| Frederick National Laboratory for Cancer Research<br>*sponsored by the National Cancer Institute* | Vaccine, Immunity, and Cancer Directorate<br><br>Standard Operating Procedure | |
|---|---|---|
| **SOP Title:** Procedure for Separating Serum and Plasma from Whole Blood | | |
| **Document ID: 20003** | Version: | 1.0 |
| **Page 1 of 16** | Supersedes: | New |
| **Effective Date: 23 Dec 21** | | |

| **Written by:** | | |
|---|---|---|
| Printed Name: | Title: | Signature/Date: |
| | | |

| **Approved by:** | | |
|---|---|---|
| Printed Name: | Title: | Signature/Date: |
| | | |
| **QA Approved by:** | | |
| Printed Name: | Title: | Signature/Date: |
| | | |

## 1. PURPOSE

1.1 The purpose of this procedure is to describe the separation of serum and plasma from whole blood samples.

## 2. SCOPE

2.1 This procedure applies to Vaccine, Immunology and Cancer Directorate (VICD) specimens to standardize separating procedures for uniform quality.

## 3. REFERENCES

3.1 10023: Good Documentation Practices

3.2 15000: Waste Disposal at the Advanced Technology Research Facility

3.3 15006: Reagent Preparation

3.4 15008: Biosafety Manual and Laboratory Standard Operating Procedures

3.5 15011: Reagent and Chemical Expiry

3.6 20003-01: Serum and Plasma Separation Procedure Form

3.7 26000: Biosafety Cabinet (BSC) Use and Maintenance

3.8 26005: Use and Maintenance of a 2-8°C Refrigerator

3.9 26009: Use and Maintenance of Pipettes

3.10 26030: Use and Maintenance of -80°C Freezers

3.11 26033: Use and Maintenance of the Thermo Scientific Sorvall XTR Centrifuge

3.12 EHS-WM-1: Disposal and Minimization of Chemical Waste

3.13 EHS-WM-2: Biological Waste Handling and Disposal

3.14 HSL_GL_020: Development Material Receiving Procedure

**3.15** VIC_GL_001: Heightened Disinfecting and Personnel Practices

## 4. RESPONSIBILITIES

4.1 All employees are responsible for understanding and complying with safety guidelines, universal precautions, and procedures.

4.2 The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.

4.3 The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.4 The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

## 5. DEFINITIONS

5.1 Biospecimen - a sample of biological material, such as urine, whole blood, blood components, tissue, cells, DNA, RNA, and protein.

5.2 BSC - Biosafety Cabinet

5.3 LIMS – Laboratory Information Management System

5.4 SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2.

## 6. REAGENTS, MATERIALS AND EQUIPMENT

6.1 01.5- 2.0 mL Sterile Screw-Top Tubes (Thomas Scientific, Cat # 1149Y78 or equivalent)

6.2 10 mL Serological Pipets (FNLCR Warehouse, Cat # 66401370 or equivalent)

6.3 15 mL Conical Tubes (FNLCR Warehouse, Cat # 66401479 or equivalent)

6.4 2” Box and 81 Position Insert (FNLCR Warehouse, Cat # 81150001 and 81150004 or equivalent)

6.5 25 mL Serological Pipets (FNLCR Warehouse, Cat # 66401361 or equivalent)

6.6 5 mL Serological Pipets (FNLCR Warehouse, Cat # 66401365 or equivalent)

6.7 50 mL Conical Tubes (FNLCR Warehouse, Cat # 66401493 or equivalent)

6.8 50 mL Serological Pipets (FNLCR Warehouse, Cat # 66401363 or equivalent)

6.9 Biosafety Cabinet II (BSC)

6.10 Bleach, Concentrated (Warehouse, Cat # 68100251 or equivalent)

6.11 Blood Collection Tubes (Vacutainers)

6.11.1 Brady Label (Anthony-Lee Associates, Cat # THT-133-461-SLIT or equivalent)

6.12 Centrifuge (Thermo Fisher Sorvall Legend XTR or equivalent)

6.13 Freezer (-65 to -90°C)

6.14 Ice Pan (Thomas Scientific, Cat # 1200R42 or equivalent)

6.15 Labels that can withstand temperatures ≥ -90°C

6.16 Parafilm (FNLCR Warehouse, Cat # 66401356 or equivalent)

6.17 Pipettes and Pipette Tips (Rainin)

6.17.1 Plasma ACD (BD, Cat # 364606 or equivalent)

6.17.2 Plasma EDTA (BD, Cat # 366643 or equivalent)

6.17.3 Plasma Heparin (BD, Cat # 367874 or equivalent)

6.17.4 Plasma Sodium Citrate (BD, Cat # 366560 or equivalent)

6.18 Refrigerator (2-8°C)

6.19 Serologic Pipettor

6.19.1 Serum tube glass (BD, Cat # 366430 or equivalent)

6.20 Wet ice

## 7. HEALTH AND SAFETY CONSIDERATIONS

7.1 Universal Precautions must be used when working with blood. Use of personnel protective equipment is mandatory. This includes, but is not limited to, lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

7.2 If SARS-CoV-2 positive samples are being processed, additional protective equipment is worn such as double layer of non-latex gloves and disposable arm sleeves.

7.3 Refer to the respective Safety Data Sheet (SDS) when working with any chemicals.

7.4 Refer to “15000: Waste Disposal at the Advanced Technology Research Facility” regarding waste disposal processes at the Advanced Technology Research Facility (ATRF).

7.5 Refer to “EHS-WM-1: Disposal and Minimization of Chemical Waste” and “EHS-WM-2: Biological Waste Handling and Disposal” regarding waste disposal processes at the Fort Detrick campus.

7.6 All contaminated BSL-2 level liquid waste must be decontaminated using 10% Clorox bleach (final concentration) with a minimum contact time of 30 minutes before sink disposal with a copious amount of water.

7.7 All work with whole blood/serum/plasma must be performed inside at least a Class 2 Biosafety Cabinet (BSC).

## 8. SERUM SEPARATION PROCEDURE

**Note: The maximum allowable time from blood collection (processing serum) to storage in a -65 to -90°C freezer is 8 hours.**

8.1 Record the steps on 20003-01: Serum and Plasma Separation Procedure Form.

8.2 Serum is obtained from blood drawn into a serum separator tube (SST, red or tiger top tube).

8.3 Allow the blood samples to clot for a minimum of 30 minutes to a maximum of 60 minutes upright at room temperature until centrifugation. A delay in centrifugation may have a detrimental effect on the sample quality. However, if the blood cannot be centrifuged immediately after the clotting time, the tubes must be refrigerated at 2-8°C for up to 4 hours.

> **Note:** Record the delay in the comments section of the form if stored 2-8°C before spinning and separating.

8.4 A minimum of 5 labels is recommended, per sample. If more tubes are needed, continue numbering sequence as needed.

> **Note:** The number of aliquots and aliquot volumes may be adjusted per tube as these are recommendations; individual studies may have other requirements defined by the Scientific Manager or designee.
>
> For example, see table below.

| Tube Number | Aliquot Volume |
|---|---|
| 001 | 50 µL |
| 002 | 50 µL |
| 003 | 50 µL |
| 004 | 200 µL |
| 005 | Remaining Serum (15- or 50-mL Tube) |
| Variable | Affix to 20003-01 |

8.5 Label at least five (5) tubes per sample donor. Place labeled 0.5-2.0 mL tubes in a rack and larger tubes on wet ice.

8.6 The label will at least include Sample ID, Sample type, and Volume in µL. Additional details such as Date and Analyst Initials are not required if printing from LIMS, but if printing from outside of LIMS then it is recommended that these details are added to the label. See Attachment 1: Serum Sample Aliquot Labels for more details.

> **Note:** Upon receipt, blood samples have a patient/ donor specific identifier on the label. Prior to processing blood, analyst must assign a unique ID for individual identification (LIMS creates a unique ID for each tube). It is the responsibility of the analyst to accurately document both specimen source identifier and unique ID assigned on form 20003-01.

8.7 In a BSC, load blood biospecimen tubes into the centrifuge buckets and add the biohazard dome.

8.8 Centrifuge blood samples for 20±1 minute at 1300±100 x g at room temperature.

> **Note:** In case of catastrophic failure such as broken rotor, bucket, or biohazard dome during centrifugation, allow the centrifuge to sit for 30 minutes after it has stopped. Prior to inspection, consult with the Safety Department for best practices of biohazard clean up.

8.9 Following centrifugation, transport the centrifuge buckets with the biohazard dome to the BSC, and unload blood biospecimens in the BSC.

8.10 After centrifugation, the serum layer will be at the top of the tube. Carefully collect the serum layer with a pipette without disturbing the buffy coat layer then place serum into a 15- mL or 50-mL conical tube depending on the total volume of serum collected. Vials from a single donor must be pooled together.

> **Note:** The sample may be centrifuged again if clots or debris are present within the sample. Repeat centrifugation for 20±1 minute at 1300±100 x g at room temperature.

8.11 Mix the pooled serum by gently inverting the 15 mL or 50 mL tube 10 times prior to making aliquots.

8.12 Aliquot appropriate amount of serum into labeled vials:

8.12.1 Three (3) 50-µL aliquots and one (1) 200-µL aliquot per donor. This process will be completed with all tubes on wet ice.

8.12.2 The remaining serum will be stored in 15-mL or 50-mL tube(s) with parafilm wrapped around the lid.

8.13 Initially, place serum in 0.5-2.0 mL tubes into designated box while the remaining serum stored in either 15 mL or 50 mL tubes will be placed into a rack. Store the serum samples at -65 to -90°C freezer. Within 48 hours, transfer the 15-mL or 50-mL tubes placed in a rack into designated box containing donors 0.5-2.0 mL serum tubes.

8.14 Record final aliquot storage location on 20003-01: Serum and Plasma Separation Procedure Form.

8.15 **Note:** A box label template file is available for generating box labels. See Attachment 2: Box Label Example, for label layout and placement.

8.16 Document sample storage information, once aliquoted, on 20003-01 form.

8.17 Document sample location in the LIMS if available; otherwise, in the freezer inventory file.

## 9. PLASMA SEPARATION PROCEDURE

**Note: The maximum allowable time from blood collection (processing plasma) to storage in a -65 to -90°C freezer is 8 hours.**

9.1 Record the steps on 20003-01.

> **Note:** Plasma specimens are obtained from blood drawn into tubes containing anticoagulants such as EDTA (purple top), sodium heparin (green top), sodium citrate (blue top), or ACD (yellow top) tubes.
>
> **Note:** Clotted specimens are not acceptable for plasma collection.

9.2 Invert the blood tubes 5 times to mix the sample with anticoagulant and inspect the flow of blood within the tube for any large clots.

9.3 In a BSC, load blood biospecimen tubes into the centrifuge buckets and add the biohazard dome.

9.4 Centrifuge blood samples for 20±1 minute at 1300±100 x g at room temperature.

> **Note:** In case of catastrophic failure such as broken rotor, bucket, or biohazard dome during centrifugation, allow the centrifuge to sit for 30 minutes after it has stopped. Prior to inspection, consult with the Safety Department for best practices of biohazard clean up.

9.5 A minimum of 5 labels is recommended, per sample. If more tubes are needed, continue numbering sequence as needed.

> **Note:** The number of aliquots and aliquot volumes may be adjusted per tube as these are recommendations; individual studies may have other requirements defined by the Scientific Manager or designee.
>
> For example, see table below.

| Tube Number | Aliquot Volume |
|---|---|
| 001 | 50 µL |
| 002 | 50 µL |
| 003 | 50 µL |
| 004 | 200 µL |
| 005 | Remaining Serum (15- or 50-mL Tube) |
| Variable | Affix to 20003-01 |

9.6 Label at least five (5) tubes per sample donor. Place labeled 0.5-2.0 mL tubes in a rack and larger tubes on wet ice.

9.7 The label will at least include Sample ID, Sample type, and Volume in µL. Additional details such as Date and Analyst Initials are not required if printing from LIMS, but if printing from outside of LIMS then it is recommended that these details are added to the label. See Attachment 1: Serum Sample Aliquot Labels for more details.

> **Note:** Upon receipt, blood samples have a patient/ donor specific identifier on the label. Prior to processing blood, analyst must assign a unique ID for individual identification (LIMS creates a unique ID for each tube). It is the responsibility of the analyst to accurately document both specimen source identifier and unique ID assigned on form 20003-01.

9.8 Following centrifugation, transport the centrifuge buckets with the biohazard dome to the BSC, and unload blood biospecimens in the BSC.

9.9 After centrifugation, the plasma layer will be at the top of the tube. Carefully collect the plasma layer with a pipette without disturbing the buffy coat layer then place plasma into a 15- mL or 50-mL conical tube depending on the total volume of plasma collected. Vials from a single donor must be pooled together.

> **Note:** The sample may be centrifuged again if clots or debris are present within the sample. Repeat centrifugation for 20±1 minute at 1300±100 x g at room temperature.

9.10 Mix the pooled plasma by gently inverting the 15 mL or 50 mL tube 10 times prior to making aliquots.

9.11 Aliquot appropriate amount of plasma into labeled vials:

9.11.1 Three (3) 50-µL aliquots and one (1) 200-µL aliquot per donor. This process will be completed with all tubes on wet ice.

9.11.2 The remaining plasma will be stored in 15-mL or 50-mL tube(s) with parafilm wrapped around the lid.

9.12 Initially, place plasma in 0.5-2.0 mL tubes into designated box while the remaining plasma stored in either 15 mL or 50 mL tubes will be placed into a rack. Store the plasma samples at -65 to -90°C freezer. Within 48 hours, transfer the 15-mL or 50-mL tubes placed in a rack into designated box containing donors 0.5-2.0 mL plasma tubes.

9.13 Record final aliquot storage location on 20003-01: Serum and Plasma Separation Procedure Form.

**Note:** A box label template file is available for generating box labels. See Attachment 2: Box Label Example, for label layout and placement.

9.14 Document sample storage information, once aliquoted, on 20003-01 form.

9.15 Document sample location in the LIMS if available; otherwise, in the freezer inventory file.

## 10. ATTACHMENTS

10.1 Attachment 1: Serum Sample Aliquot Labels

10.2 Attachment 2: Box Label Example

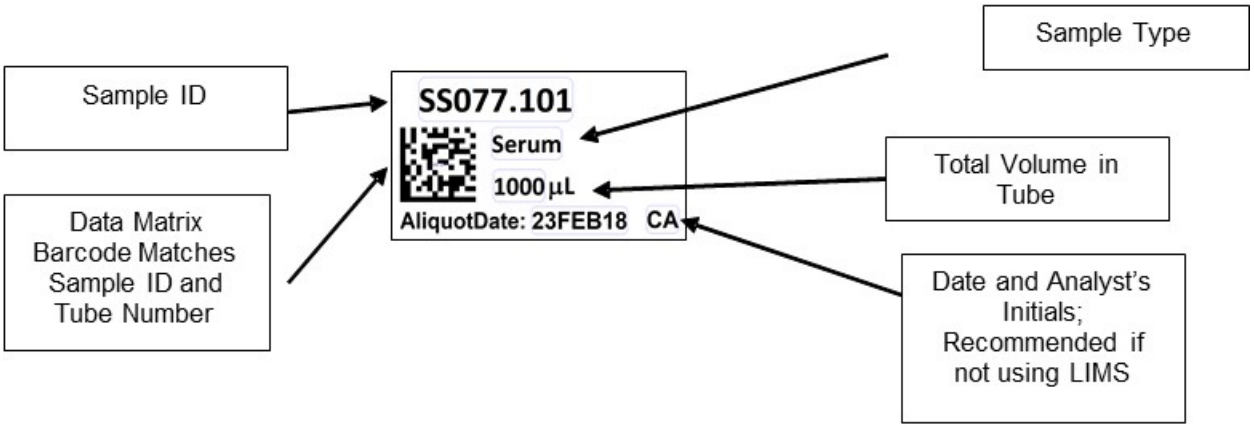
10.3 Attachment 3: 20003-01 Serum and Plasma Separation Procedure Form

**10.4** Attachment 4: Illustration and Precautionary Notes for Removing Plasma

## 11. REVISION HISTORY

| Version # | Change | Reason |
|---|---|---|
| 1.0 | 1. Renumbered SOP to 20003 from HSL_LAB_003.<br>2. Renamed Title "Procedure for separating Serum and Plasma from Whole Blood."<br>3. Minor grammar and format corrections throughout the document.<br>4. References:<br>a. Updated document titles or numbers as appropriate.<br>i. HSL_LAB_003.01 to 20003-01<br>ii. HSL_GL_001 to 15000<br>iii. HSL_GL_003 to HSL_QS_017<br>iv. HSL_GL_006 to 15006<br>v. HSL_GL_007 to 15011<br>vi. HSL_GL_008 & HSL_GL_009 combined into 15008<br>vii. HSL_EQ_001 to 26000<br>viii. HSL_EQ_003 to 26033<br>ix. HSL_EQ_008 to 26030<br>x. HSL_EQ_012 to 26009<br>b. Deleted HSL_GL_010 as the SOP is obsolete<br>c. Deleted HSL_EQ_007 now 26013 as it is not relevant<br>d. Added 26005, VIC_GL_001, HSL_GL_020, EHS-WM-1: Disposal and Minimization of Chemical Waste and EHS-WM-2: Biological Waste Handling and Disposal<br>5. Responsibilities:<br>a. Add line to generalize responsibilities<br>6. Health and Safety Considerations:<br>a. Added statement about universal precautions and combine it with personnel protective equipment.<br>b. Added references to EHS-WM-1 and EHS-WM-2.<br>7. Serum Separation Procedure:<br>a. Added information on the tubes used for blood draw<br>8. Added Plasma Separation Procedure:<br>a. Overview of the separation and aliquoting processing | 1. Reflect numbering requirement per 10000.<br>2. Procedure now covers both serum and plasma separations.<br>3. Capture information in sequential order.<br>4. Updated new reference numbers assigned as per 10000 and removed obsolete documents.<br>5. Serology samples are processed outside VICD within FNL.<br>6. Clarified based on current procedures<br>7. Added clarity to the procedure<br>8. New procedure |

**Attachment 1: Serum Sample Aliquot Labels**



**Attachment 2: Box Label Example**

Study: OHS SS001 (RDPID)
Sample Type: Serum
Date: 27AUG17
Initials: TK
Box 1 of 1

## Attachment 3: 20003-01_Serum and Plasma Separation Procedure Form

| Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute | | Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form | |
|---|---|---|---|
| **Form Title: Serum and Plasma Separation Procedure Form** | | | |
| **Document ID: 20003-01** | | Version: | 1.0 |
| Associated SOP: 20003 | | Effective Date: | 23 Dec 21 |
| Supersedes: | New | **Page 1 of 1** | |

| | | |
|---|---|---|
| Participant ID | | |
| Sample ID | | |
| Draw Date/Time | | |
| Received by Initials/Date/Time | | |
| Aliquot Completed Date/Time | | Total Aliquots:<br>Estimated Total Volume: |

**Equipment Information**

| Equipment Name | Equipment ID | Calibration Due Date |
|---|---|---|
| BSC | | |
| 2-8°C Refrigerator | | |
| Centrifuge | | |
| -65 to -90°C Freezer | | |
| Pipette µL | PIP_ | |

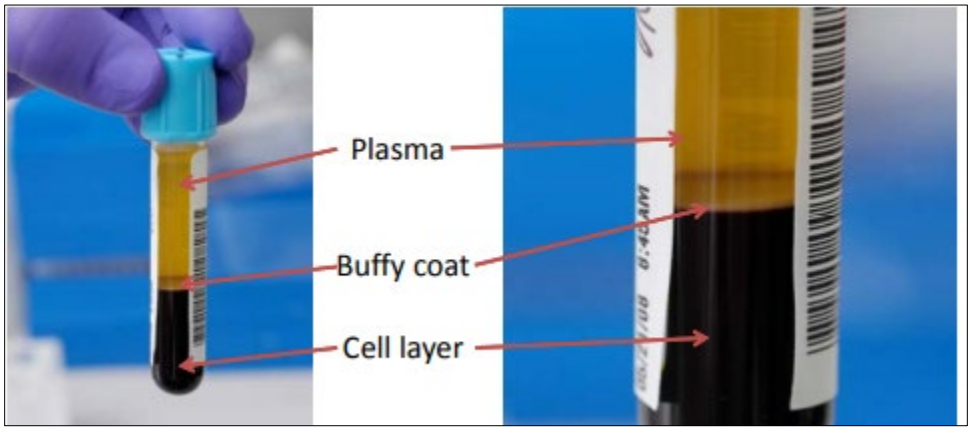
| Aliquot Label: | Comments: |
|---|---|
| ☐N/A | ☐N/A |

| | |
|---|---|
| Performed By/ Date: | |
| Reviewed By/ Date: | |

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

This document contains confidential and proprietary information. Do not copy or distribute without prior, written permission.

**Attachment 4: Illustration and Precautionary Notes for Removing Plasma**




When removing Plasma:

- Be careful not to disturb cell layers
- Never pour off plasma
- Transfer the top ¾ of plasma: Start from the top, gently draw specimen into pipette as you go further down.
- Go no further down than the marking