Laboratory tests, which measure an analyte in a specimen of blood or other body fluid, are ordered by physicians to evaluate the status of a patient. It is assumed that the analyte results obtained are representative of the analyte concentration in the patient. Unfortunately this is not always the case as several factors may invalidate this assumption. These factors are collectively referred to as preanalytical, analytical, and post analytical variables. The preanalytical variables consist of test ordering, specimen collection, and specimen processing. Analytical variables affect the actual testing process and the post analytical variables affect the reporting of the result. All of these variables have a direct affect on patient care.

Quality control has been an important part of any laboratory operation for years, but it primarily focused on analytical variables. It deals with statistics based on results reported. In the early nineties with the advent of such programs, such as Continuous Quality Improvement (CQI), Quality Assurance (QA) and in the last few years Six Sigma Quality the focus has not only been redirected, but has expanded to include what happens before, during, and after. These programs focus on the healthcare system as a whole and not just one department.

In this lecture we will focus primarily on the preanalytical variables which come into play as soon as the physician places an order and in some cases even before. Labs take many steps in minimizing sources of error such as patient preparation, sample collection, methods of sample transport, preservation and the processing of samples. A brief review of the more common specimens and specimen considerations will be presented as they apply to the clinical chemistry section of the laboratory. For additional information, Kaplan Chapter 3, "Sources and Control of Preanalytical Variables", provides extensive information on this topic.

SPECIMENS

REMEMBER: A result is only as good as the specimen from which it came.

Serum is probably the most used specimen in clinical chemistry. Serum is obtained by collecting a whole blood sample without an anticoagulant. This whole blood sample must be allowed to clot completely prior to centrifugation. Following centrifugation, the supernatant fluid is the serum. Serum does not contain any cellular components nor does it contain the protein fibrinogen. The primary advantage of serum in the clinical chemistry laboratory is that there has been nothing added to the sample that might interfere with the analysis of the sample. The primary disadvantage is the time necessary for complete clot formation, especially when the test results are needed quickly.

Plasma is obtained by adding an anticoagulant to the collection/evacuated tube prior to collecting a whole blood sample. The anticoagulant will prevent clotting of the sample thus allowing centrifugation as soon as the sample reaches the laboratory. Following centrifugation, the supernatant fluid is the plasma. Plasma does not contain any cellular components but it does contain fibrinogen. Plasma may or may not contain ions such as magnesium or calcium, depending on the anticoagulant used. Some anticoagulants prevent clotting by binding these ions. For example, sodium citrate binds calcium and magnesium in order to prevent clotting. Thus, you could not use plasma obtained using sodium citrate for calcium or magnesium measurements. Heparin, on the other hand, does not interfere with these ions allowing heparinized plasma to be used for calcium and magnesium measurements. It is important to note what form of the anticoagulant is in each tube. For example; heparin may be found in the form

of sodium heparin as well as lithium heparin. You would not use a tube with sodium heparin for electrolytes or lithium heparin for a lithium level.

There are a couple of precautions to keep in mind when using plasma. First, after the whole blood is added to the anticoagulant, the collection/evacuated tube should be mixed well by gentle inversion to prevent small clots from occurring. Secondly, the collection/evacuated tube has enough anticoagulant to prevent clotting if the tube is filled as intended. Thus, short draws result in improper ratios of blood to anticoagulant and may interfere with chemical analysis.

Whole blood is obtained by collecting the blood sample in an anticoagulant with no centrifugation. Whole blood is a two compartment system consisting of a cellular component and a fluid component. This two compartment system sometimes makes interpretation of laboratory results difficult as will be discussed when considering methods of glucose analysis. The same concerns noted for plasma are also noted for whole blood samples. In addition, mixing is not only important to prevent small clots from forming, mixing is also important to prevent settling of sample components prior to analysis. Whole blood is used primarily in the Hematology department for measurement of the cellular components of the blood.

Cerebrospinal fluid (CSF) is an ultrafiltrate of the blood which contains small amounts of protein, glucose, as well as other materials that will be considered later, and no cells. CSF is obtained from a lumbar puncture. The specimen usually arrives in the laboratory in three tubes. Different labs use different approaches here. Some say Tube #1 has the greatest chance of being contaminated with blood and should go to the microbiology section. Tube #2 typically goes to chemistry and tube #3 goes to hematology. Other labs say Tube #1 goes to chemistry, Tube #2 goes to microbiology, and Tube #3 goes to hematology. The most common chemical analyses run on CSF are protein and glucose. The significance of these, as well as other, tests will be considered at a later time.

Urine is the final sample that is commonly encountered in the clinical chemistry laboratory. Both qualitative and quantitative analyses may be run on urine. Quantitative analyses usually require that a 24-hour urine be collected. The proper procedure for collecting a 24-hour urine is as follows:

The patient should void their bladder of urine, discard the urine, and begin timing. All urine voided during the next 24 hours should be collected and added to the collection container. At the end of the 24-hour period, the patient should void their bladder of urine and add this to the collection container. The collection container should be returned to the laboratory as soon as possible after completion of the collection process.

The collection container is usually obtained from the laboratory. The laboratory is responsible for adding any preservative necessary to the container prior to issuing the container to the patient. In addition, the sample should be kept cool during collection. This may be done by placing the collection container in the refrigerator. If the patient is offended by having a jug of urine sitting in their refrigerator, you may try suggesting that they place the container in a paper bag and place this in the refrigerator. If this does not work you may suggest that they place the container in an ice chest WITH ice! The importance of keeping this sample cool must be stressed to the patient. Sometimes the patient questions if it is necessary to add any urine voided in the middle of the night. The answer is yes. At this point you may advise the patient that one of the first test run on the urine when it is returned to the laboratory is a test for completeness of collection. If this result is abnormal, the patient either has kidney problems or they did not provide a complete sample. The test typically run to check for completeness is a 24-hour urine creatinine. The significance of this test will be considered in the section on kidney function.

TIMING OF SPECIMEN COLLECTION/IMPORTANCE OF TIMING

Timing of specimen collection is also an important consideration. Problems with the proper collection, handling, and timing of the collection are all pre-analytical variables that can lead to the laboratory reporting out bad data which may adversely affect patients.

Fasting specimens are specimens collected after a specified period of fasting. Fasting means the patient can have no food or drink (with the exception of water) during the specified period of time. Fasting is different from NPO, which means the patient **cannot** have **anything** during the specified period of time, not even water.

Postprandial (pp) specimens are collected following a meal. Perhaps the most common use of this is a 2-hrpp glucose. This indicates that a blood specimen should be collected two hours after a meal for glucose measurement. Perhaps the biggest problem with this is communication between the floor and the laboratory as to when the patient has finished their meal.

Timed specimens are collected at a specific time during the day. Timed specimens (e.g., 8:00 a.m. glucose) are typically used to evaluate variation of analyte levels during the day. Timed specimens may also be ordered following certain activities.

As Soon As Possible (ASAP) specimens are typically requested when the patient is waiting on the results before leaving or when the medical staff is waiting on the laboratory results in a non-emergency situation. Many times the results of tests ordered ASAP will determine the drug and/or dosage a patient is to receive or other instructions given to the patient before they leave.

STAT specimens are ordered in emergency situations. This is an indication that the specimen ordered stat should be the next one analyzed and the results released immediately upon completion of the test. Many medical facilities set a time limit on how long it should take to report the results of analyses ordered stat. This type of order is greatly abused in some institutions.

PROBLEMS THAT CAN OCCUR

Once specimens are in the clinical laboratory, inappropriate handling could lead to changes in analyte levels which would cause the clinical laboratory to report out errant results. Six problems that would lead to changes in serum/plasma/whole blood are described below.

One problem is leaving the specimen open to the atmosphere. This is of special concern when the specimen is placed in sample containers for use on automated analyzers (which contain volumes in the microliter range) and the containers are left open to the atmosphere. With these sample containers, evaporation of only a minute amount of liquid from the sample can change the concentration of analyte from a normal level to a very elevated level. Many automated analyzers found in the clinical laboratory today that use small sample containers also provide some means to prevent evaporation.

Another problem with leaving the specimen open to the atmosphere is that gases, which are dissolved in the specimen, will begin to equilibrate with gases in the atmosphere, thus altering the levels of these gases in the specimen. Of particular concern are CO_2 and O_2 levels. Mishandling of specimens used to measure these two gases could have life threatening implications.

A **second** problem with the inappropriate handling of specimens is related to not mixing blood samples adequately when an anticoagulant is used. Inadequate mixing may allow small clots to form. As clotting occurs, CO_2 is lost. Thus, clotting leads to errant CO_2 levels being reported by the laboratory. Another problem associated with the development of small clots in the specimen is these clots may completely or partially block flow paths in laboratory analyzers. This may result in instrument downtime.

A **third** problem associated with the inappropriate handling of specimens is related to separating serum/plasma from the cells. For laboratory analyses that require serum/plasma, the serum/plasma should be separated from the cells as soon as possible after centrifugation. Some of the potential problems that may result from not separating the serum/plasma from the cells are listed below.

1. Erythrocytes will continue to carry out glycolysis. Therefore, any glucose in serum/plasma left associated with the cells will continue to decrease. As a result of glycolysis, lactate levels will increase and the pH of the serum/plasma will decrease. Also, as glycolysis continues, potassium and phosphorus levels will begin to rise in serum/plasma.

2. Another problem associated with metabolism is that erythrocytes and white blood cells continue to utilize oxygen and produce carbon dioxide. Thus, as long as the serum/plasma is in contact with the cells, the levels of these two gases will change.

3. Erythrocyte permeability will change over time. This will allow materials normally found in high concentration inside the cell to diffuse out into the serum/plasma falsely elevating the level of this material in serum/plasma. It is important to note that placing the tube containing the serum/plasma on the cells in the refrigerator will enhance this change in permeability. Perhaps the material of greatest concern in this regard is potassium. The concentration of potassium inside the erythrocyte is approximately 150 times the concentration outside of the cell. Thus, as membrane permeability changes, significant levels of potassium will move out of the cell into serum/plasma causing an errant result to be reported. To prevent this, the serum/plasma should be separated from the cells as soon as possible after centrifugation.

A **fourth** problem associated with the inappropriate handling of specimens is related to the temperature sensitivity of some analytes. There are certain analytes that must remain cold prior to analysis. These specimens should be placed on ice as soon as the specimen is collected, centrifuged in a refrigerated centrifuge, and stored in a refrigerator until time for analysis. Two examples of heat labile materials include acid phosphatase and adrenocorticotropic hormone (ACTH).

A **fifth** problem associated with the inappropriate handling of specimens is related to the light sensitivity of some analytes. These analytes are subject to photodegradation and should remain protected from direct light exposure prior to analysis. Perhaps the most common example of a photosensitive compound is bilirubin.

The **sixth** problem associated with the inappropriate handling of specimens is related to the failure to recognize hemolysis. When erythrocytes hemolyze, they release all of their cellular components into the serum/plasma. It has already been noted that potassium is much more highly concentrated inside the erythrocyte than outside the erythrocyte. Therefore, hemolysis would be associated with a significant increase in potassium levels in serum/plasma. Hemoglobin is also released giving serum/plasma a red tint which may interfere with spectrophotometric analyses. Hemoglobin is known to also inhibit certain enzymes, e.g., lipase.

A **seventh** potential problem results from allowing the tourniquet to remain tied above the venipuncture site for more than one to two minutes. Hemoconcentration is the term used to refer to this potential problem. When the tourniquet remains tied too long the blood below the site tends to pool and become more concentrated thus hemoconcentration. Most analytes in the specimen collected under these circumstances exhibit falsely elevated results. This situation may be avoided by removing the tourniquet to prepare for the venipuncture, allowing the cleansed site to dry completely and only tying the tourniquet back on immediately before the stick. The phlebotomist should always remove the tourniquet as soon as possible in any case to prevent hemoconcentration from occurring.

CHANGES OCCURRING IN SPINAL FLUID AND URINE

Changes also occur when spinal fluid and urine are allowed to sit for an extended period of time. The changes in these two specimens are similar and include the following:

Cells present begin to lyse making them impossible to identify. This is more of a problem in urine because of the hypotonic nature of urine.

1. Bacterial contamination becomes a problem in both specimens. If these specimens are allowed to sit for a period of time it becomes difficult to determine if bacteria were present when the specimen arrived in the laboratory, or if bacteria is now present due to contamination during prolonged sitting.

2. Glycolysis may continue to occur leading to a buildup of lactate and a drop in pH.