Document History:

Title:	Serum / Plasma Separation Procedure and Transport SOP	Site(s):	Shared Health Diagnostic Services – ALL
Document #:	**110-10-05**	**Version #:**	**06**
Section:	**Clinical Biochemistry**	**Subsection:**	**General**

Approved by:	L Thorlacius	**Date:**	06 July 2009

Approved by: *(Approval on File)*	L Thorlacius	**Date:**	15 Sept 2023
		Effective Date:	12 Oct 2023

#	Details of Revisions:	Approval:	Date:
1	Changed Document # for DSM Phlebotomy Manual from 100-20-28 to 100-10-20; put document in new template	L Thorlacius	6 July 2009
2	Removed references to Corvac tubes. Added point vi (chilled specimens) to section: Centrifugation cautionary notes:	L Thorlacius	6 Jul 2009
3	Changed reference to the new standardized aliquot tube. Polypropylene 13x75 mm screw cap tube (Greiner Bio-one product # 459000)	H Malvern	8 Jul 2010
4	Various updates. Deleted table 2. Updated table 1. Added figure 1	A Sokoro	19 Dec 2016
5	Updated SOP to Shared Health, removed reference to DSM. Added centrifuge spin speed in RCF and maximum spin speed; added spin temperature (p.2). Removed PTH from example of chilled specimens (p.3). Corrected document number of Phlebotomy Manual (p.4). Updated picture showing measurement of centrifuge rotor radius (p.8).	H Malvern	1 June 2021
6	Major revisions throughout.	E Petryayeva	16 Aug 2023

1.0 RELATED POLICIES AND PROCEDURES

100-10-79 Phlebotomy Collection Manual

2.0 PURPOSE

To provide instructions on centrifugation, aliquoting and transporting of serum/plasma specimens within Shared Health Clinical Biochemistry laboratories.
This document does not cover specimen preparation and transport requirements for Hematology, Immunology or Cadham laboratories.

3.0 CENTRIFUGATION

- Allow serum specimens to clot (minimum 30 min for SST and 60 min for red top serum tubes; maximum 2 hours). If clotting time is too short, fibrin is likely to be present in the sample, which may block instrument needle, impair gel barrier, lead to the variability in aspirated volume and affect patient results.
- Serum sample should be kept in a vertical position to promote clotting and reduce formation of fibrin strands that attach to the tube stopper.
- Visually inspect serum specimens for full clot retraction prior to centrifugation.
- Specimen from patients with impaired coagulation (few examples listed below) may require 60 min clotting and even then clotting may not be complete. If specimen failed to clot, notify ordering physician.
    - Patients on anticoagulant or thrombolytic therapy
    - Patients receiving high doses of heparin (specimen may not clot at all)
    - Various conditions (e.g. liver disease, autoimmune diseases)
    - Multiple myeloma – myeloma globulin inhibits all three stages of fibrin formation: (1) the proteolytic action of thrombin on fibrinogen, (2) the aggregation of fibrin monomers, and (3) stabilization of fibrin by cross-linkages in the gamma and alpha chains.
- Centrifuge all samples as soon possible, and within 2 hours of collection, unless indicated otherwise in Shared Health LIM – some analytes are more sensitive and may require prompt removal from cells within 15-30 min of collection (e.g. ammonia, neonatal glucose, ACTH, catecholamines, free fatty acids, renin). Do not remove the stoppers before centrifugation.
- Use a horizontal centrifuge head (swinging bucket) whenever possible for spinning gel tubes.
- Use the correct size centrifuge carrier for the tube to avoid breakage. Match tubes to tubes of the same fill level, diameter, type of top closure, and gel to balance the centrifuge. Failure to balance tubes could result in tube breakage.
- Ensure appropriate centrifugation speed is used. Centrifuges should be set at 1100 – 1300 RCF (×g). Consult Tables 1 & 2 as well as Figure 1 to determine the exact RPM to use for centrifugation if you do not have an RCF programmable centrifuge.
- Excessive centrifuge speeds can cause gel globules to form. These globules can clog analyzer probes. For optimal gel barrier formation and to prevent heating, if a temperature-controlled centrifuge is used, it should be set to 20 - 25°C, unless centrifuging an analyte requiring a specific temperature.
- Centrifuge samples for 10 minutes (see Table 1 for details). Have the brake setting at medium to stop the centrifuge.

3.1 Handling Chilled Specimens

- Specimens that are transported to the laboratory chilled should be centrifuged under refrigerated conditions. Centrifuge should be set to 4 °C ± 2 °C.
    - Correctly chilled specimen is placed immediately upon collection in either crushed ice, a mixture of ice and water or Crioplast® containers. Good contact between the cooling medium and the specimen is essential. Large cubes of ice instead of water are not acceptable because of inadequate contact between the coolant and the specimen. The coolant must cover the specimen level in the tube.
    - Examples of assays that require chilled collection/centrifugation include: ammonia, pyruvate, neonatal glucose and gastrin; for the most current specimen requirements always consult the Shared Health LIM.
- CAUTION:
    - Temperatures lower than 15 °C can falsely elevate potassium results. Therefore, potassium should not be analyzed on specimens chilled prior to separation from cells.
    - Specimens collected for electrolytes must not be stored at 2 to 8 °C before centrifugation.

3.2 Post-Centrifugation Handling of Specimen

- Remove tubes from the centrifuge promptly to minimize the effect of heat produced during centrifugation. Keep tubes completely upright after centrifugation until tested or aliquoted.
- Examine gel tubes after spinning. The plasma or serum must be free of red cells, fibrin and particles. The gel barrier must be of uniform thickness, no gaps, level and firmly attached to the sides of the tube. If these conditions are not met, the specimen must not be stored in the original tube. The serum/plasma must be transferred to a clean aliquot tube, re-spun, and the supernatant transferred to a new clean aliquot tube (See Section 4.1).
- Under no circumstances should a specimen be re-centrifuged in the original tube. This results in the mixing of hemolyzed material from below the gel barrier with the specimen. Transfer the specimen to a clean aliquot tube before repeat centrifugation.
- Test Vacutainer tube specimens promptly. Standard processing conditions do not completely sediment all cells, whether or not gel is present. Cell-based metabolism and degradation will especially affect LD and glucose.
- Sites performing visual assessment of hemolysis, icterus and lipemia should do so prior to loading specimen on the analyzer following procedure outlined in SOP ***110-90-49 Visual Assessment of Hemolysis, Icterus and Lipemia.***

4.0 ALIQUOTING

After centrifugation plasma/serum layer will be at the top of the tube. Carefully collect the top layer with a pipette without disturbing the gel or buffy coat layer then transfer to an aliquot tube. Amber aliquot tube may be required for light sensitive analytes or metal-free tubes for trace metal testing - consult LIM for details.

- When pipetting, avoid the very top of the sample and the area just above the gel or buffy coat, as this may result in mixing with cells and cause false results. Good practice is to leave about 1/8-1/4 inch of platelet-rich serum/plasma sitting on cells.
- Do not pour off serum/plasma (unless recommended), as it will result in mixing with cellular components.
    - Pour over of plasma is acceptable for aliquoting plasma for trace element testing to avoid cross contamination from pipettes.

When uncapping or aliquoting ensure that any red cells adhering to the cap or the side of the tube do not fall back into the sample – contamination by red cell will lead to false results.

Aliquoting of samples is required when one or more of the following apply:

1) Serum/plasma must be re-spun due to inadequate gel barrier formation.
2) Sample to be shipped to another laboratory for testing, including within province and out-of-province laboratories.
3) Freezing of specimen for storage or transport.

4.1 Aliquot tubes

Storage and air/ground transport of refrigerated and frozen serum/plasma (< 4 mL)

- Use polypropylene 13 x 75 mm tube with screw cap (Greiner Bio-one product # 459000). Send 2 aliquots for larger volumes.

5.0 STORAGE AND TRANSPORT

5.1 *Storage using original collection tube*: to be used ONLY when tubes meet the following requirements:

Examine gel tubes after centrifugation. The plasma/serum must be free of red cells, fibrin and particulate. The gel barrier must be of uniform thickness, no gaps, level and firmly attached to the sides of the tube
If these conditions are not met, the specimen **MUST NOT** be stored in the original tube. It must be transferred to a clean aliquot tube with screw cap (See Section 4.1).
Transport of serum or plasma between facilities in the original collection tube is not acceptable. Aliquot as noted above.

5.2 Temperature requirements for specimen transport

Transport conditions (i.e. room temperature, refrigerated or frozen) should be followed as per printed aliquot labels.
Requirements for miscellaneous tests should be verified in Shared Health LIM.
Any discrepancies noted between LIM and Delphic printed labels should be reported to Clinical Biochemistry Technical Director or Clinical Biochemist on Duty.

5.3 Handling of refrigerated and frozen aliquots

- Frost-free freezers are not suitable for storage, as they have freeze/thaw cycles.
- Labs receiving refrigerated or frozen aliquots must mix thawed samples and re-centrifuge aliquots, as shipped samples may contain flocculent material or droplets on the sides of the tube. This can impair the analyzer's performance and the accuracy of the results. Re-centrifugation of serum/plasma aliquots can be performed using one of the following conditions:
    - 2550 RCF, 4 min (*do not exceed 2750 RCF, see Table 2*)
    - 1100-1300 RCF, 10 min

6.0 REFERENCES

1. Vacutainer Brand Evacuated Blood Collection System package insert. Becton Dickinson, Jan 2002 (8003192).
2. Microtainer Brand Tubes package insert. Becton Dickinson, document number 400930.
3. Corvac Plasma Separator Tube package insert. Sherwood Davis & Geck, document number 5270647.
4. Corvac Serum Separator Tube package insert. Sherwood Davis & Geck, document number 5270649.
5. Green C, Krahn J. Tube handling conditions R & D Summary. St Boniface General Hospital, 1998.
6. Lin FC, Cohen R, Losada R, Bush V. Cellular Sedimentation and Barrier Formation under Centrifugal Force in Blood Collection Tubes. Laboratory Medicine October 2001; 10(32): 588-592.
7. Recommendations for sample collection and centrifugation. Roche Diagnostics, April 2002.
8. CLSI H18-A3. Procedures for the Handing and Processing of Blood Specimens; Approved Guideline – Third Edition. 2004

Table 1. Centrifuge RCF (g-force) and time for blood collection tubes.

Tube Type	RCF (g-force)	Time minutes
Vacutainer SST	1100 – 1300	10
Vacutainer PST	1100 – 1300	10
Vacutainer Red	1100 – 1300	10
Vacutainer Royal Blue	1100 – 1300	10
Vacutainer Lavender	1100 - 1300	10
Vacutainer Light Blue	Initial validation of speed/RCF, time and temperature (if applicable) must occur with each centrifuge used in coagulation sample processing to achieve consistent platelet poor plasma (PPP). PPP must then be monitored ongoing. Consult Hematology Technical Director.	
Vacutainer Grey	1100 – 1300	10
Microtainer gel	1100 – 1300	10
Microtainer non-gel	1100 – 1300	10
Vacutainer Plus	1100 - 1300	10

Note:
To find out what the recommended RCF translates into RPM for a specific centrifuge, first measure the radius of the centrifuge (Figure 1) and consult Table 2. Round of the radius measurement to the nearest whole number.

Table 2. Centrifuge speed in RPM equivalent to 1100 RCF (min), 1300 RCF (max) and re-spin 2550 RCF as a function of the centrifuge radius.

RADIUS, cm	SPEED, RPM		
	1100 (min)	1300 (max)	2550 (re-spin only)
7	3700	4000	5700
8	3500	3800	5300
9	3300	3600	5000
10	3100	3400	4800
11	3000	3200	4600
12	2900	3100	4400
13	2800	3000	4200
14	2700	2900	4000
15	2600	2800	3900
16	2500	2700	3800
17	2400	2600	3700

RADIUS, cm	SPEED, RPM		
	1100 (min)	1300 (max)	2550 (re-spin only)
18	2300	2500	3500
19	2300	2500	3500
20	2200	2400	3400
21	2200	2400	3300
22	2100	2300	3200
23	2100	2300	3100
24	2000	2200	3100
25	2000	2200	3000
26	1900	2100	3000
27	1900	2100	2900
28	1900	2000	2900

Conversion between RCF (g) and RPM units can be performed according to the following formulas:

$$RCF = \left(\frac{RPM}{1000}\right)^2 \times Radius\ (cm) \times 11.18$$

$$RPM = \sqrt{\frac{RCF}{Radius\ (cm) \times 11.18}} \times 1000$$

where *RCF* is relative centrifugal force (or G force), *RPM* is rotational speed in revolutions per minute and *Radius* is the centrifugal (rotor) radius in cm measured as distance from the center of the turning axis to the inner bottom of the extended swing-out bucket as shown in Figure 1.

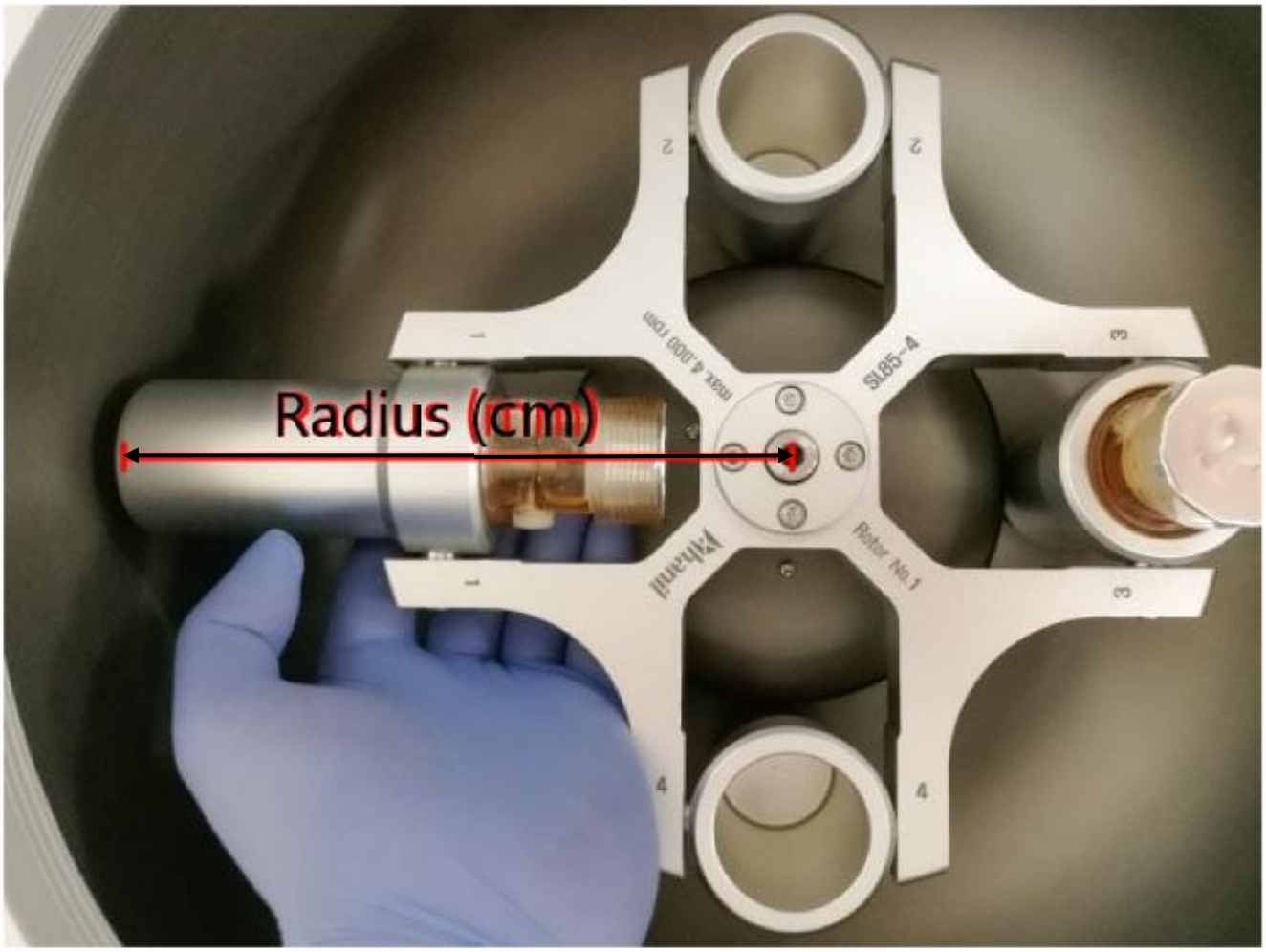

Figure 1. Measurement of the centrifuge (rotor) radius in centimeters starting at the center of the rotor to the inner bottom of the swing-out bucket (applicable for swing-bucket rotor only).