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Pearls of Laboratory Medicine
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TITLE: Phlebotomy and Tube Type Selection

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Slide 1:
Hello, my name is Prati Ranjitkar. I am a clinical chemistry fellow at the University of Washington. Welcome to this Pearl of Laboratory Medicine on "Phlebotomy and Tube Type Selection."

Preanalytical factors, such as specimen collection and the type of collection device used, are crucial in maintaining the integrity of laboratory results. In this Pearl, I will briefly describe the proper phlebotomy technique and preanalytical collection errors associated with improper draws. I will then talk about the types of blood collection tubes used for laboratory tests, their limitations, and potential errors associated with using the wrong tube type.

Slide 2:
Blood collection is typically performed via venipuncture by a trained phlebotomist. Before blood is drawn, the phlebotomist verifies the identity of the patient. Correct identification involves using two unique patient identifiers that include the patient's complete first and last name, medical record or hospital number, and/or date of birth. When applicable, the fasting state of the patient is verified.

Blood is drawn in a seated or supine position. The most common draw site in outpatients is the median cubital vein located on the crook of the elbow. In inpatients with indwelling vascular access line, the arm opposite of the line is usually preferred to prevent specimen contamination with the infusion material. The draw site is cleaned with a cotton swab saturated with 70% alcohol and the tourniquet is tied 4-6 inches above the draw site. The patient is directed to form a fist and the needle is introduced at a 30 degree angle or less. As shown in the top right figure, blood is collected typically into a vacuum extraction device, which consists of a double-ended hypodermic needle attached to a barrel. The barrel protects the phlebotomist from direct contact with blood. Once the needle is in the vein, the tube is pressed against the needle and a pre-determined volume of blood is automatically drawn via vacuum extraction. The tourniquet is then removed and the needle is withdrawn gently followed by application of a clean gauze onto the draw site.

Another common mode of sampling is with winged butterfly needles, which may also be used with vacuum extraction. As shown in the bottom right figure, this type of device has tubing attached to the needle, giving the phlebotomist greater flexibility, which is especially useful when performing difficult draws. It also allows collection of multiple tubes at once.

Capillary sampling is useful for tests that require small quantities of blood but is particularly desirable in neonates because vascular access in neonates can be difficult and because standard practices withdraw volumes of blood that are too large relative to total blood volume. The preferred sampling site is the finger, but heel or earlobe may be sampled as well. In this method, heat or topical vasodilator is applied to the sampling site to dilate the capillaries. Massaging the site for capillary sampling should be avoided as this causes hemolysis. The skin is then punctured and the first drop of blood is wiped away. Sample is then drawn into a capillary glass tube.

Slide 3:

There are several overlooked practices that should be avoided during phlebotomy.

- The tourniquet should not be left on longer than a minute as this can lead to hemoconcentration and significant elevations in certain analytes such as proteins, lipids, potassium, ammonia, hemoglobin, and calcium.
- The draw site should not be tapped to locate the vein as this will cause hemolysis and false elevations in potassium.
- Avoid fist clenching as this causes an increase in plasma potassium. In addition, fist clenching can also lead to spurious increases in phosphate and lactate levels. Lactate build-up lowers the pH, which causes ionized calcium concentration to be falsely elevated.
- In general, not using the first specimen for electrolyte measurements when obtaining multiple samples from a single patient can reduce the occurrence of pseudohyperkalemia. Of note, for non-electrolyte analytes, if multiple samples are drawn for identical tests, the first sample is always more reliable.
- Lastly, certain analytes vary due to diurnal variation or posture of the patient. For instance, a classic example of an analyte that exhibits diurnal variation is cortisol, which is at its highest in the morning, decreasing gradually throughout the day. Therefore, the time of draw for such analytes should be done within the clinical context. For therapeutic drug monitoring, samples are generally drawn at trough but peak or random draw may be indicated for some drugs.
- An upright to supine position has a dilutional effect due to fluid shift from the interstitial compartment to the intravascular compartment. For example, supine draw has been observed to decrease albumin concentration. Cholesterol or triglycerides decrease by 10-12% in supine position.

Slide 4:

A number of other preanalytical collection, transport, and processing errors exist that I have listed on this slide. While each of these errors merits its own discussion, due to the scope of this Pearl, I will focus on three specific issues shown on the left.

Stability of specimens should be determined by the laboratory and practiced accordingly. For example, bilirubin is light sensitive and should be protected from light until analysis. Failure to do so could lead to falsely lowered results. Potassium specimens in whole blood should be transported at room temperature. This is because refrigeration inhibits the Na+K+ ATPase pump, resulting in potassium leaking from inside the cells into plasma and causing pseudohyperkalemia.

Ideally, if serum or plasma is needed, whole blood specimens should be processed within two hours of being collected. Two analytes that are significantly impacted due to the delay in separation of cells from serum or plasma are glucose and potassium. Blood drawn into a collection device continue to undergo metabolism, thereby consuming glucose, and continue to pump K+ into the cells and Na+ out of the cells. When plasma or serum is not separated from the cells in a timely fashion, glucose is depleted causing a falsely lowered result. Furthermore, the Na+K+ ATP pump ceases to function as the glucose is depleted, resulting in equilibration of electrolytes across the cell membrane, and causing a spurious increase in potassium.

Hemolysis, lipemia, and icterus are three major interferants of accurate measurement of chemistry analytes. The degree of interference is dependent on the analyte and the method being used and could be physiologic or analytic:

- Analytic interferences occur primarily with spectrophotometric techniques in which hemolysis, lipemia, and icterus interfere by virtue of overlapping light absorption within the measuring wavelength of the assays. For instance, hemoglobin and bilirubin have overlapping absorption spectrum, which would lead to erroneous bilirubin results in hemolyzed specimens. Lipemia can cause false reduction of sodium concentrations when measured using indirect ion-selective electrode methodology. This is due to the fact that this method requires a sample dilution step and makes the assumption that plasma is 93% water. However, lipemic samples have decreased water content and the assumption that plasma is 93% water is no longer applicable, resulting in calculating a falsely lowered sodium concentration.
- Physiologic interferences can occur primarily with hemolysis. For instance, hemolysis releases intracellular potassium and enzymes like LDH and AST that results in false elevation of these analytes. To this end, most laboratory instruments are equipped with optical systems that detect all three types of interferants.

Slide 5:

Next, I will talk about commonly used blood collection tubes for chemistry analytes and consequences of using inappropriate collection devices. Proper understanding of tube additives and how they affect test results are important for laboratorians to know. Early blood collection tubes were made of glass but they have long been replaced with plastic tubes. Plastic tubes are not only cheaper and safer but also allow for reduced centrifugation time and longer sample stability. Furthermore, they can be used as primary sampling tube in laboratories with automated instruments. A disadvantage of using plastic tubes is that the tube material is generally made out of hydrophobic polymers and do not activate the coagulation cascade efficiently. As a result, more gelatinous clots are formed making serum separation difficult. Glass tubes in contrast make a “cleaner” clot because the interior surface of glass tubes itself plays a key role in activating blood coagulation. However, the conveniences of using plastic tubes far out-weigh the detractors. To facilitate “cleaner” coagulation, plastic tubes are coated with clot activators such as silica, surfactants, or water soluble polymers. The tube walls may also be coated with additives such as anticoagulants, clot activators, or surfactants, and the stopper is made of plastic or rubber, which may contain lubricants. Some tubes contain gel separator at the bottom of the tube which acts as a barrier between serum and clot or plasma and cells following centrifugation.

Slide 6:
Components of the rubber or plastic stoppers can contaminate blood specimen. For instance, metals like calcium and magnesium used in manufacturing rubber stoppers can leach into blood upon contact, falsely elevating these divalent cation measurements. Drugs adhere to some plasticizers and can be falsely decreased. To avoid these preanalytical issues, stoppers are usually made from materials that are low-extractable. Commonly used stopper lubricants are silicone oils and glycerol, but silicone is preferred as they are less likely to interfere with assays. Specifically, glycerol containing tubes should not be used when measuring triglycerides because most assays require triglycerides hydrolysis to glycerol and contamination from the lubricant will falsely elevate triglyceride levels.

Slide 7:
Separator gels are made from viscous liquids that contain gelling agents such as dibenzylidene sorbitol. These materials are thixotropic, meaning that they are solid under static conditions but liquefy under sheer stress such as during centrifugation. The specific gravity of the liquid state under centrifugation is such that it partitions between the serum and clot or plasma and red cells. This allows for safer and easier handling, shorter processing time, and eliminates the need for aliquoting serum or plasma into a separate tube. Gel separator tubes, however, should be avoided when testing for drugs that require monitoring. Hydrophobic drugs like phenytoin, carbamazepine, and lidocaine adsorb onto the gel separator, falsely decreasing drug concentrations.

Slide 8:
As shown in the picture here, stoppers are color-coded to designate the type of additives and the presence of gel separator contained within the tube. It is important to pay attention to the type of tube being used for blood draw as a variety of different additives are found in these devices and tests are typically validated on samples obtained from some but not all types of tubes. Almost all testing is done on plasma, serum, or whole blood. Testing done on plasma or whole blood require anticoagulation of blood. Plasma can be subsequently obtained by separating red cells out. Heparin, ethylenediaminetetraacetic acid (EDTA), and citrate are the most commonly used anticoagulants. Alternatively, serum can be obtained from clotted blood after centrifugation of the clot. Serum is usually the preferred specimen as it is a much cleaner matrix than plasma. However, plasma is a useful alternative when rapid turn-around time is needed. Serum can be obtained from tubes that contain materials that activate coagulation such as silica or thrombin.

Slide 9:
Green tops contain sodium heparin, lithium heparin, or ammonium heparin. Depending on the type of metal complex or the presence of gel separator, heparin tubes are color-coded with shades of green. Heparin-containing tubes produce whole blood or plasma samples by inhibiting antithrombin. Lithium heparin tube is more commonly used than sodium heparin as it is less likely to interfere when measuring ions. Blood gases, pH, electrolytes, and ionized calcium should be measured on heparinized sample but not sodium if sodium heparin is the additive, as this would result in false elevation of sodium.

There are certain assays for which heparinized samples are not suitable. For instance, heparinized samples should not be used for cryoprotein measurement as it precipitates cryofibrinogen. Insufficient mixing of heparin can cause samples to be unevenly anti-coagulated resulting in micro-clot formation. Micro-clots can obstruct probes in automation lines as well as interfere with immunoassays. A well-documented example of micro-clots interference is the troponin immunoassay, which causes false positive results.

Slide 10:

Lavender tops contain potassium EDTA coated onto the tube wall. EDTA blocks the coagulation cascade by chelating calcium and producing whole blood or plasma sample. It is the preferred tube for hematology and genetic testing. EDTA also chelates other divalent metal cations. Therefore, it should not be used for the measurement of metals such as calcium, zinc, and magnesium. These metals would be falsely decreased if sample is drawn into EDTA containing tubes. Alternatively, potassium would be falsely elevated if measured in samples drawn into lavender tubes. It should also be noted that EDTA containing tubes should be avoided if the assay requires divalent cations. For instance, alkaline phosphatase is a commonly used enzyme in immunoassays, which requires magnesium for activity. EDTA would interfere with the activity of alkaline phosphatase producing a false negative result.

Light blue top tubes contain buffered sodium citrate solution, theophylline, adenosine, and dipyridamole. These components inhibit platelet activation by binding calcium and producing whole blood or plasma sample. The blue top is the preferred tube type for coagulation testing. It is important that sufficient sample is collected to achieve 1 part citrate to 9 parts blood because excess citrate will subsequently falsely prolong the prothrombin or partial thromboplastin time.

Slide 11:

The royal blue and gray tops are specialized tubes used for specific tests.

The royal blue top is made up of glass and usually do not have any additives. It is certified to be heavy metal free and used for trace element analysis in whole blood, plasma, or serum.

The gray top is used for glucose and lactate testing when specimen transport time to the lab is an issue. Glucose in whole blood is stable for approximately two hours and lactate for 15-30 minutes. Gray tops contain potassium oxalate as anticoagulant and sodium fluoride as preservative. Sodium fluoride inhibits glycolytic enzyme enolase, which limits ex vivo consumption of glucose in the specimen. Therefore, glucose in the gray top is stable for up to 24 hours. However, metabolism does occur at a rate of 5-7% for about 4 hours from the time of draw before complete inhibition of glycolysis. More recently, acidification of blood with citrate has been shown to be more effective at inhibiting glycolysis than NaF. Tubes containing citrate buffer with NaF are capable of blocking glycolysis immediately, preventing any consumption of glucose during preanalytic steps and allowing the most accurate measurement of glucose. These tubes are currently only available in Europe. Oxalate in gray tubes also inhibits the conversion of pyruvate to lactate, preventing false elevation of lactate. Lactate stability in heparinized plasma requires specimen transport on ice and the analyte is only stable for about 30 minutes. In the gray top tube, lactate is stable for up to 8 hours at room temperature.

Slide 12:
The red, gold, and orange tops contain clot activators that produce serum samples. Red and gold tops contain siliceous substances that activate the clotting cascade. Serum is a cleaner sample matrix and has been shown to reduce false positive lab results. However, some limitations to be aware of include ineffective clotting that can produce fibrin clots after centrifugation, which can interfere with pipetting accuracy or with immunoassays, producing spurious results. In addition, complete clotting of blood typically takes 30 minutes, which impacts test turn-around time. To facilitate faster clotting, orange tops or rapid serum tubes can be used. Rapid serum tubes are coated with thrombin that activates the coagulation cascade and produces a clot in 5 minutes, significantly improving turn-around time. However, these tubes are not appropriate for patients on high-doses of heparin or warfarin. High concentrations of anti-coagulants presumably prolong clotting process, producing latent clots that may obstruct sample probes or produce erroneous results. Most chemistry analyte concentrations are similar in serum and plasma. However, there are differences that exist between the two matrices. For instance, clotting depletes fibrinogen and releases potassium during platelet lysis. Therefore, potassium is higher in serum compared to plasma, and total protein is lower in serum than in plasma. Similarly, drug levels in heparinized plasma may differ from serum. If the drug is bound to fibrinogen, plasma level would be higher than in serum. However, if the drug is distributed to platelets and granulocytes, such as the antimalarial drug cholroquine, serum concentration is expected to be higher than plasma due to the release of cholroquine during the coagulation process.

Slide 13:
Sample collection for blood gas measurement requires special syringes that are air-tight and contain ion balanced lyophilized heparin. Historically, glass syringes were used but standard devices today are made of plastic. Plastic syringes should not be chilled on ice during transport as was practiced for glass syringes because cooling the plastic syringe can alter the measured pO_2. The mechanism of how this occurs has been hypothesized to be due to the contraction of plastic in the cooling process that results in widening of pores large enough to allow O_2 but not CO_2 to escape or enter the tube.

Air bubble introduction during sample draw is another preanalytical issue to consider. Equilibration of the sample with the air bubble often leads to elevation or drop of pO_2 to atmospheric pressure depending on the initial pO_2 value. pCO_2 typically drops resulting in an increase in pH. An increased pH could then affect other blood gas analytes, such as ionized calcium, which decreases due to increased protein binding.

Other preanalytic concerns are hemolyzed specimens that would produce falsely elevated potassium. The pH and partial pressures, however, remain unaffected. The sample collection tube itself contains lyophilized heparin that has been pre-titrated with physiologic concentration of cations. This is because heparin is a polyanion and can bind cation electrolytes. Therefore, pre-loaded cations will minimize errors in measurement due to cation exchange with heparin. Liquid heparin is not recommended, as insufficient sample collection will affect the blood to anticoagulant ratio, diluting out analytes such as bicarbonate and pCO_2, and falsely lowering the results.

Slide 14:
An additional important issue to consider is the variability observed in analyte concentration from one tube type to the next and the subsequent effect on reference intervals or published diagnostic cut-offs. For instance, there are published cut-off values for plasma glucose levels used to diagnose, manage, and perform risk assessment of diabetic patients and patients with impaired glucose metabolism. Therefore, accurate glucose results are very important. Selection of the appropriate tube type has a huge impact on the stability of glucose. Gambino et al. compared four different types of glucose collection devices as shown on this table, which were processed under different conditions. In this study, the reference concentration of glucose was defined based on the current guidelines, which recommends placing specimens in ice-water immediately after collection and separating plasma from cells within 30 minutes. As you can see, fluoridated samples vary significantly from the reference method. The notion that NaF is effective at inhibiting glycolysis is a mistaken belief and as such, the American Diabetes Association no longer recommends using these tubes for glucose measurement in the clinical care of diabetic patients or patients at risk for diabetes. The use of NaF tubes increases the intra-individual variability of glucose, which could lead to false negative diagnoses or hinder accurate quantification of patients at risk for diabetes. A notable caveat is that the current cutoffs for diabetes were generated using studies performed using NaF tubes. Laboratories need to be aware of these discrepancies and if the most current recommendation for glucose sample collection and handling is practiced, current diagnostic cutoff points need to be re-evaluated.

Slide 15:
To conclude, blood collection tubes contain a variety of different additives. Not only is it important to draw blood into the right tube but it is also important to draw samples in a specific order if more than one type of blood draw is needed when non-vacutainer tubes are being used. This is because additives from the first tube may carry over to the next due to back flow during blood draw. For instance, blood drawn into a K2EDTA top can cause EDTA to carry over to the next tube causing calcium in the next sample to be falsely decreased and potassium to be falsely increased. To this end, the Clinical and Laboratory Standards Institute (CLSI) has developed guidelines to standardize the order of blood draw. It is recommended that the order of draw start with the blood cultures for microbiology testing, royal blue tube that doesn't contain any additives, followed by the light blue top for coagulation tests. The serum generating tubes are drawn next which is followed by heparinized plasma, EDTA plasma, and finally, fluoridated plasma tubes. However, if vacutainer tubes are used, the order of draw has been shown to have negligible effect on sample quality.

Slide 16: References

Slide 17: Disclosures

Slide 18: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Phlebotomy: Tube Type Selection." My name is Prati Ranjitkar.