University of Vermont College of Medicine Department of Pathology Laboratory for Clinical Biochemistry Research Standard Operating Procedure: **Serum & Plasma Sample Clarification Via Centrifugation**	Doc. No **LCBR_SOP 017**

1. Distribution:

Personnel	Training Date	SOP Version	Signature

Date:	02-21-2020		Effective Date: 02-21-2020
Written By:	Danielle Parent, MLS		
Approvals:			
	Laboratory Director	Laboratory Coordinator	
Related Documents & attachments:			
Revision History:			
Rev #	Effective Date	Description of Change	

2. Background and Principle:

2.1 Background: In accordance to the Kit Instructions for the Simoa N4PA Advantage Kit (Catalog Number: 102153), excess fibrin, lipids and particulate matter may interfere with the accuracy of the assay. The following procedure efficiently removes these interfering substances.

2.2 Principle: High speed centrifugation will separate lipids (top layer) and fibrin/particulate matter (pellet formation) such that a clarified serum or plasma sample can be plated for use on the Quanterix HD-1 or HD-X analyzer.

3. Specimen Handling and Collection:

3.1 Specimen Handling:

3.1.1 Blood-borne pathogen safety training (offered by UVM ESF) must be completed before any work in the laboratory commences. **Use Universal Precautions and treat blood and blood products as potentially infectious materials. It is not known if specimens contain HBV, HIV, and other blood borne pathogens.**

3.1.2 Wear proper protective equipment, lab coat and gloves.

3.1.3 Dispose all tips and materials that come in contact with biological agents into proper biological waste containers.

3.2 Specimen Collection:

3.2.1 **Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at < -20 o C. Avoid repeated freeze-thaw cycles.

3.2.2 **Plasma:** Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at < -20 o C. Avoid repeated freeze-thaw cycles.

4. Reagents, Recipes and Equipment

4.1 **Reagents:** 10% Bleach for work area decontamination

4.2 **Equipment:**

4.2.1 Vacuum Apparatus
- Bench Top Built in or equivalent

4.2.2 1L - 2L Vacuum flask
- Thermo Fisher Catalog DS4101-1000 or equivalent

4.2.3 One hole Stopper to fit vacuum flask
- Fisher Scientific Catalog 14-135M (Stopper size 8)

4.2.4 Tygon Tubing
- ¼ - $^3/_8$ inch or equivalent to fit Vacuum Flask.
- Thermo Fisher Catalog 8702-0065 or equivalent

4.2.5 Vacuum VacTrap (Alternate to individual pieces listed above)
- Fisher Scientific Catalog 50-148-9320

4.2.6 Adjustable Pipette (50 – 300uL & 100 – 1000uL)

4.2.7 200 – 300uL Pipette Tips

4.2.8 1000uL Pipette Tips

4.2.9 Vortexer/Mixer
- Thermo Fisher Catalog 88880017TS or equivalent.

4.2.10 Centrifuge/Micro-centrifuge:
- Thermo Fisher Catalog 75002437 or equivalent, capable of 14,000g.

4.2.11 Bright Work Lamp
- Thermo Fisher Catalog 11-990-108 or equivalent

4.2.12 Cryovial, 12x75 Test Tube or 15mL Conical or other suitable temporary container to mix clarified sample.
- 1.5mL Conical Tube
  4.2.12..1 Fisher Scientific Catalog 50-754-1456
- 12x75 Polystyrene Test Tubes
  4.2.12..1 Fisher Scientific Catalog 22-171-604
- 15mL Conical Centrifuge Tube
  4.2.12..1 Fisher Scientific Catalog 14-959-53A

5. Procedural Steps:

5.1.1 Method 1: Low Sample Volume
- **Step 1:** Thaw sample at 37°C for 5-7 minutes
- **Step 2:** Vortex sample to mix well
- **Step 3:** Centrifuge sample(s) at 14,000g for 10 minutes
- **Step 4:** Carefully remove the vial from the centrifuge such that the lipid layer and pellet formed is not disturbed.
- **Step 5:** Carefully insert pipette into the clarified serum or plasma. Take care to minimally disturb any lipid layer and/or pellet. Tilting the cryo may be helpful in exposing clarified serum.
- **Step 6:** Aliquot to desired receptacle: cryovial, HD-1/HD-X plate.
- **Step 7:** Freeze aliquots at -80°C until further use. And/or Run on HD-1/HD-X analyzer

5.1.2 Method 2: High volume and/or multiple aliquot procedure

- **Step 1:** Thaw sample at 37°C for 5-7 minutes, or until sample is fully thawed.
- **Step 2:** Vortex Sample to mix well.
- **Step 3:** Centrifuge sample(s) at 14,000g for 10 minutes
- **Step 4:** Place 200uL or 300uL tip onto the end of the vacuum tubing
- **Step 5:** Slowly and carefully insert vacuum tip into the centrifuged sample and remove lipid layer. (Expect to lose ~200uL – 250uL depending on amount of lipids). Replace tip between samples.
- **Step 6:** Carefully remove all clarified serum or plasma to a NEW temporary vial (cryo-vial, 12x75 tube, 15mL conical tube etc.)
- **Step 7:** Ensure that serum or plasma is visually clear of lipids or other debris or particulates. If any debris remains, centrifuge again at 14,000g for 10 minutes.
- **Step 8:** Vortex clarified serum or plasma
- **Step 9:** Transfer clarified serum or plasma into desired daughter aliquots.
- **Step 10:** Cap and freeze aliquots at -80°C until further use.

Certificate of Review

Title:					
Prepared By:			**Date Prepared:**	__/__/____	
Distribution:			**Location:**		
Supersedes:		**Date Initiated:**	__/__/____	**By:**	
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