

EXERCISE 15: SPECIMEN PROCESSING

Skills: 15 points

- Objectives:
1. Explain how a centrifuge works.
 2. Name five types of centrifuges and tell what each is used for.
 3. Safely and correctly demonstrate the proper use of a clinical centrifuge.
 4. Distinguish between serum and plasma.
 5. Define hemolysis, icterus, and lipemia and explain their effects on laboratory tests.
 6. Explain the importance of verifying the blood specimen identification with the requisition slip.
 7. Demonstrate proper accessioning of laboratory samples by correctly filling in the accessioning log sheet.
 8. Demonstrate appropriate specimen processing by comparing requisitions with samples and noting any discrepancies, problems with sample suitability, and/or missing samples.
 9. Safely and accurately separate serum and/or plasma from cells using appropriate PPE..

Discussion

Serum versus Plasma

Whole blood is composed of cellular elements (red blood cells or RBC, white blood cells or WBC, and platelets or PLT) and the liquid component, which is either serum or plasma. In general, adult blood has about 40% cellular elements and 60% serum or plasma.

Serum is the liquid expressed from **clotted** blood (blood drawn into a tube with no additive). In this case, blood is allowed to clot and fibrinogen along with some of the other coagulation factors are used in the formation of the clot. Serum, therefore, does not contain fibrinogen or other coagulation factors. Serum is the preferred specimen for most chemistry, blood bank and serology tests because fibrinogen may cause interference in the test procedure performed, removing it prevents this problem. Some additives may also interfere with these analyses and it is best that they not be present.

Plasma is the liquid portion of the blood present in **anticoagulated** specimens. Plasma contains all the coagulation factors, including fibrinogen. Calcium, which is considered to be a coagulation factor, is not present in plasma as most anticoagulants prevent coagulation by binding (chelating) the calcium. Heparin is a naturally occurring anticoagulant which acts as an anti-thrombin. Thrombin is another essential component of the coagulation mechanism. The effects of heparin are short lived, blood drawn in heparin will begin to clot in approximately 48 hours. Plasma tends to be somewhat more hazy than serum.

Centrifuges

Centrifuges are instruments used to spin samples of blood or other body fluids at high rates of speed, forcing the heavier particles to the bottom of the container. The most frequent laboratory use of the centrifuge is to separate the cellular components of blood from the liquid so that the liquid may be used for testing.

Centrifuges vary in size, capacity, and speed capability. **Clinical centrifuge** is the name given to tabletop models which can be used for urinalysis or serum separation. These usually have a speed capacity of up to 3000 rpms (revolutions per minute), and will hold tubes ranging from 5 to 50 mL sizes, depending on the adapters. A **serofuge** is a small centrifuge used in blood banking a serology to spin serological tubes.

Microcentrifuges or microfuges are also becoming widely used. These will spin special microtubes 1.5 cm capacity at high speeds, usually about 12,000 rpm. The **microhematocrit** centrifuge is a variation of the microfuge; it spins capillary tubes at high speeds so that hematocrits can be measured.

Other types of centrifuges include **high-speed centrifuges** which rotate at speeds up to 20,000 rpm and **ultracentrifuges** which rotate at speeds over 50,000 rpm. These centrifuges are specially equipped so that samples may be kept cool while being centrifuged. Centrifuges such as these are typically used only in research laboratories and are not required for routine clinical testing.

Operating the Centrifuge

The manufacturer's instructions must always be followed when using a centrifuge or any other type of equipment. Some general rules to follow in the operation of all centrifuges are:

1. Do not operate centrifuges with the lids open.
2. Balance the contents of the centrifuge before operating. For example, if there is only one sample to be centrifuged a tube identical in size and volume of solution contained must be placed in the rotor opposite the sample tube. The rotor is the part of the centrifuge which holds the tubes and rotates during the operation of the centrifuge. For every sample placed in the rotor, there must be a balancing sample placed directly opposite.
3. Do not open the centrifuge lid until the rotor has stopped spinning.
4. Spin samples with lids on to avoid creating aerosols.
5. Use only tubes that are specified as appropriate for that particular centrifuge.

Preventative Maintenance

Centrifuges should be checked and have maintenance performed on a regular basis. The routine checks of revolutions per minute and time of centrifugation using either a tachometer or a stroboscope are absolutely essential for consistent results that meet quality control standards.

Separation of Serum

Whole blood that is anticoagulated is most often not centrifuges and separated, but used as whole blood for such tests as cell counts, hemoglobin/hematocrit (H&H) determinations, etc. Clotted blood must be centrifuged and the serum separated from the cells so that it can be used in various analyses. It is critical that the serum **not** be contaminated with red blood cells as this could cause falsely increased or decreased results.

Serum can be separated from the cells in a variety of ways:

1. The tube is centrifuged and the serum is carefully separated to another clean, properly labelled test tube with a clean pipet.
2. The tube is centrifuged and then a serum separator tube is gently pushed into the blood tube. The serum separator tube contains a filter which allows serum through but keeps out the cells.
3. Before the tube is centrifuged a "serum separator" is added to the blood. When the tube is centrifuged,

the serum separator or gel forms a layer between the serum and the cells. then the serum can stay in the original tube physically separated from the cells or the serum can be pipetted into a clean, properly labelled test tube.

4. A serum separator tube (SST) is used. The SST already has the serum separator in it and the blood is drawn on top of it. The tube is then centrifuged and the serum can be handled as in #3 above.

Specimen Appearance and Causes of Rejection

If serum or plasma is transferred, it should be into a clean, dry, properly labelled glass or plastic tube with a clean, dry glass or plastic pipet. Observe the serum or plasma - it should be clear to hazy and pale yellow in color. There are several different appearance one should be aware of:

1. **Hemolysis** is a red or reddish color in the serum or plasma which will appear as a result of red blood cells rupturing and releasing the hemoglobin molecules. Hemolysis is **usually** due to a traumatic venipuncture (ie, vein collapses due to excessive pressure exerted with a syringe, "digging" for veins, or negative pressure damages innately fragile cells. Gross hemolysis (serum or plasma is bright red) affects most lab tests performed and the specimen should be recollected. check the specific lab test to see what effect hemolysis has on it. Slight hemolysis (serum or plasma is lightly red) affects some tests, especially serum potassium and LDH (lactate dehydrogenase). Red blood cells contain large amounts of both of these substances and hemolysis will falsely elevate their measurements to a great extent.
2. **Icterus**. Serum or plasma can be bright yellow or even brownish due to either liver disease or damage or excessive red cell breakdown inside the body. Icterus can, like hemolysis, affect many lab tests, but unfortunately, recollection is not an option since the coloration of the serum or plasma is due to the patient's disease state. However, appearance should be noted on the lab report as "**serum icteric**". Handle icteric serum with extreme caution due to the possibility that the patient may have hepatitis.
3. **Lipemia**. Rarely serum or plasma may appear milky. slight milkiness may be caused when the specimen is drawn from a non-fasting patient who has eaten a heavy meal. A thick milky appearance occurs in rare cases of hereditary lipemia. As with icterus, the appearance should be noted on the lab report as "**serum lipemic**".

Specimen Storage

If the specimen is to be stored in the refrigerator or freezer, the tube should be tightly capped or tightly sealed with parafilm. If the specimen is to be frozen, the ideal freezer is a true laboratory freezer which maintains constant temperature. Regular kitchen refrigerator freezers are generally frost free and go through freeze and thaw cycles, which may adversely affect the specimen.

Accessioning Specimens

Every lab has a procedure to accession specimens in the lab. Patient names and identification numbers are logged in on a computer or on a log sheet or book along with the test(s) to be performed. This accessioning serves as a record of tests done and is a convenient way to check if the specimen has actually arrived in the lab for testing in the event that the physician or nurse calls to check on the results. It is extremely important that all patient information is correctly transcribed, that includes spelling of the patient's first and last names and the correct identification numbers. Remember, most lab errors are clerical in nature, someone misspelled a name or transposed names or numbers. Be careful! You **will** be graded down if you make clerical errors.

Procedure

1. The instructor will give each student requisition slips and specimens.
2. Check the names and identification numbers on **each** tube with the names and identification numbers on the requisition slip. If any errors are identified, note the problem on your "Specimen Accession Log" and describe the action which must be taken to resolve the problem.
3. Make sure that you have the correct specimen for each test ordered. Note on the log if specimens are missing or inappropriate specimen was drawn and describe the action which must be taken to resolve the problem.
4. Fill in the date/time columns with today's date and the current time. Write the patient's name, identification number, and list each test ordered on the specimen on the Specimen Accession Log.
5. Centrifuge each of the tubes in one of the centrifuges. Blue coagulation tubes must be centrifuged in the Serofuge, all others in the large table top centrifuge by the sink. Make sure each tube has one directly opposite from it with identical size and blood volume. If no match is found create a balance tube.
6. Once the centrifuge is full spin for 7 - 10 minutes.
7. Label a test tube for each specimen to be separated with patient's name and identification number, and whether it is serum or plasma.
8. Carefully separate the serum and plasma from the original tube into the labeled tube with a clean pipet making sure not to contaminate the serum/plasma with red blood cells.
 - If the red blood cells are resuspended the tube must be respun.
 - Use a different pipet for each specimen.
 - **Take great care not to put the wrong specimen in the wrong tube.** This could have devastating consequences since these results are used to diagnose and treat patients.
9. Parafilm the top of each tube.

Name _____

SPECIMEN ACCESSION LOG

Date	Time	Patient Name	Patient ID #	Tests Ordered	Problems	Initials

6. What is a serum separator? (1 point)
7. Define and explain the clinical significance of each of the following terms(3 points):
- hemolysis**
- icterus**
- lipemia**
8. Why is a regular kitchen refrigerator not the most desirable one to use to freeze a clinical specimen? (1 point)
9. Why is plasma from an EDTA tube not be used for a plasma calcium level. (1 point)
10. What action must be taken if a discrepancy exists between the information on the tube of blood and that found on the requisition slip? (1 point)