

Specimen Collection and Preparation

Laboratory test results are dependent on the quality of the specimen submitted. It is important that all specimens and requisitions be properly labeled with the full name of patient, collection date, and origin (source) of specimen, when applicable.

If there is any doubt or question regarding the type of specimen that should be collected, it is imperative that Saint Francis Medical Center be called to clarify the order and specimen requirements.

Specimen Labeling

All specimens submitted to the laboratory for testing must be properly labeled. Minimally, all specimens must be labeled with patient's full name and date of birth. Inpatient specimens must also include a medical record number and initials/tech code of person procuring the specimen.

Blood Collection

Most laboratory tests are performed on anticoagulated whole blood, plasma, or serum. In general, specimens should be refrigerated until they are transported to the laboratory. Please see the alphabetical test listing for specific requirements.

- **Plasma:** Draw a sufficient amount of blood with indicated anticoagulant to yield necessary plasma volume. Gently mix blood collection tube by inverting 6 to 10 times immediately after draw. If required, separate plasma from cells by centrifugation within 20 to 30 minutes.
- **Serum:** Draw a sufficient amount of blood to yield necessary serum volume. Allow blood to clot at ambient temperature. Separate serum from clot by centrifugation within 20 to 30 minutes.
- **Whole Blood:** Draw a sufficient amount of blood with indicated anticoagulant. Gently mix blood collection tube by inverting 6 to 10 times immediately after draw.

Draw Order

When multiple tubes are drawn, it is important to prioritize drawing order to help prevent contamination. The accepted draw order is to fill sterile blood culture tubes followed by blue-top tubes filled completely, then clot tubes (eg, red-top tube, gold-top tube), and anticoagulated tubes next (eg, green-top tube before lavender-top tube and pink-top blood bank tube).

After lavender-top and pink-top tubes are drawn, black-top and grey-top tubes are drawn last. A discard tube must be drawn prior to a blue-top (citrate) tube when only a coagulation test is to be drawn and the blue-top tube is always filled to the target volume shown on the tube.

Specimen Collection Tubes Available

The following is a list of tubes referred to in the alphabetical test listing:

- **Green-Top (Sodium Heparin) Tube:** This tube is used for drawing heparinized plasma or whole blood for special tests.
Note: After tube has been filled with blood, immediately invert tube several times in order to prevent coagulation.
- **Grey-Top (Potassium Oxalate/Sodium Fluoride) Tube:** This tube is used to preserve glucose in whole blood and for some special chemistry tests.
Note: After tube has been filled with blood, immediately invert tube 6 to 10 times in order to prevent coagulation.
- **Lavender-Top (EDTA) Tube:** This tube is used for most hematology tests.
Note: After tube has been filled with blood, immediately invert tube 6 to 10 times in order to prevent coagulation.
- **Light Blue-Top (Sodium Citrate) Tube:** This tube is used for drawing blood for coagulation studies.
Note: It is imperative that tube be filled to fill line. The ratio of blood to anticoagulant is critical for valid prothrombin time results and other coagulation tests. Immediately after draw, invert tubes 6 to 10 times to activate anticoagulant.
- **Mint Green-Top (Lithium Heparin Gel) Tube:** This tube is used for drawing some chemistry tests such as electrolytes, BUN, and creatinine.
Note: After tube has been filled with blood, immediately invert tube 6 to 10 times in order to prevent coagulation.
- **Pink-Top (K₂ EDTA) Tube:** This tube is used for immunohematology (Blood Bank).
Note: After tube has been filled with blood, immediately invert tube 6 to 10 times in order to prevent coagulation.
- **Red-Top Tube:** This tube has no additives and is used for drawing serum for selected chemistry tests as well as clotted blood for immunohematology (Blood Bank).

- **Royal Blue-Top Tube:** There are 2 types of royal blue-top tubes—one with anticoagulant EDTA and the other with no additive. These are used for drawing whole blood or serum for trace element analysis. Refer to individual metals in the alphabetical test listing to determine tube type necessary.
- **Serum Gel Tube:** This tube is used for routine chemistries.
Note: Invert tube to activate clotting; let stand for 20 to 30 minutes before centrifuging for 10 minutes. If frozen serum is required, pour off serum into plastic vial and freeze. Do not freeze gold-top VACUTAINER® tubes.
- **Special Collection Tube:** Some tests require specific tubes for proper analysis. Please contact the laboratory prior to patient draw to obtain correct tubes for metal analysis or other tests as identified in the alphabetical test listing.
- **Yellow-Top (ACD) Tube:** This tube contains ACD—used for drawing whole blood for special tests.

Serum Collection

When serum is the required specimen, use of a barrier tube will provide the most accurate results with exception of therapeutic drugs. Draw blood specimen using usual venipuncture technique (see “Venipuncture/Fingerstick/Heelstick Collection” in “Specimen Collection and Preparation” in “General Information”) of filling tube completely. Gently invert barrier gel tube 6 to 10 times to mix clot activator with blood. Allow blood to clot in a vertical position for 30 minutes. This insures complete clot formation. An incomplete clot will allow latent fibrin to contaminate serum and inhibit flow of gel.

- **Referral Clinics:** Centrifuge at 3,000 rpm to 3,500 rpm for 10 to 15 minutes. Remove specimen from centrifuge. A gel barrier will have formed, separating cells from serum. All of the separation gel should have moved from bottom of tube to form a barrier layer. The specimen is now ready to be transported to the laboratory. Do not re-centrifuge or remove stopper if a barrier tube is requested as specimen of choice. If multiple aliquots or specially prepared specimens must be submitted, use plastic serum vials to submit specimen.
- **Saint Francis Medical Center Patient Care Zones:** Please transport tubes as soon as possible to the laboratory via pneumatic tube system or manually if tube system is unavailable.

- **Frozen Serum:** Draw and prepare serum as described above. Pour off serum into plastic serum vials. **Submit separate aliquots for each frozen test requested.** Freeze plastic serum vials immediately. **Do not freeze glass vacuum tubes. Submit frozen specimens on a separate requisition and in a separate envelope from non-frozen specimens.**
- **Therapeutic Drug Serum:** Preferred specimen for therapeutic drug levels is serum from a plain, red-top tube. **(No barrier gel.)** Gel in the barrier tube may result in falsely lowered drug levels if serum sits on gel for an extended period of time (>1 day). If a specimen is drawn in a barrier tube, centrifuge after a 30-minute clotting period and separate serum immediately into a plastic vial to minimize binding of the drug to barrier gel.

Plasma Collection

Draw blood specimen in anticoagulated tube specified by test requirements. Gently mix blood collection tube by inverting 6 to 10 times immediately after draw. Centrifuge at 3,000 rpm to 3,500 rpm for 10 to 15 minutes. Separate plasma from cells as soon as possible. Avoid transferring cells with plasma. Mint-top barrier tubes do not have to be separated, only spun. Insure patient identification information is on the transfer tube and that the tube is tightly capped.

Whole Blood Collection

Draw a sufficient amount of blood with indicated anticoagulant. Gently mix blood collection tube by inverting 6 to 10 times immediately after draw. Specimen is now ready to be transported to laboratory.

24-Hour Urine Collection

All 24-hour urine collections must be labeled with patient name and date of birth. Because proper collection and preservation of a 24-hour urine specimen are essential for accurate test results, patients should be carefully instructed in the proper procedure.

Patient should consume a normal amount of liquids unless otherwise instructed by the physician. No alcoholic beverages should be consumed during this period.

- Patient should empty his or her bladder in the morning, discard urine, and note exact time.
- From this point on, all urine voided must be collected in the clean, labeled container provided by the laboratory containing proper preservatives. Do not remove any powder, liquid, or tablets from container before beginning collection.

- Exactly 24 hours after time noted previously, bladder should be emptied and this urine added to collection container.
- Urine should be kept refrigerated during collection, and delivered to the laboratory as soon as possible.
- Bring request form given to you by your physician or the laboratory when bringing the specimen back to the laboratory.

Cultures

- *Abscess, Body Fluid, and Wound Drainage*: Specimens are processed for anaerobic bacteria when submitted for routine culture and susceptibility testing. Fluid in a sterile container or syringe is the preferred specimen. If swabs must be sent, include an *Eswab*. **Do not refrigerate** these specimens as many anaerobes are sensitive to cold temperatures.
- *Blood*: Whole blood should be drawn into both an aerobic and anaerobic *BacT-Alert* blood culture bottle using a sterile technique (eg, use Chloro-Prep® scrub and allow to dry). Blood cultures are usually drawn when patient is spiking a temperature and at 2 different times to verify an infection (ruling out any contamination). Common practice is to draw 2 specimens 15 to 30 minutes apart, unless otherwise specified.
- *Body and Joint Fluid*: Serous and joint fluid tend to clot shortly after collection, making laboratory analysis difficult. For basic laboratory analysis (eg, cell counts with differential and crystal identifications), a lavender-top (EDTA) tube and a red-top tube are required. These should be processed as soon as possible. Outreach clients should contact the laboratory for specimen pick-up.
- *Genital (Urethral, Vaginal, and Cervical)*: JEMBEC® plates for *Neisseria gonorrhoeae* and small urethral swabs are available from the laboratory. Send Jembec® plates in its CO₂ bag and culture transport tube or urethral swab to the laboratory. **Do not refrigerate.**
- *Nasopharyngeal Specimen for Influenza and Respiratory Syncytial Virus (RSV) Testing*: These tests require a mini-tip swab available from Microbiology Department.
- *Spinal Fluid and Other Body Fluid*: Specimen should be collected in a sterile container and delivered to the laboratory immediately. Outreach clients should contact the laboratory for pick-up as soon as possible.
- *Sputum*: An **early-morning** specimen is preferred for routine culture and sensitivity, acid-fast bacillus, or fungus culture. Have patient rinse mouth with water, discard, and then collect a deep-cough sputum into a sterile, screw-capped container. Refrigerate specimen if there is a delay in transport to the laboratory.
- *STD Specimen for Chlamydia and Neisseria gonorrhoeae*: Specimen requires a *XPert* swab collection or first 15 mL to 20 mL of “dirty” urine. Swab must remain at ambient temperature and urine must be kept refrigerated.
- *Stool Specimen for Clostridium difficile Toxin (CDT)*: Specimen should be collected in a plastic stool or urine container and refrigerated until delivery to the laboratory.
- *Stool Specimen for Culture/Pathogens*: Stool specimen for culture should be sent to the laboratory and be kept at ambient temperature until delivered. Since stool is not sterile, it is unnecessary to collect specimen in a sterile container. If collected in a bedpan or bedside toilet, do not allow urine contamination of stool specimen. Stool culture specimen may also be collected in a urine or stool container, Cary Blair (a pink-colored liquid) media, or a feces laden swab. Swab is the least preferred specimen and should remain at ambient temperature until delivery to the laboratory.
- *Stool Specimen for Fecal Fats and Fecal Leukocytes*: Specimen should be collected in a plastic stool or urine container and be kept at ambient temperature.
- *Stool Specimen for Giardia/Cryptosporidium Enzyme Immunoassay (EIA)*: Stool specimen for *Giardia* and/or *Cryptosporidium* should be collected in a urine or stool container or in *Cary Blair* or (PVA-formalin fixative sets. PVA- formalin fixative sets are the preferred media and should be kept at ambient temperature. Acceptable for substitute.)
- *Stool Specimen for Ova and Parasites*: Ova and parasite stool specimen should be collected in a urine or stool container or in *Ecofix* and *Cary-Blair* (PVA-formalin fixative sets and must be kept at ambient temperature. Acceptable for substitute.)
- *Urine Specimen*: Urine specimen collected for culture, sensitivity, and colony count should be placed in the refrigerator until delivered to the laboratory. (This is necessary because microbial organisms multiply at ambient temperature with a 2-fold increase in numbers at 20 to 30 minute intervals.) This helps to produce an accurate colony count.
- *Viral Specimen*: Specimen should be collected in viral/*Chlamydia* transport media following collection instructions.

Submission of Tissue for Histology/ Cytology

Surgical pathology request forms are used for all tissue/body fluid submitted to Pathology Department. The following is the procedure for submitting specimen to Pathology Department:

- A “Surgical Pathology Request Form” will accompany each tissue specimen for pathological review.
- Request form must include the following information on requisition:
 - Patient’s name (first, last, and middle initial), age, and date of birth
 - Clinical diagnosis or pre or post diagnoses
 - Location in or on body from which specimen was obtained (source)
 - Surgical procedure performed
 - Date and time specimen was collected and who it was collected by
 - Submitting physician
- If specimen is being sent from a Saint Francis Medical Center area such as surgery or patient care zone, the medical record number and account number must be included on the “Surgical Pathology Request Form.”

Tissue Specimen Collection and Handling

- All specimens for pathological review, excluding tissue for frozen section diagnosis and tissue for special studies such as electron microscopy; immunofluorescence; and flow cytometry, shall be submitted in a properly labeled tissue specimen container containing 10% buffered formalin in the ratio of 20 volumes of formalin to 1 volume of tissue.
Note: Large specimens must not be forced into small containers. Please do not place specimens from more than 1 site in a surgical container.
- Each container must be labeled with the following information:
 - Medical record number and account number
 - Patient’s name (first, last, and middle initial)
 - Body site from which specimen was collected (source)
 - Physician’s name
 - Date and time of collection
- If specimen is being sent from a Saint Francis Medical Center area such as surgery or patient care zone, the
- Amputated limbs are placed in the walk-in refrigerator by surgery staff. The requisition is taken into the pathology laboratory and technicians are notified of limb in cooler.
- If after hours, put the requisition in the pathology laboratory on the appropriate counter.
- Every specimen submitted to the Pathology Department must have a pathology requisition form that is accurately and completely filled out for the tissue to be accepted.

medical record number and account number must be included on the container:

- Submission of unfixed specimens must be brought to the attention of a laboratory/histology technician.
- When identification of special tissue or areas of an excised specimen are marked by a suture or clip, verbal or written notice must be given to the histology technician.

Surgical Specimen Submission

- At the time of removal, each specimen container shall be labeled with the following information:
 - Patient’s name (first, last, middle initial)
 - Medical record number
 - Hospital account number
 - Body site from which specimen was collected
 - Submitting doctor
 - Date and time of collection
- Specimens are placed in appropriate preservative (10% buffered formalin at a ratio of 20 volumes of formalin to 1 volume of tissue), unless a frozen section has been requested. Refer to Saint Francis Medical Center’s Operating Room (OR) policy on “Frozen Section Specimens.” A formalin biohazard sticker must be placed on all containers using formalin as a preservative.
- A “Surgical Pathology Request Form” shall be completed with the following information.
 - Patient’s name (first, last, and middle initial)
 - Age and date of birth
 - Hospital account number
 - Medical record number
 - Clinical history
 - Pre-op and/or post-op diagnoses
 - Surgical procedure performed
 - Type of specimen submitted
 - Location from which the specimen was removed
 - Date and time specimen was collected
 - Submitting doctor
 - If submitting more than 1 specimen, a detailed and itemized listing of each specimen is required
- If several biopsies or specimens are obtained from the same patient, care should be taken that each is placed in a separate jar and individually labeled. Each specimen should be identified on the requisition form.

Venipuncture/Fingerstick/Heelstick Collection

Please find link on left pane for procedure

Collection Variables

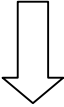
The person drawing the specimen from the patient must be certain that the correct individual is drawn. Correct identification must be recorded on the specimen tube and on the request form. Specimen tube and request form will be verified upon receipt in laboratory. A discrepancy between name on request form and name on specimen tube is grounds for specimen rejection.

- ***Diurnal Variable:*** In evaluating clinical significance of a result, time of day specimen was drawn needs to be considered. Some tests demonstrate significant diurnal variation.
- ***Fasting:*** An overnight fast is required for most fasting specimens. Some tests, however, require further dietary restrictions. For these tests, nothing should be eaten for 12 hours prior to specimen draw. The evening before, the meal should contain no fatty foods and be finished by 6 p.m.
- ***Hemolysis:*** Hemolysis refers to abnormal lysis of red blood cells. Factors contributing to in vitro hemolysis include: a) wider bore needle, b) mixing with an oxalate anticoagulant, c) vacuum tubes, d) not allowing alcohol applied to skin to dry, e) centrifugation and separation steps. Hemolyzed serum or plasma varies in color from faint pink to bright red, rather than normal straw color.
- ***Icteria:*** Bilirubin present in serum will result in a noticeable “jaundice” color. This ranges from a dark to a bright yellow rather than normal straw color. Bilirubin will interfere with certain assays.
- ***Lipemic (Turbidity):*** Turbidity caused by elevated triglyceride concentration in serum will cause elevation in substances whose measurements are based on absorbance. A lipemic serum specimen will be cloudy or milky. A recent meal produces a transient lipemia, so fasting before specimens are drawn is desirable.
- ***Stasis:*** Prolonged use of a tourniquet may elevate laboratory results. This results in a stasis or pooling of blood above constriction. Significant elevation of serum constituents may be seen with as short as a 3 minute application of a tourniquet.
- ***Storing/Transport:*** Specific instructions for storing and shipment of specimens for individual tests are listed under the individual test listings. Please follow them carefully.

Phlebotomy Order of Draw

GUIDE FOR PHLEBOTOMY TUBE TYPES IN ORDER OF DRAW

Note: If more than 4 tubes indicated to draw, call laboratory for consultation at 573-331-5147.

Tube Color Draw Order	Number of Gentle Inversions After Drawing
<div style="text-align: center;">  </div> Blood Culture Vials: x Aerobic—Purple X Anaerobic—Red	
Blue (Sodium Citrate)	3-4
Gold (Clot Activator With Gel)	5
Red (Clot Activator/Plastic)	5
Mint Green (Lithium Heparin)	8-10
Dark Green (Lithium Heparin - No Gel)	8-10
Pink (EDTA)	8-10
Lavender (EDTA)	8-10
Grey (Potassium Oxalate/Sodium Fluoride)	8-10
Black (Snake Venom)	3-4

Always use 2 identifiers to verify patient identification. These same 2 identifiers must be included on the tube(s) and the identifying label must be attached to the specimen container(s) at the time of draw by the collector. Mix all tubes as they are being drawn. Failure to do so may jeopardize the specimen.

If using Saint Francis' barcode labels, please include all labels if 1 tube is drawn for multiple tests.

Appendix A

Maximum Amounts of Blood to be Drawn on Patients <14 Years of Age			
Patient's Weight in Pounds	Kg/(approx)	Maximum Amount to be Drawn at Any 1 Time	Maximum Amount of Blood (Cumulative) During Given Hospital Stay (1 Month or Under)
6-8	2.7-3.6	2.5 mL	23 mL
8-10	3.6-4.5	3.5 mL	30 mL
10-15	4.5-6.8	5 mL	40 mL
16-20	7.3-9.1	10 mL	60 mL
21-25	9.5-11.4	10 mL	70 mL
26-30	11.8-13.6	10 mL	80 mL
31-35	14.1-15.9	10 mL	100 mL
36-40	16.4-18.2	10 mL	130 mL
41-45	18.6-20.5	20 mL	140 mL
46-50	20.9-22.7	20 mL	160 mL
51-55	23.2-25.0	20 mL	180 mL
56-60	25.5-27.3	20 mL	200 mL
61-65	27.7-29.5	25 mL	220 mL
66-70	30.0-31.8	30 mL	240 mL
71-75	32.3-34.1	30 mL	250 mL
76-80	34.5-36.4	30 mL	270 mL
81-85	36.8-38.6	30 mL	290 mL
86-90	39.1-40.9	30 mL	310 mL
91-95	41.4-43.2	30 mL	330 mL
96-100	43.6-45.5	30 mL	350 mL

Adapted from Becan-McBride K: Textbook of Clinical Laboratory Supervision, New York, Appleton-Century-Crofts, 1982