

**COLLECTION OF BLOOD AND
CEREBROSPINAL FLUID**

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COLLECTION OF BLOOD AND CEREBROSPINAL FLUID

SAFETY¹⁻⁵

The principal risk associated with the collection of blood and cerebrospinal fluid (CSF) is infection with blood-borne pathogens. Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are the pathogens of principal concern. See also Chapter 2 (Laboratory Biosafety) and Centers for Disease Control and Prevention (CDC) recommendations for universal precautions.²

1. CDC recommends that phlebotomists and other health care workers handling or processing blood or blood products be vaccinated against HBV.¹
2. The likelihood of infection after skin exposure to blood containing a viral pathogen depends on the concentration of virus (e.g., viral concentration is much higher for hepatitis B than for HIV), the duration of contact, the presence of skin lesions on the hands of the health care worker, and for HBV, the immune status of the health care worker.² Gloves reduce the incidence of blood contamination of hands during phlebotomy (drawing blood samples), but they do not prevent penetrating injuries caused by needles or other sharp instruments. Some institutions have relaxed recommendations for using gloves for phlebotomy in settings where the prevalence of blood-borne pathogens is low. Institutions that consider routine glove-wearing by all phlebotomies as unnecessary should periodically reevaluate their policy.²

Gloves should always be available to health care workers who wish to use them during phlebotomy.² In addition, the following general guidelines apply. Gloves should be worn: (1) while performing phlebotomy when the health care worker has cuts, scratches, or other breaks in the skin; (2) in situations in which the health care worker judges that hand contamination with blood may occur (e.g., when performing phlebotomy on an uncooperative patient); (3) while performing finger and/or heel sticks on infants and children; and (4) while receiving training in phlebotomy.

3. Wear laboratory coat, and where appropriate, mask, goggles or glasses.
4. Take care not to spill blood or body fluid in collection areas.
5. Examine the outside of collection tubes before removing gloves and remove any contamination with an alcohol platchet.
6. Never recap needle by hand; needle recapping, removal, and protection devices are available commercially.

7. Discard used needles and syringes or needles and holders as a unit in needle-proof (Sharps) containers.
8. Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids (See Chapter 2). Should a needlestick occur, immediately remove gloves and wash hands. If blood splashes onto mucous membranes or into the mouth, rinse immediately. Secure permission to test patient's blood for HBV and HIV. Report needlestick to supervisor or appropriate infectious disease personnel. (See Chapter 2.)
9. Hands should be washed immediately after gloves are removed and when leaving the work area. Any time you leave the immediate workplace, remove and discard latex gloves and wash hands.
10. For detailed instructions for cleaning up after a spill see Chapter 2. Briefly, collect paper towels and gloves used to clean up spills and/or wipe work areas and place in discard container, autoclave- or double-bag, label as infectious, and incinerate or dispose of according to institutional policies. Decontaminate all laboratory glassware, disposable material, and waste material suspected of containing, or known to contain HIV, preferably in an autoclave, before they are washed, discarded or recycled. Glassware, pipette tips, and needles may be soaked in a disinfectant before autoclaving.
11. Always store tubes containing serum samples in an upright position.
12. When shipping a sample to a reference laboratory, secure the top and seal with tape or Parafilm. Indicate on the serum sample whether it has been heated. Place sample in a leakproof, sealable plastic bag inside a container containing absorbent tissue impregnated with a germicide. Place specimen documentation around the outside of the container in a sealable plastic bag and place both in a container that cannot be crushed.
13. Specimen documentation and laboratory records must be considered contaminated after any contact with serum or blood. These papers should remain in the laboratory area.
14. Specimens received in shipping containers should be unpacked by properly trained and protected personnel. Any specimens in containers whose internal pressures may be different from ambient pressures should be opened in a biological safety cabinet (Class I or II). Otherwise, open packages in an appropriate area of the laboratory (a package may contain a broken container; see step 4). Masks and safety glasses with side shields offer protection and are encouraged for routine laboratory work.

BLOOD COLLECTION⁶

Serum is the specimen of choice for all tests for syphilis. Plasma samples cannot be used in tests requiring heated samples, and their use has not been evaluated in the microhemagglutination test

for *Treponema pallidum* antibodies (MHA-TP). Plasma may be used in the macroscopic card tests;^{7,8} however, their use should be limited to:

- 1) Specialized laboratories, such as blood banks,
- 2) Field laboratories without centrifugation facilities,
- 3) Situations in which a serum sample cannot be obtained.

Plasma specimens should be used only for qualitative testing.⁸ See specific test directions in manufacturer's product insert for the anticoagulants that may be used in a particular macroscopic card test.

Equipment

1. Red top or serum separator vacuum collection tubes without anticoagulant. Holder to secure evacuated tube and sterile blood collection needle 18-21 gauge for adults or 21-23 gauge for children

or

2. Plastic or glass syringe and sterile blood collection needle 18-21 gauge for adults, or for children, 21-23 gauge needle or 20-23 gauge winged infusion set with attached tubing
3. Tourniquet
4. 70% alcohol-and-gauze pad or alcohol prep pads
5. Dry gauze pads and adhesive tape
6. Safety face shield, goggles or glasses
7. Latex gloves
8. Protective clothing
9. Test tube rack or leakproof plastic bags
10. Discard containers for collection apparatus (Sharps container) and planchets or gauze
11. 1:10 dilution of household bleach

Venous Blood

1. Label collection tubes to identify specimens. Special warning labels need not be used since all tubes containing blood should be handled as if they contain infectious material, according to universal precautions.^{2,5}
2. Have the patient form a fist so that veins become more prominent. The larger superficial forearm veins are used most frequently.
3. Palpate the path of the vein with the index finger.
4. Use an alcohol pad or swab to cleanse the venipuncture site with a circular motion and allow area to dry.
5. Assemble needle and vacuum tube holder while the needle is still sheathed.
6. Apply tourniquet about 3 to 4 inches above the venipuncture site.
7. Insert the blood collection tube into the holder and onto the needle up to the guideline on the needle holder.
8. Remove sheath from needle.
9. Grasp the patient's arm firmly, using your thumb to draw skin taut. Your thumb should be 1 to 2 inches below the venipuncture site.
10. With the bevel up, line up needle with the vein and enter vein. Immediately grasp the flange of the holder and push the tube forward until the butt end of the needle punctures the stopper.
11. Remove the tourniquet as soon as blood flow is established.
12. Fill tube until vacuum is exhausted. Draw additional tubes if necessary.
13. Open the patient's hand and lightly place dry gauze over the venipuncture site.
14. Apply slight pressure to the pad and slowly remove the needle.
15. Slip the gauze over the puncture site, continuing mild pressure, and apply an adhesive tape.
16. Instruct the patient to leave the bandage on for at least 15 minutes.
17. Dispose of the blood collection needle and holder as a unit in a puncture resistant container. Never bend, break, or recap needles.

Note: If a syringe is used to collect blood from the patient, slight modifications need to be made. After drawing the patient's blood, draw back slightly on the syringe plunger to remove blood from the needle. Remove and properly discard the needle. Remove the top from the tube and gently expel the blood from the syringe into the tube. Do not fill the tube more than 2/3 full. Replace the top of the tube.

18. Should blood be on the outside of collection tube, decontaminate with alcohol or household bleach on gauze.
19. Place tube upright in test tube rack.

Cord Blood

The ideal specimen for neonatal testing is the infant's serum as obtained by heel stick procedure, if the mother's serologic result is reactive. However, cord blood may be used for baseline screening when no other specimen is available. Since cord bloods are easily contaminated with Wharton's jelly, which can cause false-positive nontreponemal test results, the laboratory should accept only those specimens that have been properly collected. **Serology on a cord blood specimen *cannot* be used instead of serology on a specimen of maternal venous blood.**

Additional Equipment

1. Clamps
2. Tube with opening ≥ 25 mm and stopper

Procedure

1. Wear gloves, protective garment, and safety glasses.
2. Clean the outside of the umbilicus, double-clamp, and make a fresh cut.
3. Collect blood in a tube with an opening large enough that blood does not run down the outside of the tube.
4. Decontaminate the outside of the tube with diluted household bleach, disinfectant or alcohol planchet, if necessary; and stopper tube.
5. Place tube upright in test tube rack.

BLOOD PROCESSING¹² AND HANDLING

Equipment

1. Centrifuge with or without refrigeration (When using a refrigerated centrifuge, set the temperature at 20E - 25EC.)
2. Tubes and closures for separated serum (If specimens are to be sent to a reference laboratory, use screw-cap tubes with external threads.)
3. Gloves, safety glasses, and protective clothing
4. Mechanical pipetting devices
5. Self-adhesive labels (Multiple sets of numbered labels that can be transferred from tube to tube with the specimens as it is being processed are preferred.)
6. Tube rack
7. Leakproof, sealable plastic bags
8. Discard containers

Serum

1. Wear laboratory coat and gloves to process blood and body-fluid specimens.
2. After blood collection, stand the collection tube upright for a minimum of 20-30 minutes at room temperature (23° - 29°C) so that a clot will form.
3. In general, centrifuge the specimen within 60 minutes after collection. Do not rim clot before centrifugation. Properly balance centrifuge and use safety cups with lids, if possible. With tube stopper in place, centrifuge clotted blood at 1000-1200 x g for 10 ± 5 minutes.
4. If a tube should break during centrifugation, remove and wipe unbroken tubes with disinfectant impregnated paper towels. Place centrifuge carrier in germicide so that carrier is covered. Do not attempt to remove broken glass until carrier has been immersed in diluted household bleach for at least 10 minutes. Follow any specific decontamination recommendations provided by the centrifuge manufacturers. Remove broken tubes with forceps and place in waste containers for disposal.
5. After centrifugation, remove stoppers from tubes, working either in a biological safety cabinet (Class I or II) or in a designated area of the laboratory. If the stoppers must be removed outside a biological safety cabinet, wear eye protection and cover the stopper with an absorbent paper or cloth and gently twist the stopper to remove. After use, place protective papers and the stopper in discard containers.

6. Use a mechanical pipetting device to carefully transfer serum off the cells to the labeled test or storage tube; stopper the tube. Do not pour serum off cells. Do not mouth-pipette. If the outside of the tube is contaminated, wipe it with disinfectant or a 1:10 solution of household bleach.
7. Discard tube containing clot and cellular debris.
8. As a safety precaution, serum samples may be transferred to a clean tube and heated for 20 minutes at 56°C in a waterbath without affecting the results of syphilis serologic tests. It is not certain how effectively heating at 56°C for 20 minutes destroys HIV in serum, but heating small volumes of serum samples reduces residual infectivity to less than detectable levels.^{9,10} The use of heated samples should not alter or replace adherence to universal precautions.¹⁰ Heating of serum samples may preclude their use in later serologic tests for HIV-1 antibody.¹⁰
9. When heating serum samples, label the transfer tubes for samples. Serum should be at room temperature before heating and testing (10-30 minutes).
10. Store serum at refrigerator temperature (2° - 8°C), if testing is to be delayed more than 4 hours. If a delay of more than 5 days is anticipated before testing, freeze the specimen at ≤-20° C. Avoid repeated freeze-thawing of specimens.

Plasma

Processing and handling of plasma is identical to that for serum with the following exceptions:

1. Plasma may be retained in the original collection tube if the test is to be performed immediately. Otherwise, plasma should be removed from cellular elements. Specimens collected with anticoagulant can be centrifuged within minutes after they are collected.
2. Plasma specimens must be used within 48 hours.^{7,8}
3. Store plasma specimens at refrigerator temperature (2° - 8°C) if testing is to be delayed more than four hours. Plasma samples must be at 23° - 29°C (73° - 85°F) at the time of testing.
4. Plasma specimens cannot be heated.

Criteria for Acceptable Serum and Plasma Specimens

1. An acceptable specimen should not contain particulate matter that would interfere with reading test results. Specimens that are excessively hemolyzed, grossly contaminated with bacteria, chylous or otherwise extremely turbid are unsatisfactory for testing. A specimen is too hemolyzed for testing when printed matter cannot be read through it.
Note: Hemolysis may be caused by transporting blood in freezing or extremely hot weather without proper insulation.

2. Not all unsuitable samples should be discarded or not analyzed. When an unsatisfactory sample is received in the laboratory, notify the requesting physician and discuss whether testing is appropriate for that specimen. If a test result is still desired by the ordering physician, then the condition of the sample must be stated on the report, and a notation made on any limitation in test result interpretation.¹¹

COLLECTION OF CEREBROSPINAL FLUID¹³⁻¹⁵

Lumbar puncture must be done ONLY by qualified medical personnel. Certain risks are associated with lumbar puncture, including, uncommonly, serious complications such as brain herniations, infection, and persistent pain, and more commonly, transient headache and paresthesia. Lumbar puncture may be contraindicated for patients with increased intracranial pressure or coagulation deficits because of the increased risk of herniation or local bleeding respectively. The Venereal Disease Research Laboratory (VDRL)-CSF test is the only standard serologic test for CSF. The fluorescent treponemal antibody-absorption (FTA-ABS) test, Western blot for *T. pallidum* and polymerase chain reaction (PCR) are experimental test procedures in which CSF may be used.

Equipment

1. Needle (use of 20 gauge or less may reduce the frequency of headaches)
2. Stylet
3. Three leakproof tubes
4. 2% tincture of iodine
5. Dry gauze pads and adhesive tape
6. Safety face shield, goggles or glasses
7. Latex gloves
8. Protective clothing
9. Test tube rack or leakproof plastic bags
10. Discard containers

Procedure¹³⁻¹⁵

1. Have the patient lie curled in the lateral recumbent position on a firm surface with the back just at the edge of the table. The vertical plane of the back should be perpendicular to the table surface.
2. Disinfect site with 2% tincture of iodine.
3. Insert needle with stylet at L3-L4 or lower for adults. In small children and infants, the conus medullaris extends lower than in adults; insert needle with stylet at L4-L5 or lower.
4. Upon reaching subarachnoid space, remove stylet.
5. Collect 1-2 ml of fluid into each of 3 leakproof tubes.
6. The risk of headache may be reduced by placing the patient in a prone position for several hours after the lumbar puncture.¹⁵

Processing

1. Perform total protein determinations according to standard procedures.
2. Cell counts must be done within 2 hours after the lumbar puncture.
3. Centrifuge at 1000-1200 x g for 10 ∇ 5 minutes and transfer to a clean, labeled tube.
4. If testing is to be delayed more than 4 hours, store the CSF sample at refrigerator temperature (2° - 8°C). If testing is to be delayed more than 5 days, freeze the sample at temperatures \leq -20° C. Avoid repeated freezing - thawing of specimens
5. Specimens must be at room temperature, 23° - 29°C (73° - 85°F), at the time of testing.
6. Do not heat spinal fluid before testing.

Criteria for Acceptable CSF Specimen

1. An acceptable CSF specimen should not contain particulate matter that would interfere with reading test results.
2. CSF specimens with traces of blood (\geq 0.8 μ l of blood/1 ml of CSF¹⁶) are unsuitable for testing, except for the VDRL-CSF test. For the VDRL-CSF, samples that are light red (pink) (\geq 3 μ l of blood/1 ml of CSF¹⁶) are unsuitable.
3. Not all unsuitable samples should be discarded or not analyzed. When an unsatisfactory sample is received in the laboratory, notify the requesting physician and discuss whether testing is appropriate for that specimen. If a test result is still desired by the ordering

physician, then the condition of the sample must be stated on the report, and a notation made on any limitation in test result interpretation.¹¹

Note: ASpecimen Collection and Processing of Lesion Material and Tissue Samples@ for direct microscopic examination are described in detail in Chapters 5 and 7.

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