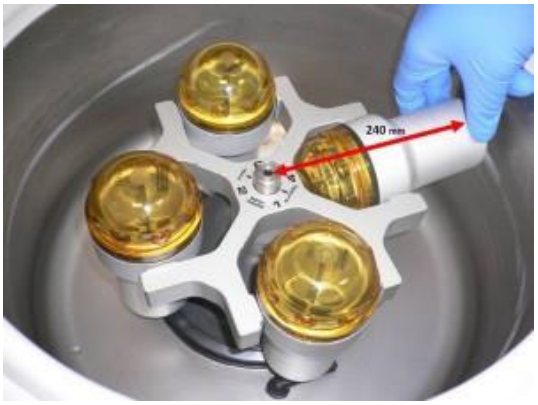
How to prepare a specimen for special coagulation testing

Special coagulation testing is often batch tested from frozen aliquots. Whenever plasma needs to be frozen for future testing it must be made platelet poor. To ensure the best possible specimens, follow this guideline as closely as possible.

For certain tests, the patient cannot be receiving anticoagulant medications (Heparin or Warfarin/Coumadin®); see individual test information for requirements.

1. Appropriately collect the required number of light blue top (3.2% sodium citrate), Becton Dickinson vacutainer tube(s) from the patient. 4.5 mL or 2.7 mL tubes are available through the Allina Health Laboratory supply catalog.
2. Prepare a platelet free (<10,000/µL) plasma specimen by double centrifuging the specimen.
    a. Centrifuge the citrate tube for 15 minutes at 1500-2000 rcf/G-force. Wait for the centrifuge to stop completely; DO NOT APPLY BRAKE.
        i. Follow the link below to identify RPM/rcf for site specific centrifuge: http://insilico.ehu.es/mini_tools/rcf_rpm.php
        ii. Length of radius in mm is required. Measure from rotor center to the bottom of extended swinging bucket as shown in picture below.



Example: Eppendorf 5702 (4 x 85 round bottom swinging bucket) with a radius of 140 mm. Enter 2000 rcf/G-force and radius of 140 mm into calculator = 3571 round down to 3500 RPM.

    b. Inspect spun sample:
        i. Review hematocrit, if the patient's hematocrit is ≥ 55%, the sample should be redrawn using tubes that have had their anticoagulant volume adjusted. Refer to Hematocrit – Anticoagulant Adjustment procedure.
        ii. Review for the presence of hemolysis. Reject sample as necessary.
    c. Using a plastic transfer pipet, remove the plasma leaving approximately 0.5 mL of plasma above the cell layer in the spun tube (do not pick up or disturb any of the buffy coat or red cells). Place in a labeled plastic centrifuge tube and cap.
    d. Using a wooden applicator stick, check the cells remaining in the blue top tube(s) for clots. DO NOT submit plasma from tubes in which a clot was detected.
    e. Centrifuge the plasma from step c. for another 15 minutes at 1500-2000 rcf/G-force.

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f. Using a plastic transfer pipet, remove the plasma from the middle of the tube leaving 0.5 mL of plasma on top of the spun red blood cells. Do not disturb the plasma and cells in the bottom of the tube. Place in labeled plastic micro-centrifuge / transfer tubes with approximately 0.75-1.0 mL per aliquot.

   Notes:
   - *Aliquots with visible red cells or hemolysis (pink plasma) are not acceptable.*
   - *Prepare one aliquot for each blue top tube received. Each tube should yield 0.75-1.0 mL of plasma. Sending all available plasma ensures duplicate testing can be performed and helps prevent recollection.*
   - *Von Willebrand requires a minimum of four aliquots.*

g. Label aliquots samples with the NaCi Plasma PPP label as well as the patient label (minimum of 2 patient identifiers required). Cover the label with clear tape to ensure label adherence during the thaw process.

h. Freeze plasma immediately. Sample(s) must remain frozen during transport.

i. Transport 2 micro-centrifuge tube aliquots in urine conical tube with cap. Rubber band multiple conical / transfer tubes together if same patient.

Additional notes:

- Patient specimens should be frozen at ≤ -20°C, if possible, and sent together in the same container. They must arrive in a frozen state.
- *Do NOT send specimens with the courier if they are not completely frozen; wait for the next available courier***.**
- Please include the requested information (see individual test description) as the testing and interpretations are dependent on clinical history.
- Careful specimen handling will most often ensure acceptable specimen and valid results.