"Do's & Don'ts" for Reliable Specimen Quality

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Basic Concept:

Red cells (RBCs) often have a much higher concentration of analytes than the liquid portion (serum/plasma) of blood. For example, RBCs have 20x more potassium (K) than serum/plasma, so prolonged contact and/or contamination with red cells will significantly change K levels. The practices below help ensure acceptable samples for all analytes.

Obtaining the Sample:

DO **use a 21, 22, or 23-gauge needle & do pull back gently on plunger when using a syringe.**
WHY? Small gauges (25 g or higher needles or butterflies) and suction or transfer practices (excessive pulling or pushing on plunger) may damage red cells and release analytes from the cells such as K. Note: Difficult draws or young children may require a smaller needle gauge and will then have a higher risk of hemolysis.

DO **follow prescribed order of draw (blood cultures, blue tops, red or gold tops, green tops/with or without gel, lavender tops, and gray tops).**
WHY? Contamination from anticoagulant from preceding tubes can affect results.

DO **use a discard tube (blue or white top tube) if a butterfly is used and a blue top tube for coagulation testing would otherwise be the first tube drawn.**
WHY? The first tube in the series will be under filled, so a blank (discard) tube is needed prior to drawing the coagulation tube to ensure the proper blood to anticoagulant ratio.

DO **ensure that tubes with an indicated fill line or minimum fill line (such as blue top tubes) are appropriately filled.**
WHY? Over or under filled tubes may cause inaccurate results due to improper blood to anticoagulant ratios.

DO **invert tubes gently that contain anticoagulant 8 times (blue tops for coagulation 3-4 times), red top or gold top tubes gently 5 times—no shaking*. Note: One complete inversion is top up, top down, top up.**
WHY? Shaking tubes can break fragile red cells and release analytes from the cells into the serum/plasma. Immediate mixing assists with clot activation in serum tubes and anticoagulation in plasma tubes.
*Exception: Shake tubes for Quantiferon vigorously 5 seconds to create frothing and ensure optimal results.

DON'T **instruct the patient to clench/pump the fist before or during draw; may ask patient to close the hand to distend veins, then release. Don't leave tourniquet on for more than 60 seconds.**
WHY NOT? Fragile red cells may be damaged and release K or other cell components.

Handling the Sample:

DO **allow red top tubes to clot in an upright position at room temperature for 60 minutes, gold top for 30 minutes. Centrifuge for the preprogrammed time or 10 minutes for red or gold top tubes, 10 minutes for green, and 15 minutes for BD Blue top tubes. If minimum g-force (1300g) cannot be achieved, spin for 20 minutes. Do not use a manual brake on the centrifuge. Only un-spun tubes may be placed in pneumatic tube system.**
WHY? Spin time and force of centrifugation are related. Harsh braking or pneumatic tube systems will re-suspend particles causing false results. Laboratories meeting special centrifuge conditions may be able to spin green top tubes for a reduced time.

DO **spin tubes within 2 hours of draw. Inspect gel barrier: no gaps, uniform thickness (unless slanted from a fixed angle centrifuge). If gel has gaps or is uneven (gel should either be horizontal or a sealed slant), remove an aliquot and respin the aliquot.**
WHY? A proper gel seal between the cells and the serum/plasma is needed to prevent leakage into sample. Re-spinning tubes that contain gel will contaminate the sample, so remove an aliquot for the re-spin.

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DO **keep tubes completely upright after centrifugation until tested unless an aliquot is sent in a transport tube. If transport is needed, use a pipette to remove sample into aliquot tube, avoiding the very top of the sample and the area just above the gel.**
Why? Mixing with cellular components occurs in tubes if not kept upright or if sample is poured instead of pipetted. Sample mixed with cells (from top of sample, stopper, just above gel) causes false results.

DO **ensure that any red cells adhering to the cap or the side of the tube do not fall back into the sample when the tube is uncapped.**
WHY? Contamination by red cells will falsely affect testing.

DO **mix by gentle inversion and remove an aliquot of refrigerated (or frozen) plasma sample then respin (5 minutes) before testing (most analytes stable 48 hours maximum in plasma).**
WHY? Cell aggregates or microclots form which must be removed prior to testing.

DO **bring lock boxes inside in the morning & place specimens out only at the end of the day.**
WHY? Hot or cold temperatures adversely affect samples & cause erroneous results.

DON'T **respin blood in a gel tube after it has been centrifuged to recover additional sample.**
WHY NOT? Proper gel migration can only occur upon initial spin and subsequent spins will be contaminated with material from cell layer. If a respin is needed, remove an aliquot of serum/plasma and respin that.

DON'T **combine serum/plasma from a difficult draw with that from another stick to "get enough".**
WHY NOT? Serum/plasma from difficult draws will contaminate the sample from a "good" draw.

DON'T **freeze an aliquot if red cells are present, respin and remove the sample from cells.**
WHY NOT? Freezing will release K and other components from the red cells.

DON'T **refrigerate unspun blood samples that are collected for K levels.**
WHY NOT? Leakage of K from red cells into the serum will occur faster at refrigerated temperatures

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