site in a concentric fashion beginning at the center of the site and moving outward

Wait 1 minute for the iodine to dry (disinfection does not occur immediately). Do not palpate the vein again after disinfection. If such is required, sterile gloves should be used or the finger used to palpate the vein must also be disinfected.
Disinfect tops of blood culture bottles or tubes with alcohol only. (Iodine damages the diaphragm)
Draw required amount of blood using needle and syringe and, without changing the needle, inoculate appropriate culture bottles or tubes (use SafSite Transfer Set provided in the Blood Culture Kit.
See following Requirements for Blood Cultures. Use of a butterfly may be needed for patients difficult to draw.

Blood cultures should be transported promptly to the Laboratory. Do not refrigerate.

Back To Top

Requirements for Blood Cultures

Routine Cultures

Obtain 20 mL of blood per culture. (One culture set consists of one bottle of aerobic culture medium and one bottle of anaerobic culture medium or two aerobic bottles)
Inoculate 10 mL into each bottle

Obtaining less than 20 ml per culture compromises recovery of organisms and is not recommended

If less than 20 ml but more than 5 ml is obtained for the first set of bottles, divide the blood evenly into the aerobic and anaerobic bottle. If less than 10 ml is obtained for the second set of bottles, inject the entire volume into one bottle.
always note on the requisition the volume of blood in each bottle if less than the recommended 10 ML.
The Laboratory must report this to alert the physician about reason for possible false negative result.

BLOOD CULTURE COLLECTION GUIDELINES
Adult Chart

Amount Obtained	Blood Culture
<10 ml	Inject specimen into aerobic bottle only. Attempt repeat venipuncture. Note volume of blood obtained.
11 – 19 ml	Set 1 – Transfer 10 ml to the aerobic (blue top) bottle and the remainder to the anaerobic (gold top) bottle in the set Set 2 – (Both aerobic bottles) Transfer 10 ml to the first bottle and the remainder to the second aerobic bottle. Important: Write on the requisition that only ____ ml of blood could be obtained. It is important for the physician to know that less than the optimum amount was obtained, because this decreases the sensitivity of the culture.

VOLUME OF BLOOD BY WEIGHT GUIDELINES

Pediatric Chart by Weight

Weight of Patient		Vol/stick (Put in 1 bottle if <5ml)	Total/two (2) Cultures	mL's that = approx 1% volume
Lbs	Kgs			
<19	<9	1 ml	2 ml	2 ml
19-30	9-14	3 ml	6 ml	6-10 ml
31-60	15-27	5 ml	10 ml	10-20 ml
61-90	28-41	10 ml	20 ml	20-30 ml

>90	>42	20 ml	40 ml	>30 ml

Pediatric IsolatorTM tubes are available and hold 1.5 mL of blood
If less than 5ml is collected use only one (1) aerobic bottle unless the patient is an Oncology patient or an immunocompromised patient; in which case divide blood equally into the aerobic & anaerobic bottles
If greater than 5mL blood is collected divide blood equally into both bottles
Always note the volume of blood inoculated into each bottle on the requisition

Reference 1

Special Blood Cultures

Fungal Cultures for Histoplasma and other molds, Malassezia

Obtain 10 mL per culture.
Inoculate 10 mL into Isolator™ tube. Mix well to avoid clots, which compromise organism recovery. Note: Pediatric Isolator™ tubes are available and hold 1.5 mL of blood.

Mycobacterial (AFB) Cultures

Obtain 20 mL per culture
Inoculate 10 mL into each of two heparin tubes (green top)

Other Cultures: Customer Service at 1-877-717-3733 and request the Clinical Microbiology Laboratory for instructions

Transport promptly to the Laboratory - never refrigerate

Communicate with the laboratory when special culture protocols may be needed to recover unusual, slow growing, or fastidious pathogens. Special protocols are infrequently needed, but are available for limited indications

Fungal Blood Cultures (for molds such as, Histoplasma, and in infants, Malassezia).
Notify the laboratory if Histoplasma is suspected
Routine blood cultures are comparable to fungal blood cultures for detecting yeasts.
Notify the laboratory if Cryptococcus is suspected. Note: Recommend also ordering a Foccal serum latex test.
Combination Aerobic/Fungal Cultures
Mycobacterial (AFB) Blood Cultures
Quantitative Blood Cultures
Rule out N. meningitidis, N. gonorrhoeae, Brucella, Legionella, Bartonella
Culture Negative Endocarditis/Fever of Unknown Origin

Fungus

Fungus Specimen collection can only be performed by the ordering client. Specimens for fungus cannot be collected at a Patient Service Center.

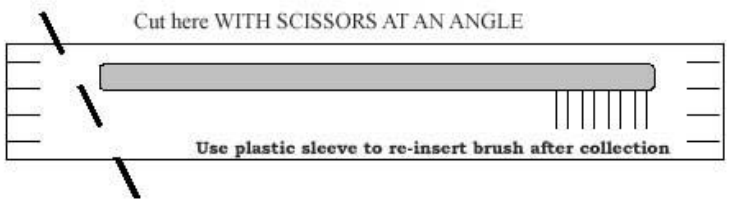
Specimens submitted for fungal culture require tissue sample or fluid, unless yeast only is suspected. Most viable invading organisms are found in the region of the most proximal involvement. Collect a sample of sufficient volume. If only yeast is sought, a swab may be sent.

Brush Collection for Fungus Culture

The best method for collecting samples from skin or nails for fungal detection by direct visual microscopic examination and/or culture is controversial. Nail scrapings, nail cuttings, subungual curettings, and subungual debris have all been collected. Use of a sterile brush (similar to a toothbrush) was recently recommended based on a prior study.

Note: Use scissors to cut the plastic sleeve at an angle to facilitate re-inserting the brush after specimen collection. Order from the supply department.

Special Fungus Collection Brush



Specimen Collection

Skin
Clean skin with 70% alcohol and allow to dry completely. At the peripheral edge of a lesion, brush firmly with the sterile brush. Gently rub the area in circles to collect scales on all bristles. Infected scales will attach to the brush.

Scalp and Hair:
Wipe the infected area with 70% alcohol and allow to dry completely. Use the sterile brush to firmly brush and rub the peripheral edge of the affected region in a circular motion. Rub all bristles of the brush.

Nails
Wipe the top and underside of the nail with 70% alcohol and allow to dry completely. Rub the infected area firmly with the sterile brush, using vigorous back and forth motion.The underside of the infected nail is preferable, as this is softer and more likely to shed infected material.

Label with patient ID label directly on the brush and place the brush back into the plastic sleeve and then into the plastic, biohazard specimen transport bag with the matching requisition form in the outside pocket.

Other specimens may be submitted in a sterile container, tube or envelope; do not add saline or any other fluid to the container.

Submit specimens within 24 hours. Store and transport specimens at room temperature; do not refrigerate or incubate.

Note: nail clippings should also be collected and sent in a small sterile tube or container.

Materials

- 70% alcohol wipes
- Sterile brush (similar to a toothbrush)
- Sterile envelope or container

Precautions:
Inadequate rubbing or brushing may fail to pick up enough infected specimen. Inadequate cleaning prior to specimen collection may result in an overwhelming growth of bacteria, which could in turn inhibit the growth of mold or yeast.

Body Fluid for fungus culture

Collect 50 mL body fluid, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid, ascitic fluid, or thoracentesis fluid in a sterile container. Do not refrigerate or incubate. For optimum recovery, send directly to the laboratory.

Blood for fungus culture:

Stanford's routine blood culture system is adequate for detection of yeast. If mold (Histoplasma, Fusarium, etc.) is suspected, an Infectious Diseases consultation is required to obtain special collection materials. See Special Blood Culture Collection Section for Instructions.

Note: Indicate specimen source, collection date/time, current antibiotic therapy, and clinical diagnosis. If an unacceptable specimen is received, the client will be notified and another specimen will be requested before disposal of the original specimen.

Reference 2

Sputum

Give patient clear instructions. This will enhance the probability of obtaining a good sample of sputum rather than saliva. The specimen should be evaluated and recollected if sample appears to be saliva.

Routine Sputum Culture

- Early morning specimens are preferred since they are usually free of gross oral and nasal secretions. The hours of rest have allowed pooling of sputum in the patient's lungs
- Obtain the specimen when the cough is productive. This represents lung rather than pharyngealsecretions
- Attempt to obtain sputum specimens before antibiotic therapy is initiated
- Do not obtain a sputum specimen immediately after a patient has eaten, chewed gum, smoked, brushed teeth, uses mouthwash, or does any activity that increases oral or nasal secretions
- If the patient is able, encourage rinsing mouth with fresh tap water to remove gross saliva before attempting to cough. (Do not rinse with tap water if Legionella or AFB cultures are ordered)
- If the patient has difficulty coughing, have the patient take a deep breath and hold it as long as possible. Then, while taking the next breath (inhaling), have the patient attempt a deep cough. During the period of holding the breath, the bronchial secretions pool and trigger the coughing mechanism
- Send all specimens to the laboratory immediately in sterile screw-cap containers. Many respiratory pathogens are fragile and may die or be overgrown with storage or delay in transport

Mycobacterium tuberculosis (TB) Sputum Culture

Submit three first morning expectorate sputum samples, collected on three different days. Refrigerate the samples. Gastric aspirates cannot be collected in an outpatient setting; the patient must not have been up and moved around before the procedure is performed. At least 3 mL sputum is required for AFB cultures.

Back To Top

Urine Specimens: Collection of Clean Catch Urine Specimens

Male

- Clean catch urine specimens without cleansing the urethral meatus have been found to be reliable in males
- The hands should be washed thoroughly with soap and water and then dried with a paper towel
- The initial portion of urine is passed into the toilet bowl. A portion of the remaining urine should be passed into a sterile, screw-cap plastic cup

Aliquot a portion to a Urine Culture Transport tube or transport the specimen to the laboratory immediately. Refrigerate if transport is delayed

Female

The patient should remove her undergarments

- The hands should be washed thoroughly with soap and water and then dried with a paper towel
- With one hand the patient should spread her labia and keep them continuously apart until the urine is voided into a sterile screw-cap container
- The patient should cleanse the urethral meatus from front to back
- The patient should void the urine and after the first portion of the urine is passed, a specimen should be caught in the sterile container without stopping the stream.
- The sterile container should be held in such a way that contact with the legs, vulva, or clothing is avoided

Aliquot a portion to a Urine Culture Transport tube or transport the specimen to the laboratory immediately. Refrigerate if transport is delayed.

Urine Specimen for Acid-Fast Bacilli (AFB)

The procedure is the same as for the clean catch urine

- Ideally, the first morning specimen should be taken and approximately 50 mL of urine should be collected from the patient in a sterile screw-cap container.
- Refrigerate
- Smears are not performed.

Back To Top

Wounds, Abscesses, Sinus Tract, Skin Lesions

For reliable culture results, good techniques for site preparation and specimen collection are essential. Tissue taken from the base or leading edge of the wound is more likely to recover viable clinically significant organisms than sampling of necrotic material.

- Swabs are accepted for aerobic cultures only.
- Anaerobic culture is limited to tissue and aspirated material in an anaerobic transport tube. See Microbiology Brochure for more information.

Avoid sampling mucosal flora and use an anaerobic transport device. Only tissue or aspirated material is acceptable for anaerobic cultures.

Transport all microbiology specimens promptly to the Laboratory.

Other Cultures: Call Customer Service at 1-877-717-3733 and request the Microbiology Laboratory.

Back To Top

Scabies Collection

Call 724-8632 for collection supplies if needed.

Place a drop of mineral oil on a sterile scalpel blade.
Scrape top of papule 6-7 times.
Transfer oil, flecks of blood, and scraped material to a clean glass slide.
Place slide in sterile container or petri dish.
Hand-deliver to the lab undisturbed in an upright position.
Swabs are not accepted.

Reference 1

Baron, E.J., M.P. Weinstein, W.M. Dunne, P. Yagupsky, D.F. Welch, and D.M. Wilson. 2005. Cumitech 1C. Blood cultures IV. E.J. Baron, Coord. editor. In Cumitech Series, ASM Press, Washington, DC.
Garza Diana, Becan–McBride Kathleen. Phlebotomy Handbook Blood Collection Essentials Fifth Edition, Appleton & Lange 1999
The National Committee for Clinical Laboratory Standards. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, Approved Standard-Fourth Edition, 1998
The National Committee for Clinical Laboratory Standards, Procedures and Devices for the Collection of Diagnostic Blood Specimens by Skin Puncture, Approved Standard-Fourth Edition. 1999
Lewis, Warren H. Gray's Anatomy of the Human Body Twentieth Edition Revised, New York: Barleby.com 2000

Reference 2

Hubbard, T.W. and J. M. de Triquet. 1992. Brush-culture method for diagnosing Tinea capitis.
Pediatrics 90:416-18. Larone, Davise; Medically Important Fungi, 4th edition, 2002, ASM Press

Back To Top