

Procedure for Double Spinning Coagulation Specimens (For Freezing and Transport)

It is required by the College of American Pathologists that plasma specimens for coagulation testing have a plasma platelet count of less than 10,000. This is especially true for specimens which are being frozen for any kind of Lupus Anticoagulant testing. The time and speed at which the coagulation specimens are spun should be set to achieve a plasma platelet count of less than 10,000. It is recommended that specimens for any type of lupus anticoagulant testing also be double spun to ensure that the platelet count is below the 10,000 limit. **Do Not Filter Plasma.** While filtering of the plasma has been used in the past, it has been reported in the literature that filtering removes the larger molecules of factors and vonWillebrand antigen. Therefore, double centrifuging is the method of choice. Since centrifuges vary, each site should validate that their centrifuges are set at a time and speed which will give them a plasma platelet count of less than 10,000.

The reasons for plasma to be platelet poor before freezing are as follows:

1. Platelets contain Factor 8 and Von Willibrand Antigen. These two factors play a big role in activating and continuing the coagulation process. If platelets are lysed when frozen and then thawed, these two factors will activate the coag process and may falsely shorten the patient's results.
2. Platelets contain a large amount of phospholipids. Lupus anticoagulant antibodies are phospholipid dependent. If you release these phospholipids into the plasma by freezing the platelets, the phospholipids will mask the presence of that antibody and may give falsely normal results.
3. Platelets contain Platelet Factor 3 which will neutralize heparin, including low molecular weight heparin (Lovenox). Therefore freezing the platelets may give a falsely lower result when trying to monitor the use of heparins.

Specimen Collection

Citrated blood 9:1 (blood to anticoagulant) in 3.2% sodium citrate. No other anticoagulant is acceptable. Samples that are under-filled, clotted or hemolyzed may yield incorrect results and **will not be accepted**. Expired collection tubes also will not be accepted.

Processing of Specimen:

1. Centrifuge the specimens at the appropriate time and speed to ensure a plasma platelet count of less than 10,000.
2. **Time and speed must be determined for each centrifuge.** If you do not have the means to check your plasma platelet count, please contact Tulsa RML/SJMC for assistance at 918-744-2500 x15513. We will ask you to send us a random double spun plasma specimen, **Not Frozen**. We will perform a platelet count for you which will be reported to your office with appropriate instructions.
3. Clients using the Horizon Mini B centrifuges should use 20 minutes spin times for both the initial spin and the second spin to ensure proper removal of platelets from the plasma.
4. After the first spin, using a plastic transfer pipette, remove the plasma to just above the cell line and place the plasma in a plastic tube. You must have a plastic tube for each blue top tube. You cannot pool the plasma from several tubes into one tube.
5. Spin this plastic tube at the speed and time to achieve a platelet count of less than 10,000. Horizon Mini B users need to spin for 20 minutes.
6. Using a plastic transfer pipette, slowly remove the plasma from the first plastic tube, leaving about 0.25 ml in the bottom of the first tube, and place the plasma into a second plastic tube. Discard the first tube. You should have 1.0 to 1.5 ml of plasma in the second tube.
7. Cap the second tube, label with patient's name, date and time of collection, and freeze. **DO NOT combine** the plasma from multiple tubes. Each plastic tube should contain **ONLY** the plasma from **ONE** blue tube.
8. Please transport frozen specimens on dry ice to the RML Coagulation lab in Tulsa. Specimens that thaw during transport cannot be tested.
9. **Please record the patient's anticoagulant history on the paperwork that is sent with the specimens. This will aid the pathologist in their interpretation and will alert the technologists to possible testing issues.**

We sincerely hope that the information presented here will be helpful to you. Please do not hesitate to call the RML/SJMC Coagulation Department with any questions.