Best Practices in Sample Handling: Part 1
Responses to Participant Questions

1. Can Sodium Fluoride tubes be stored in the freezer for a lactic acid test?

   This has not been tested by Greiner Bio-One. Please refer to the assay package insert for information regarding appropriate sample types and storage for method specific recommendations.

2. What is the effect of freezing on SSTs and other tubes prior to use?

   It is recommended that VACUETTE® Blood Collection Tubes be stored at 4-25°C. Short term variations in temperature do not typically impact product performance but this is dependent on the storage temperature and length of time.

3. Is the royal blue top the same as the light blue top? If not, where is the royal blue placed in order of draw?

   Blood collection tubes with a light blue cap contain sodium citrate and are typically used for routine coagulation testing. Tubes with royal blue caps are for testing of trace elements and additive varies depending on manufacturer. Greiner Bio-One offers two tubes with different additives for trace element testing and recommends that the trace element tube be included in the order of draw according to additive. However, institutional policy should be followed.

4. If the additive is spray dried, must the tube still be filled the whole way? What is the difference between the spray-dried additive and liquid additives?

   Spray-dried additives are commonly used in plastic evacuated tubes. Additive is sprayed onto the walls of the tube and then allowed to dry to the surface. Liquid additives were more common when glass was used. If liquid additive is used in plastic tubes, certain accommodations must be made to prevent evaporation as with the tube-within-a-tube configuration used for sodium citrate tubes where the inner tube is polypropylene, which prevents evaporation, and the outer tube is Polyethylene which maintains the vacuum.

5. In regards to emergency department specimens, we are centrifuging too early but we know time is of essence. How quickly should we process and put on the machine to avoid rejected and recollected specimens?

   Samples can be processed immediately following collection with the exception of serum tubes, which should be placed in a rack in an upright position to allow time for clot formation. Tube manufacturers typically recommend a 30 minute time period. If samples are processed too soon, there is a risk of fibrin formation in the serum, which may interfere with testing.

One researcher (Csako) found centrifuging samples without stoppers increased mean potassium levels 132% (4.7 versus 6.2mmol/L). We are unaware of the mechanism involved in unstoppered centrifugation that would have resulted in the increase, but speculate evaporation during high-speed centrifugation may be a contributor.


7. Is second centrifugation still a problem if the serum/plasma is removed to a new vial (no additives) and no longer exposed to the cellular material?

No – this concern is primarily related to re-centrifugation of gel tubes.

8. Does Drucker have any educational material they can share with us? We have several outreach clinics that need to be educated and the material would help out. Our facility uses Drucker centrifuges.

Yes, education material is available on Drucker Diagnostic's website. Contact Drucker customer service directly with specific requests.

9. What would you recommend for mobile phlebotomists with regards to storage, transport and centrifugation?

The requirements for sample collection and handling are relevant to all circumstances. Therefore, procedures should be developed in accordance with manufacturer instructions and CLSI standards and guidelines.

10. How important is balance in the mini centrifuges, i.e. can you balance a blue top tube with an SST tube?

Centrifuges are designed to handle a fair amount of imbalance. However, to maximize the life of the centrifuge and to minimize risk of hemolyzing cells due to excessive vibrations, it is recommended that tubes of equal size and weight are used for balancing.

11. Do you need to collect a discard tube if you collect the blue top first?

A discard tube should be collected if a winged collection set/needle is used for the venipuncture and the sodium citrate tube is the first tube drawn. This will compensate for the dead space in the tubing so that the full draw volume with the coagulation tube is possible.
12. Can placing filled lithium heparin tubes in the refrigerator unspun affect K⁺ levels?

Yes – as explained during the webinar, cold inhibits the ATPase pumps allowing potassium to leak from the red blood cells into the plasma. This can result in pseudohyperkalemia.

13. How can you maintain the 9:1 blood to diluent ratio in a citrate tube when you only draw 90% of the full volume?

According to ISO 6710 and CLSI GP39 (Tubes and Additives for Venous and Capillary Blood Specimen Collection) standards, evacuated tubes must draw within ±10% of the stated draw volume for the duration of the stated shelf life. Specific to coagulation and according to the CLSI H21 guideline (Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays), a minimum of 90% fill is recommended for sodium citrate evacuated tubes.

14. How does a pneumatic tube system affect sample integrity?

Sample integrity is dependent on many factors. In general, a pneumatic tube that is functioning correctly should not have any impact on sample integrity unless the patient has a condition where the cells are particularly fragile. However, it may be beneficial to verify that pneumatic tube transport does not impact analytical results by conducting a study comparing tubes sent through pneumatic tube with those manually transported.

15. I like the tidbit about potassium increasing when tubes are centrifuged without the stoppers. Is this re-centrifugation or on initial centrifugation?

It is our understanding samples were only centrifuged once.

16. What is the best transportation temperature between different sites (for non and centrifuged specimens and urine)?

This is dependent on the analytes to be measured and methodologies used for testing. Please refer to assay package inserts for specific information.

17. I like your answer regarding concentration of anticoagulant and under filled tubes with spray-dried or liquid anticoagulants. Any current literature/study regarding liquid vs spray affects on under filled tubes that I can read/reference to?

Many studies have been published that establish the effect of under filling citrate tubes (Reneke). Many others have established the effect of under filling spray/dried anticoagulant tubes on lithium heparin (Donnelly, Tietz, Tamechika, Lippi), and EDTA tubes(Donnelly).

- Tietz Guide to Clinical Laboratory Tests WB Saunders, St. Louis, MO (2006)
- Tamechika Y, Iwatabni Y, Thoyama K, Ichihara K. Insufficient filling of vacuum tubes as a cause of microhemolysis and elevated serum lactate dehydrogenase levels. Use of


18. Since centrifuging a second time is not good practice, what do you suggest if you notice RBCs present in the serum after centrifuging once and the patient has already left?

The best solution in the case would be to aliquot off the serum or plasma and centrifuge the aliquoted sample.

19. Why are most blood culture bottles glass? Why is it difficult to find a plastic bottle?

Many blood culture bottles are glass due to the high degree of vacuum that must be applied to draw the correct quantity of blood. There are some manufacturers that offer plastic alternatives, such as bioMérieux.

20. Quest says that the PTT is good for longer than 4 hours. Should we spin and freeze these before sending?

CLSI H21 does instruct that, if PTT cannot be completed within 4 hours, platelet-poor plasma should be removed and frozen. However, this document also indicates that laboratories can perform studies if testing is delayed more than 4 hours. Recently, studies indicate longer stabilities, and Quest may have conducted internal studies that support longer transit times. Please contact Quest for specific information and directions.

21. Are fibrin clots the only side effect of not storing tubes upright?

Clots adhering to the stopper are primarily a concern with serum tubes. However, plasma tube left in a horizontal position may result in red cells being trapped around the stopper so that, when tubes are uncapped, red cells may run down the tube walls and mix with plasma.

22. How do you ensure integrity of samples in the ED for immediate use on iStats?

Unfortunately, this question requires more specific information in order to provide an appropriate answer.

23. What place does the BLACK tube go in the process?

If this is in reference to the citrate tube for ESR, CLSI standards do not specifically address this tube type so institutional policy should be followed.
24. Should gel tubes be transported upright after centrifugation?

An upright position is always preferred whether before or after centrifugation.

25. You said earlier that the inversion test wasn’t the correct method for testing if a tube was clotted. What is?

There is no effective subjective measurement to assure clotting is complete. The recommendation to wait 20-30 minutes assures complete clotting unless the patient is overmedicated or has a coagulopathy.

26. What is the effect on HIV and HCV molecular testing if centrifugation happens later than 2 hours after draw?

Please refer to the package insert for the assay/method in use for specific information on sample handling and storage.

27. Is there a specific minimum volume for a “discard” tube draw?

With regard to drawing blood with a winged collection device for coagulation testing, it is only necessary to draw enough blood to fill the tubing and eliminate the dead space, i.e., when blood enters the discard tube, you can remove it and proceed with collection of the sodium citrate tube.

28. In regards to keeping a tube upright after the draw (before centrifugation) - what do you suggest for nurses who draw, put them in a biohazard bag and then transport while it’s horizontal or sent through the pneumatic transport system to the lab?

This is a common practice, but not a best practice. Transporting tubes in other than an upright position creates a risk of the clot attaching to the stopper and yielding serum with fibrin strands and trapped red cells. If you experience samples that come out of the centrifuge with fibrin strands that need to be removed, you should explore a process improvement that permits tubes to be stored and transported upright.

29. Does the type of needle affect the potassium level, meaning does it matter if you use a butterfly or straight needle when drawing a potassium level?

The type of needle should not impact potassium levels as long as the needle gauge is appropriate for the circumstances of the draw and good phlebotomy technique is followed.

30. There are centrifuges available for automobile application?

Yes, mobile centrifuges are available. Contact Drucker customer service directly for product specific information. 1-877-231-3115
31. How do we get information on portable centrifuge equipment?

Contact Drucker customer service directly for product specific information. 1-877-231-3115

32. What's the difference between the pink tube and lavender tube? Are both versions of EDTA the same additive? Can lavender go to BB?

Greiner Bio-One offers both K₂EDTA and K₃EDTA in the lavender and pink cap tubes. The information on the specific EDTA type is on the product label. If both the lavender and pink cap tubes in your facility are the same, i.e. both K₂ or both K₃, or your blood bank methodologies are validated for both versions, then the lavender can be used in the blood bank.

33. In a plain red top tube, does a serum filter separator have the same effect on stopping potassium crossover as the gel does or would the serum be best taken off the cells?

This may depend on the type of filter used. Please refer to manufacturer instructions for use.

34. Does butterfly usage have any impact on the specimen when centrifuged?

Use of a winged collection set should not impact centrifugation as long as the device used is appropriate for the circumstances of the draw and utilized following proper phlebotomy technique. If the collection device is not appropriate and results in a traumatic draw, i.e. needle gauge too small, syringe plunger pulled too fast, etc., this can impact the integrity of the cells and ultimately sample quality.

35. Do high altitude tubes state that on tube or do the manufactures automatically send the proper tubes to the proper locations? I live at 7000 ft and have never seen or known about the altitude tubes being available.

High altitude tubes are appropriately labeled but must be specifically ordered from the manufacturer. They may not be required as long as adequate fill is obtainable.