Montreal Neurological Institute and Hospital
McGill University

CBIG-02-003

SERUM ISOLATION FROM WHOLE BLOOD

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1. REVISION HISTORY

Version	Summary of revisions	Effective Date
1.0	Initial	01-May-2020

2. SCOPE AND APPLICATION

This protocol is to be used for the isolation of human serum from whole blood.

3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-02-003 Serum Isolation from Whole Blood*

3.1 Reference to Other C-BIG SOPs or Documents

1. C-BIG Repository: CBIG-03-002 RPM Conversion

3.2 Reference to External SOPs or Documents

1. ETP Standard Operating Procedures: ETP-P-0013 Serum Isolation from Whole Blood, 1X high speed
2. EA. Plumhoff *et al.* 2008: Preanalytic Laboratory Errors: Identification and Prevention. Mayo clinic communiqué 33 (12)

4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who isolate serum from whole blood. All personnel who read this SOP should sign the form found in the training log binder.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
C-BIG	Clinical Biological Imaging and Genetics Repository
Min	Minutes
mL	Milliliters
QA	Quality Assurance
QC	Quality Control
RT	Room Temperature
RTT	Red Top Tube (vacutainer)
SOP	Standard Operating Procedure
µL	Microliters
°C	Celsius

6. MATERIALS AND EQUIPMENT

NOTE: All disposable items are sterile (gamma-irradiated) unless otherwise specified. All equipment, disposables or reagents can be substituted with equivalent materials following evaluations and approval, unless specified otherwise. Use clean gloves at all times to prevent inadvertent contamination during processing.

NOTE: All sample contact materials must be suitable for RNA work (i.e. RNAse-free). Use clean gloves at all times to prevent inadvertent RNAse contamination during processing. All equipment, disposables or reagents can be substituted with equivalent materials following evaluation and approval, unless specified otherwise.

The materials and equipment listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for site-specific task or procedure.

Material/Equipment	Material/Equipment (site specific)
Blood collection tubes, 10 mL, red top	BD Diagnosis; Cat # 366430
Disposable transfer pipets	
Polypropylene conical tubes, 15 mL	
Pipets tips, 1000 µL	
Cryovials, 1 mL, conical bottom	Nunc Biobanking and Cell Culture Cryogenic Tubes, Thermo Scientific; Cat # 366656
Micropipets, range 200 µL to 1000 µL	
Centrifuge with swinging bucket rotor	Eppendorf centrifuge 5810, Cat # 022625101; with rotor S-4-104, Cat # 5820759003
-20°C freezer	
-80°C freezer	
Class II biological safety cabinet	

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7. PROCEDURES

PRECAUTIONS: All biological samples derived from a human source are considered to be biohazardous. Use appropriate precautions when working with such samples (i.e. personal protection equipment such as gloves, lab coat and safety glasses). All waste (samples and related contact materials) must be placed in marked biohazardous waste containers and disposed under hospital guidelines.

NOTE: Best results are obtained when whole blood is processed promptly. When possible, processing should be done on the same day as the blood draw. If it is not possible, put the vacutainers in the credo box RT overnight for a next day processing.

7.1 Isolation of human serum from whole blood

1. Keep RTT upright at RT for a minimum of 30 min and a maximum of 120 min to allow blood to clot, unless received by external courier service.
2. Centrifuge for 10 min at 2500Xg using a centrifuge with a swinging bucket.
3. Transfer supernatant (serum) to a new 15 mL conical tube without disturbing the pellet. In the event of more than one RTT from the same donor, pool the supernatant of similar appearance from the different tubes (as per figures 1 and 2 found in section 9). If a tube is hemolyzed, do not mix with normal samples and aliquot it separately.
4. Prepare aliquots of chosen volume into 1 mL conical cryovials.

5. Serum samples can be frozen at -20˚C for up to a week (temporary storage) and then transferred to a -80˚C freezer for long term storage.

7.2 Summary Table

Steps
1. Keep RTT upright for a minimum of 30 min and a maximum of 120 min
2. Centrifuge for 10 min at 2500Xg
3. Transfer supernatant (serum) to a new 15 mL conical tube
4. Make appropriate number of aliquots in 1 mL cryovials
5. Store samples temporarily at -20˚C and then for long term storage at -80˚C

8. QUALITY CONTROL / QUALITY ASSURANCE

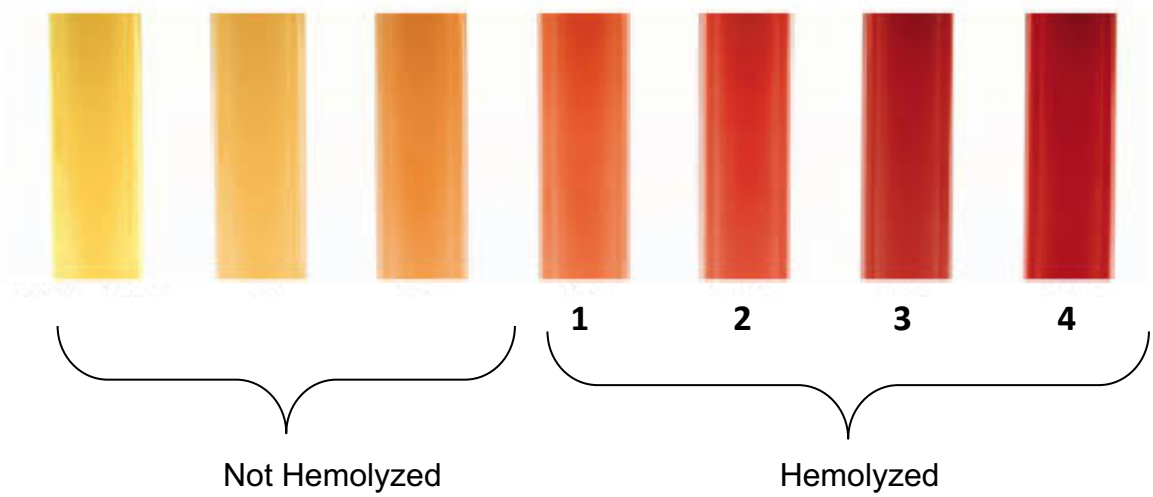
All the equipment used should be monitored, cleaned and calibrated per their specific SOPs.

Reagents with an expiry date should be monitored and used before this date. If used after expiry date, it should be recorded.

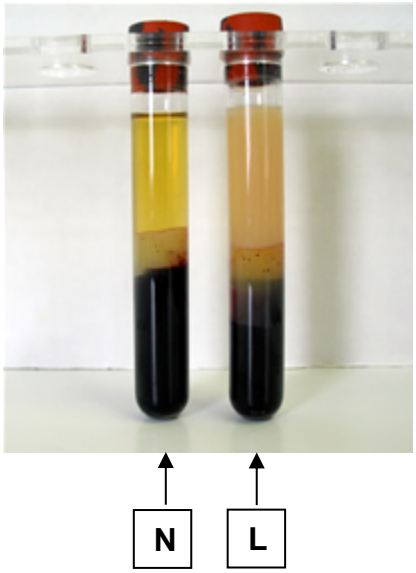
8.1 Sample Quality

Hemolyzed and lipemic serum samples must be considered as suspect. The degree of hemolysis must be specified.

8.1.1 Figure 1 - Hemolysis Index



8.1.2 Figure 2 - Lipemic Serum



N = normal serum; L = lipemic serum

9. APPENDICES/FORMS

9.1 Appendix A – Sample Processing Form: Serum

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