**BD Vacutainer Recommendations for Venous Blood Collection**

**Frequently Asked Questions**

Venous Blood Collection - General Tube Questions

* **What type of plastic is used to produce BD Vacutainer® PLUS Blood Collection Tubes?**BD Vacutainer® PLUS Tubes are made of a special formulation of the plastic, polyethylene terephthalate (PET). This special formulation is extremely strong, dimensionally stable, and resistant to chemicals and breakage.
* **What speed and time should the BD Vacutainer® Blood Collection tubes be spun at?** All non-gel blood collection tubes, including those that contain heparin, EDTA and non-gel serum tubes can be centrifuged at ≤1300 RCF for 10 minutes.  The BD Vacutainer® SST**™**and PST™ gel tubes should be spun at room temperature at a speed of 1000 to 1300 RCF for 10 minutes in a swinging bucket centrifuge and 15 minutes in a fixed-angle centrifuge.
* **What coatings layer the walls of BD Vacutainer® Plus Serum and SST™ Tubes?**BD Vacutainer® Plus Plastic Serum and SST™ Tubes are coated with silicone and micronized silica particles to accelerate clotting. A silicone coating reduces adherence of red cells to tube walls. The silica coating can sometimes cause the inner tube wall to appear cloudy and/or filmy.  This cloudy appearance does not make the tubes unacceptable for use.
* **What are the proper number of inversions for the various BD Vacutainer® Blood Collection Tubes?**An inversion is one complete turn of the wrist, 180 degrees, and back.  Tubes should be inverted according to the following recommendations:
	+ SST and serum tubes – 5 inversions
	+ Additive tubes (EDTA, heparin, etc) – 8-10 inversions
	+ Sodium citrate tubes (blue top) – 3-4 inversions
* **What is a BD Hemogard™ closure?**The BD Hemogard™ closure has a unique rubber stopper that seals BD Vacutainer® Blood Collection Tubes. It is recessed within and covered by a plastic shield to help protect laboratory personnel from contact with blood on the stopper or around the outer rim of the tube. The BD Hemogard™ tube closure also helps prevent blood from splattering when the tube is opened.

Gel Tubes

* **What is the purpose of the gel in BD Vacutainer® SST™ Serum Separation Tubes and BD Vacutainer® PST™ Plasma Separator Tubes?**
The gel forms a physical barrier between serum or plasma and blood cells during centrifugation. It is important to note that after collection, BD Vacutainer® SST™ Serum Separation Tubes should be inverted five times, allowed 30 minutes clotting time, and centrifuged for 10 minutes at 1000-1300 RCF (g) in a swing bucket centrifuge. BD Vacutainer® PST™ Plasma Separation Tubes should be inverted 8 times, and centrifuged for 10 minutes at 1000-1300 RCF (g) in a swing bucket centrifuge.
* **What is the difference between BD Vacutainer® SST™ and PST™ Blood Collection Tubes?**SST™ refers to the Serum Separator Tube containing clot activator and serum separator gel. PST™ refers to the Plasma Separator Tube containing lithium heparin and plasma separator gel.
* **What is the gel composed of?**
The gel is composed of inert components, which are part of a polyester-based proprietary formulation.
* **What is the clot activator in BD Vacutainer® SST™ Serum Separation Tubes?**The silica particles that coat the walls of the BD Vacutainer® SST™ tube are the clot activator. Initial activation occurs when blood enters the tube and contacts the particles on the tube wall. To continue the activation process, it is necessary to thoroughly mix the blood and particles by inverting the tube five times.
* **Why do the inside walls of the BD Vacutainer® SST™ Tubes appear white and cloudy? Are the tubes still all right to use?**The walls of BD Vacutainer® SST™ Serum Separation Tubes are coated with silica particles as a clot activator. The coating process creates a film on the tube surface that appears white and slightly cloudy. The tubes are fine to use. However, it is important to remember to invert the SST™ Tube at least five times after filling. This ensures adequate mixing of silica particles with the blood, which is required for optimal performance.
* **How soon after collection should BD Vacutainer® gel tubes be centrifuged?**Gel separation tubes should be centrifuged no longer than 2 hours after collection.
* **Can I re-centrifuge BD Vacutainer® gel tubes?**BD does not recommend re-centrifuging gel tubes once the barrier has formed.
* **Can the serum and plasma be frozen on the gel, in the original BD SST™ and PST™ Tubes?**It is not recommended to freeze the sample in the primary blood collection tube, on the gel barrier.  The gel may separate when it is frozen and thawed, resulting in red cell contamination of the sample.

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### EDTA Tubes

* **What does EDTA stand for and how does the anticoagulant work?**EDTA stands for Ethylenediaminetetraacetic acid.  EDTA functions by binding calcium in the blood and keeping the blood from clotting.
* **What is the minimum and maximum fill volume in the EDTA tubes?**The minimum and maximum acceptable blood volumes should be established by each facility, to ensure that accurate hematology results are obtained.  BD Vacutainer® Blood Collection Tubes are designed to draw the appropriate volume to ensure a proper blood to additive ratio.
* **Does BD offer an EDTA tube that contains gel?**Yes, the BD Vacutainer® PPT™ Tube (reference number 362788) contains EDTA anticoagulant and an inert polyester gel.  The Hemogard closure color is pearl white and the blood draw volume is 5mL.

Sodium Citrate Tubes

* **What is the minimum volume of blood that should be collected into a BD Vacutainer® sodium citrate tube?**
BD tubes are designed to yield within ± 10% of the stated draw volume on the label of the additive tube throughout the entire shelf life. (CLSI guideline - Evacuated Tubes and Additives for Blood Specimen Collection-Fifth Edition H1-A5, 2003)
* **What are the recommended centrifuge speed and time for the sodium citrate tubes?**
CLSI recommends that sodium citrate tubes be spun at 1500 RCF for 15 minutes to achieve platelet poor plasma (platelet count < 10, 000) and accurate coagulation test results.
* **What do the lines mean on BD Vacutainer® Plus sodium citrate tubes?**The etched fill indicator on the plastic citrate tubes indicates the minimum acceptable blood volume in the tube.
* **With such low draw volume Plus Plastic citrate tubes, will we have enough volume of plasma for testing?**
The following bullet points demonstrate that there is enough plasma to perform coagulation testing in a patient with an average hematocrit of 45%.
Tests in a 1.8 mL Plastic Citrate Tube Assuming a Patient Hematocrit of 45:
	+ Total plastic citrate tube blood volume is 2.0 mL
	+ Packed Cell Volume (PCV) = 2.0 mL x 0.45 = 0.9 mL
	+ Subtract PCV from whole blood volume to get volume of available plasma (2.0 - 0.9 = 1.1 mL)
	+ Allow 0.4 mL plasma to remain to avoid disturbing the buffy coat (1.1 - 0.4 = 0.7 mL)
	+ This still leaves 0.7 mL of plasma available for testing
	+ Automated instruments require an average of 100 µl per test for PT and APTT tests, 50 µl for Fibrinogen, and 20 µl for Factor Assays
	+ This volume, 0.27 mL, allows for a repeat test, if needed

For 2.7 mL Plus Citrate Tube Assuming a Hct of 45:

* + Total plastic citrate tube blood volume is 3.0 mL
	+ PCV = 3 mL x 0.45 = 1.35 mL
	+ Subtract PCV from whole blood volume to get volume of available plasma (3.0 - 1.35 = 1.65 mL)
	+ Allow 0.4 mL plasma to avoid disturbing the buffy coat cells (1.65 - 0.4 = 1.25 mL)
	+ This leaves 1.25 mL of plasma available for testing
	+ Automated instruments require an average of 100 µl per test for PT and APTT, 50 µl for Fibrinogen, and 20 µl for Factor Assays (i.e., PT, APTT, Fibrinogen Plus 2 assays = 0.29 mL)
* **Is a discard tube needed if the only tube being collected is a coagulation tube?**
There have been recent articles indicating that drawing a discard tube is not necessary before drawing the sodium citrate tube. In the CLSI coagulation document (H21-A5) it is still recommended that the citrate tube is the second or third tube drawn. However, in the CLSI guideline for Venipuncture Collection (H3-A6) it states that for routine PT and APTT, the first tube drawn may be used for testing.
It is also important to ensure a discard tube is drawn when a blood collection set is used and only a citrate tube is ordered, as the tubing may contain up to 0.5 mL of air that will be drawn into the tube and displace blood volume.
* **When and why must the air be purged from the BD Vacutainer® Safety-Lok™ Blood Collection Set (SLBCS) and the BD Vacutainer® Push Button Blood Collection Set (PBBCS) tubing?**
It is critical to purge the air from the tubing of a blood collection set when collecting a sodium citrate tube for PT and/or APTT testing. A plain discard tube or another sodium citrate tube should be used first to purge the air from the tubing and allow a full draw into the citrate tube. Purging the air from the tubing is also recommended before drawing any additive tube. CLSI recommends that all additive tubes should be filled to their stated volume.
* **How can the 1.8 mL and 2.7 mL BD Vacutainer® Plus Coagulation Tubes be differentiated?**
The 1.8 mL tube has a light blue rubber stopper covered with a translucent shield and the 2.7 mL tube has a light blue rubber stopper covered with a solid light blue Hemogard™ closure.
* **What is the blood to additive ratio in the sodium citrate tubes?**The blood to additive ratio is 9 parts blood to 1 part sodium citrate.
* **What is the additive volume in the BD Plus sodium citrate tubes?**The 1.8 mLdraw Plus sodium citrate tube contains 0.2mL of sodium citrate, and the 2.7 mL draw contains 0.3 mL of sodium citrate.
* **What is the effect of hemolysis on coagulation results?**Hemolyzed specimens should not be processed since there could be activation of the clotting factors. Lipemic or icteric specimens may also interfere with the instrumentation's optical system affecting the coagulation specimen result.
* **What constitutes a tube inversion?**An inversion is one complete turn of the wrist, 180 degrees, and back.  BD recommends that citrate tubes be inverted 3 to 4 times.
* **How long should the tourniquet be left on for drawing coagulation specimens?**Placing the tourniquet for longer than 1 minute can lead to changes in the concentration of coagulation protein in the plasma as well as platelet activation which can result in erroneous coagulation results.
* **Can the sodium citrate tube be used when a patient exhibits platelet clumping in an EDTA tube?**Yes, the sodium citrate tube can be used to get an accurate platelet count if a patient’s platelets clump in the EDTA tube.  The platelet count from the sodium citrate tube should be multiplied by 1.1 to account for the different blood to additive ratio in the citrate tube.

## **Molecular Diagnostics**

**The product inserts for these products are available on-line at:** [**http://www.bd.com/vacutainer/referencematerial/**](http://www.bd.com/vacutainer/referencematerial)

### BD Vacutainer® PPT™ Plasma Preparation Tube

* **What is the intended use of the BD PPT™ Tube?** The BD Vacutainer® PPT™ Plasma Preparation Tube (BD PPT™ Tube) is a plastic evacuated tube for the collection of venous blood which, upon centrifugation, separates undiluted plasma for use in molecular diagnostic test methods (such as but not limited to PCR - polymerase chain reaction and/or bDNA - branched DNA amplification techniques) or other procedures where an undiluted plasma specimen is required as determined by the laboratory.
* **Is spray-dried EDTA the preferred anticoagulant for quantitative molecular diagnostic tests?**Yes. Spray-dried K2EDTA does not dilute plasma and does not interfere with downstream molecular diagnostic assay protocols such as viral nucleic acid extraction, gene or signal amplification or sequencing.
	+ Liquid additives dilute plasma, limiting their use for quantitative tests.
	+ Heparin is not indicated because it can inhibit target sequence amplification during PCR.
	+ ACD (acid citrate dextrose) will dilute plasma and can cause hemolysis.
	+ Serum samples are not preferred for molecular test methods.
* **What is the concentration of the additive in the BD PPT™ Tube?**
The reference number 362788 contains 9 mg of dried K2EDTA, and reference number 362799 contains 15.8 mg of  K2EDTA yielding a concentration of 1.8 mg EDTA/mL of blood when the evacuated tube is filled correctly to its stated draw volume. The tube also contains a polyester gel that upon correct centrifugation (1,100xg for 10 minutes, swing-out bucket rotor) forms a barrier between the plasma and most of the cellular elements, allowing for transportation of the BD PPT™ Tube without removal of the plasma. Only the inside of the tube is sterile. The tube is not pyrogen free.
* **What is the recommended storage temperature for the BD PPT™ Tube?**Store unfilled tubes at 4-25°C (39-77°F).
* **What are the recommended collection and handling procedures for the BD PPT™ Tube?**
	+ The BD PPT™ Tube should be at room temperature (18-25°C) and properly labeled for patient identification.
	+ Collect blood into the BD PPT™ Tube using your institution’s recommended procedure for standard venipuncture technique and sample collection.
	+ After collection of whole blood in the BD PPT™ Tube, gently invert the BD PPT™ Tube 8 - 10 times.
	+ After mixing, store the BD PPT™ Tube upright at room temperature until centrifugation. Blood samples should be centrifuged within two (2) hours of blood collection for best results. Centrifugation of a sample at a period greater than 2 hours may require validation by your institution or testing laboratory. Centrifuge the BD PPT™ Tube/blood specimen at room temperature (18-25°C) for a minimum of 10 minutes at 1,100 RCF (Relative Centrifugal Force).
	WARNING: Excessive centrifuge speed (over 10,000 RCF) may cause tube breakage and exposure to blood and possible injury. To calculate the correct centrifuge speed for a given RCF, use the following calculator. Simply enter two of the three values in the calculator and press the calculate button.<http://www.bd.com/vacutainer/products/molecular/ppt/procedure.asp#forcecalc>
	+ To obtain an undiluted plasma sample, remove the BD Hemogard™ Closure and aliquot plasma into a separate vessel using a transfer pipette. NOTE: When using a transfer pipette be sure NOT to disturb the barrier with the tip of the pipette.