Specimen Collection and Preparation

**Introduction:**

The laboratory must have a written or electronic request for patient testing from an authorized person. The quality of results from laboratory testing depends greatly on the proper collection and handling of the specimen submitted for analysis. Correct patient preparation, specimen collection, specimen labeling, specimen packaging and transportation are essential factors for quality results.

Specific specimen requirements for each determination, including sample size, are provided in the BBPL Directory of Services Test section. To avoid additional expense and inconvenience, please make sure that you have submitted at least the quantity specified for the test requested.

All specimens must be submitted with a test request form with the patient information, billing information and any pertinent clinical information necessary to interpret patient results. Patient History forms and Patient Consent forms are also required for specific testing. Tests that require these forms are identified in the Test List section and can be downloaded on the BBPL website under Specimen Inquiry for the specific test. General Test Requisitions are available to order with the supplies. Specialty Test Requisitions are available to order with supplies or can be downloaded on the BBPL website.

**Specimen Labeling:**

All specimens submitted to Boyce and Bynum Pathology Laboratories, P.C. (BBPL) for testing must be appropriately labeled. This requirement assures positive identification and optimum integrity of patient specimens from the time of collection until testing is complete and results reported. The College of American Pathologists requires that all specimens must be labeled with two (2) identifiers at the time of collection. Ideally, a name-number system is desirable so that there are at least 2 person specific identifying items on each sample. Person-specific identifiers may include accession number, patient’s first and last name or patient’s initials, unique identifying number (e.g., medical record number), or date of birth. When insufficient or inconsistent identification is submitted, BBPL may recommend that a new specimen be obtained if feasible.

**Instructions for Completing Manual Test Requisitions:**

1. Use a **black pen** to complete the form as BBPL will scan the request for image retrieval.
2. Complete the patient demographic information at the top of the form.
3. Be sure to note any pertinent clinical information that will be used to interpret the test results i.e. fasting status, last dosage of medications if applicable, total volume for 24 urines etc.
4. Indicate the requested tests by marking an “X” in the box to the left of the test name or write both the complete test name and order code at the bottom of the requisition.
5. The specimen container must have the patients first and last name plus a unique identifier. If using the General Test Requisition the label affixed to the requisition fulfills the unique identifier requirement.
6. If sending frozen specimens, complete a separate requisition for all frozen tests or affix a frozen sticker and specify how many frozen specimens are being submitted.
7. Keep the second copy of the requisition for your records.

**Blood Components:**

Different blood components are used depending on the test ordered. Specimen requirements will specify whether serum, plasma, or whole blood should be submitted and how to obtain the specific component needed for testing.

Whole blood is obtained by collecting blood in a vacuum tube with an anticoagulant. To prevent clots from forming, thorough mixing of blood with the anticoagulant is necessary. Gently invert tube (do not shake) four to eight (4-8) times (depending on the specimen tube being used) immediately after collection. Do **not** freeze unless specified for the specimen handling requirements.
Plasma is the liquid portion of the blood in which particulates such as platelets and fibrinogen, a clotting protein, are present. Plasma normally appears as a hazy yellowish liquid and is obtained by centrifugation when blood is drawn in a tube containing an anticoagulant. The tube should be gently inverted immediately at least four to eight (4-8) times depending on the specimen tube used. Separate the cells within one-half hour by centrifugation and transfer the plasma into a clean vial for delivery to the laboratory.

Serum is the cell-free portion of blood from which the fibrinogen and particulate components have been separated in the process of clotting. Serum usually appears as a clear yellowish liquid and is obtained by centrifugation after the blood clots. Draw a full 7 mL gel-barrier tube for each 2 mL of serum required. Special processing techniques are explained in the section “Order of draw”.

**Collection Tube Types/ Transport tubes:**

BBPL provides special vacuum tubes, containers and transport tubes for collection and transport of specimens. Vacuum tube stoppers are color-coded and each color has a corresponding use and/or additive. Check which tubes need to be drawn in the BBPL Directory of Services by each test and profile. To provide the most accurate results, it is necessary to use the indicated tube(s) for the requested test(s). The following are general guidelines for processing the various vacuum tubes. More specific instructions are listed in the specific test requirement section. Be sure to submit the specific blood component listed under “Preferred Specimen.”

**Order of draw:**

To prevent contamination of tubes with additives from other tubes, it is important to draw tubes in a specific order called “the order of the draw”. The sequence of collection of evacuated tubes in a multi-draw should be in this order:

- **Clear or White Top Tube:** Non-additive tube (no clot activator in tube). Waste tube. Fill before filling blue-top tube when using butterfly set.
- **Light Blue Top Tube:** Contains sodium citrate as an anticoagulant. Fill completely and invert three to six (3-6) times in order to facilitate mixing.
- **Gel-Barrier Tube:** Contains no anticoagulant and is to be used for the collection of serum. Also known as serum separator tubes (SST) or gel separator tubes. A gel substance is present at the bottom of the tube, which upon centrifugation moves upward to the serum clot interface. The gel then acts as a barrier between the serum and the cells until the serum can be transferred for transport. Plastic gel-barrier tubes contain a clot activator.
- **Red Top Tube:** Contains no anticoagulant and yields serum. Plastic red top tubes contain a clot activator.
- **Green Top Tube:** Contains sodium or lithium heparin as an anticoagulant. After tube has been filled, invert eight (8) times in order to facilitate mixing. Refer to specimen requirements for the type of heparin that is acceptable.
- **Lavender Top Tube:** Contains ethylenediamine-tetraacetic acid (EDTA) as an anticoagulant. Fill completely and invert eight (8) times in order to facilitate mixing.
- **Yellow Top Tube:** Contains ACD anticoagulant (type A or B). Refer to specimen requirements for the type of ACD that is acceptable.
- **Gray Top Tube:** Contains sodium fluoride as a preservative and potassium oxalate as an anticoagulant. After tube has been filled with blood, immediately invert eight (8) times in order to facilitate mixing.

**Please follow these instructions when using the gel-barrier tube:**

1. Collect blood specimen using the normal venipuncture technique. Fill tube completely. Invert the tube gently.
2. Allow to stand at room temperature for 30 minutes to clot. Never allow serum to remain on cells more than two hours before centrifugation as chemical changes may occur, which could render some results invalid.
3. Check the centrifuge type. The type of centrifuge will determine how long tubes will need to be spun.

   **Fixed angle centrifuge** – Gel-barrier tubes must be spun for 15 minutes. The gel will be on an angle when tube is removed from the centrifuge.
   **Drucker Model 642E, electronically controlled horizontal centrifuge** – Programmable run time is factory preset to 10 minutes. Gel will be flat across the top of the cells.

4. Remove from centrifuge. Barrier will have formed. Verify that the gel has formed a complete barrier.

5. Allow tube to remain upright for a minimum of 15 minutes to allow gel barrier to adhere to plastic tube wall.

6. If a complete barrier has not formed in the tube, transfer the clear serum to a plastic transport vial for transport to the laboratory.

**To obtain plasma or serum without using gel-barrier tubes, follow these instructions:**

1. Draw 12 mL of blood for each 5 mL serum or plasma needed. Collect in an appropriate collection tube.

2. If serum is required, allow sample to clot for at least 30 minutes before centrifugation.

3. Centrifuge all samples within 1 hour of collection at 2200-2500 RPM for 10 minutes.

4. Pipet the serum or plasma into a clean plastic transport vial and attach the label. Do not transfer red cells to the vial.

   **Royal Blue Top Tube**: This tube is designed for collection of specimens for trace elements testing. Royal blue top tubes are available with EDTA, heparin and without anticoagulant. Refer to individual tests for specific requirements. Royal blue top tubes with EDTA should be drawn right before the lavender top tube and royal blue top tubes with heparin should be drawn right before the green top tube. Royal blue top tubes with no additive should be drawn before a gel-barrier tube or red top tube.

**Transport Tubes:**

   **Standard plastic transport tube**: These containers have been evaluated and are not known to cause analytical interference in the associated assays. The tube’s threaded cap provides a leak-proof seal when screwed on properly.

   **Amber transport tube**: Amber transport tubes are provided for specimens that require protection from light. If amber tubes are unavailable, the standard transport tube should be completely wrapped in aluminum foil, top and bottom and the patient’s name placed directly on the transport tube and on the outside aluminum foil.

**Frozen Specimens:**

   **Important Note for Frozen Specimens**: For tests requiring frozen serum or plasma, remove the serum or plasma from cells and transfer into a plastic transport vial. Specimens should be frozen as soon as possible after centrifugation and separation. If more than one test is requested on a frozen specimen, please split the specimen prior to freezing and submit separately. Indicate if specimen is plasma on transport tube and test request form (eg. “Plasma, Sodium Citrate”, “Plasma, EDTA”). Do not freeze glass vacuum tubes.

**Blood Smears:**

To obtain the best possible specimens for leukocyte differentials, we request that blood smear slides be made at the time of collection. By following this procedure, red cell morphology is preserved and the deterioration of platelets and white cells is prevented. Blood smears may be made from either a fingerstick specimen or the blood drawn into a lavender top tube. Clean slides must be used for making the smears. Contact your laboratory marketing representative if you would like assistance in blood smear preparation.
Urine:

**Random Urine:** The normal composition of urine varies considerably during a 24-hour period. Most reference values are based on analysis of the first urine voided in the morning. This specimen is preferred because it has a more uniform volume and concentration, and its lower pH helps preserve the formed elements.

To reduce contamination, the specimen submitted for urinalysis should be a clean catch "midstream sample."

Submit a first morning specimen whenever possible. Urine for pregnancy testing should be a first morning voiding or a random specimen with a specific gravity of at least 1.010. Note the time of collection of the specimen on the test request form and on the label of the container.

If a frozen specimen is required, freeze the urine immediately after collection. Pack in dry ice for shipment to the laboratory.

**24-Hour Urine:** Because proper collection and preservation of 24-hour urine specimens are essential for accurate test results, patients should be carefully instructed in the correct procedure. Printed instructions for the patient are available from the laboratory.

**Note:** For those analyses requiring the addition of 6N HC1, add the acid at the start of collection. Have the patient collect each voiding in a smaller container and carefully pour the urine into the 24-hour container to avoid any possible acid burns to the patient. **Be sure to mix the urine thoroughly before removing the aliquot.**

Follow these instructions if **someone other than the patient is to collect the urine:**

1. Follow the physician’s directions regarding food, drink, or drugs before and during collection.

2. During the collection period, place the 24-hour urine container provided by Boyce and Bynum in a refrigerator or cool place, to prevent growth of microorganisms and possible decomposition of urine constituents.

3. On the day of collection, have the patient empty his/her bladder in the morning into the toilet (not to be included in the 24-hour collection). Write the date and time of this voiding on the container and label as the “start” date and time.

4. Collect the patient’s next voiding and add it as soon as possible to the 24-hour container.

5. Add all subsequent voidings to the container as in (4). The last sample collected during the 24-hour period should be the first specimen voided the following morning at the same time as the previous morning’s first voiding. Record the date and time of this last voiding on the container and label as “finish”.

6. Mix the contents of the container gently but thoroughly. Examine to ensure that the contents appear homogeneous.

7. Measure the total volume.

8. Transfer the required aliquot to the screw-cap plastic urine containers provided by Boyce and Bynum. Add any additional required preservative and mix well.

9. Record the 24-hour urine total volume and hours of collection on the specimen container and on the test request form before sending to the laboratory.

10. Refrigerate the aliquot until it can be sent to the laboratory. For frozen specimens, freeze before packing in dry ice for shipment.

**If the patient is to collect the urine,** give the patient the clean, labeled container provided by Boyce and Bynum, and instruct him/her not to remove any preservatives (powder, liquid or tablet) that may be in the container. **Alert the patient that the preservatives are hazardous chemicals.**
1. The patient should follow their physician’s directions regarding food, drink, or drugs before and during collection.

2. Have the patient carry out steps 3-5 above and submit the 24-hour collection in the container.

**Feces:**

Carefully read the specimen requirements for special patient preparation and fecal specimen collection and handling. Special containers and aliquot containers for the collection and processing of fecal specimens are supplied by Boyce and Bynum Pathology Laboratories.

**Guidelines for stool collection of timed specimens:**

1. Review special specimen requirements with the patient, such as collection duration and diet requirements or restrictions.

2. Collect timed specimens in a pre-weighed, well-sealed container. Do not collect in metal cans.

3. Determine weight of total sample.

4. Mix contents of timed sample well to obtain a homogeneous mixture.

5. Transfer the required aliquot to a clean screw-cap plastic container and seal well.

6. Record the total weight and collection time of the sample on both the sample container and the test request form. Do not send the entire collection unless instructions for specific test indicate otherwise.

**Common Causes of Unacceptable Specimens:**

**Hemolysis:** Hemolysis occurs when the membrane surrounding red blood cells is disrupted and hemoglobin and other intracellular components escape into the serum or plasma.

Hemolyzed serum or plasma varies in color from faint pink to bright red, rather than the normal straw color.

Grossly or moderately hemolyzed specimens may be rejected and even slight hemolysis will alter certain test results.

**Hyperbilirubinemia:** Icteric serum or plasma varies in color from dark to bright yellow, rather than the normal straw color. Icterus may affect certain determinations. Upon receipt of such specimens, we may request a new sample to assure results of diagnostic value.

**Turbidity (Lipemia):** Turbid, cloudy, or milky serum (lipemic serum) may be produced by the presence of fatty substances (lipids) in the blood. Bacterial contamination may also cause cloudy serum. Moderately or grossly lipemic specimens may alter certain test results.

A recent meal produces transient lipemia; therefore, we recommend that patients fast 14-16 hours before a blood specimen is obtained.

**Radioisotope interference:** Diagnostic procedures or therapy involving radioactive compounds may invalidate radioisotope assays. Please obtain specimens for anticipated radioisotope assays before administering isotopes to patient. Please indicate on the test request form if radioisotopes have been administered before specimen was obtained.