**Released by / Effective Date:** Angelina C. Richards -S (Affiliate) Digitally signed by Angelina C. Richards -S (Affiliate) Date: 2020.11.24 17:34:25 -05'00'

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## 1. PURPOSE

1.1. This GUIDANCE DOCUMENT is designed to explain how to process serum biospecimens.

1.2. This GUIDANCE DOCUMENT is intended to convey the process parameters and practices to be followed by each institute associated with the National Cancer Institute (NCI) Serology Network (SeroNet).

## 2. SCOPE

2.1. This document applies to all institutes associated with SeroNet through collaborations, grant funding, subcontracts, etc. that perform biospecimen processing.

2.2. This procedure does not describe the biospecimen collecting process. The biospecimen collecting process is dictated by the institute's protocol.

## 3. REFERENCES

3.1. VIC_GL_002: Shipping SARS-CoV-2 Associated Specimens to the FNL Central Repository (NCI SeroNet Guidance Document)

3.2. VIC_GL_003: Key Entity Identifier Assignment (NCI SeroNet Guidance Document)

## 4. RESPONSIBILITIES

4.1. It is the responsibility of the institute performing the serum biospecimen processing to:

4.1.1. Perform biospecimen processing using the indicated reagents, materials, equipment and process parameters in this guidance document.

4.1.2. Ship the processed biospecimens to the Frederick National Laboratory for Cancer Research (FNL) Central Repository following "VIC_GL_002: Shipping SARS-CoV-2 Specimens to the FNL Central Repository (NCI SeroNet Guidance Document)."

4.2. It is the responsibility of the Vaccine, Immunity and Cancer Program (VIC) to:

4.2.1. Generate, review and approve the biospecimen processing guidance document.

4.2.2. Distribute the most current version of this guidance document to each institute associated with SeroNet.

## 5. DEFINITIONS

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



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5.2. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

## 6. REAGENTS, MATERIALS AND EQUIPMENT

6.1. Equipment

6.1.1. Class II Biosafety Cabinet (BSC)

6.1.2. -80°C Freezer

6.1.3. 2-8°C Refrigerator

6.1.4. Benchtop Centrifuge

6.1.5. Serologic Pipette

6.1.6. Pipette

6.2. Consumables

**Note:** Consumables requiring approval for use as “equivalent” by the NCI SeroNet are indicated with an Asterisk (*).

6.2.1. SeroNet specified 5 mL sterile tubes (Fisher Scientific, Cat #12-565-291 or equivalent*)

6.2.2. 125 mL Media Storage Bottle (Thomas Scientific, Cat # 19A00M420 or equivalent)

6.2.3. 250 mL Media Storage Bottle (Thomas Scientific, Cat # 19A00M421 or equivalent)

6.2.4. Pipette Tips

6.2.5. Serological Pipets

6.2.6. Blood Collection Tubes (Vacutainers)

6.2.6.1. Serum tube glass (BD, Cat # 366430 or equivalent*)

6.2.7. 4” Box and 81 position insert, or equivalent

6.2.8. Labels that can withstand temperatures ≤ -80°C

6.2.8.1. Example: Brady Label (Anthony-Lee Associates, Cat # THT-133-461-SLIT)

## 7. HEALTH AND SAFETY CONSIDERATIONS



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**Note:** Each institute's Environment, Health, and Safety department will provide definitive measures for safety when processing human biospecimens as these considerations are provided only as a guideline.

7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as 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. A face mask is part of the standard personal protective equipment for the laboratory during the SARS-CoV-2 pandemic.

7.4. Follow the institute governed Biosafety Level 2 (BSL-2) requirements for handling and processing human biospecimens.

7.5. All human biospecimen processing work is performed inside of a Class II BSC.

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

7.7. Refer to the institute's processes for disposing of biohazardous and chemical waste.

## 8. PROCEDURE PRINCIPLES

8.1. Refer to "VIC_GL_003: Key Entity Identifier Assignment (NCI SeroNet Guidance Document)" for process of assigning IDs to biospecimens and biospecimen aliquots.

8.2. Image of form "VIC_LAB_002.01, Serum Biospecimen Processing Form" is attached for institute's reference. The minimum information requiring documentation during the performance of the processing of the serum biospecimen is included in this form. See Attachment 1.

8.3. Image of form "VIC_LAB_002.02, Serum Biospecimen Collection Form" is attached for institute's reference. The minimum information requiring documentation during the collection of the blood biospecimen for serum processing is included in this form. See Attachment 2.

8.4. It is preferred that all equipment used in this process be maintained, at minimum, per the equipment manufacturer's recommendations.

8.5. It is preferred that all Pipettes, Laboratory Freezers and Refrigerators, and Benchtop Centrifuges used in this process be calibrated by a vendor or other qualified party.

8.6. It is preferred that all Laboratory Freezers and Refrigerators used in this process be monitored for temperature by a temperature monitoring system.



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8.7. All human biospecimen handling is performed in a Class II Biosafety Cabinet (BSC) except for centrifugation and storage.

## 9. SERUM SEPARATION

**Note: The maximum allowable time from blood collection (processing serum) to storage in a -80°C freezer is 8 hours.**

9.1. Once blood biospecimen is received, allow the blood to clot upright at room temperature for 30-60 minutes.

9.2. If the blood biospecimen cannot be centrifuged immediately after the clotting time, refrigerate tubes at 2-8°C for up to 4 hours.

9.3. Label 5 mL sterile tubes for each serum biospecimen being processed. Use Attachment 3 for label specifications.

**Note:** The labels are expected to be printed by each Capacity Building Center (CBC) according the example in Attachment 3.

9.3.1. Biospecimen Aliquot ID: Refer to VIC_GL_003 for biospecimen aliquot ID assignment process. **Use Deidentified Biospecimen Aliquot ID Only**.

9.3.2. Biospecimen Type: Human Serum

9.3.3. Volume in milliliters (mL).

9.4. Rack the labeled tubes and set aside.

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

9.6. Centrifuge blood biospecimen tubes for 20 minutes at 1300 x g at 20-25°C.

**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 clinic's Safety Department for best practices of biohazard clean up.

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

9.8. Carefully collect the top serum layer with a pipette. Do not disturb the buffy coat layer.

**Note:** Be very careful not to pick up red blood cells. Keep pipette above the red blood cell layer and leave a small amount of serum in the tube.



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9.9. Place serum into a sterile Media Storage bottle. Vials from a single research participant are pooled together.

9.10. Mix by inverting the bottle 10 times.

9.11. Pipette 4.5 mL serum into the labeled sterile 5 mL tubes.

9.12. Label box(es) using label specification in Attachment 3.

9.13. Place aliquots into labeled box(es).

9.14. Store in -80°C freezer.

9.15. Ship specimens on dry ice to the FNL Central Repository following VIC_GL_002.

## 10. ATTACHMENTS

10.1. Attachment 1: VIC_LAB_002.01, Serum Biospecimen Processing Form

10.2. Attachment 2: VIC_LAB_002.02, Serum Biospecimen Collection Form

10.3. Attachment 3: Vial Label and Box Label

## 11. REVISION HISTORY

| Version | Change | Reason |
|---|---|---|
| 1.0 | New guidance document for specimen processing by SeroNet organizations. | Currently no procedure; new initiative requiring communication of expectations. |
| 2.0 | 1. Changed “specimen” and “sample” to “biospecimen” throughout document.<br>2. Minor formatting and grammatical changes throughout document.<br>3. Added Biospecimen and SARS-CoV-2 to new Definitions section.<br>4. Added VIC_GL_003 to References section.<br>5. Added SARS-CoV-2 and pandemic specific health/safety guidelines to Health and Safety Considerations section.<br>6. Added reference to VIC_GL_003, reference to new form, reworded equipment requirements to be “preferred” in the Procedure Principles section.<br>7. New form VIC_LAB_002.02 to capture biospecimen collection. | 1. Consistency between documents and database verbiage.<br>2. Clarification.<br>3. Clarification.<br>4. Referenced in body of procedure.<br>5. Clarification.<br>6. Clarification.<br>7. Ease of use. |



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| | | |
|---|---|---|
| | 8. Revised form VIC_LAB_002.01 to capture serum processing. Reformatted to accommodate processing of more than one biospecimen at one time. | 8. Ease of use. |



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## Attachment 1: VIC_LAB_002.01, Specimen Processing Form

| Frederick National Laboratory for Cancer Research<br>sponsored by the National Cancer Institute | | Vaccine, Immunity and Cancer Program<br>Standard Operating Procedure<br>Form | |
|---|---|---|---|
| **Form Title:** Serum Biospecimen Processing Form | | | |
| **Document ID: VIC_LAB_002.01** | | Version: | 2.0 |
| Associated SOP: VIC_LAB_002 | | Effective Date: | |
| Supersedes: | 1.0 | **Page 1 of 3** | |

Biospecimen Receipt

| Serum Biospecimen Processing Laboratory Name: | | | | |
|---|---|---|---|---|
| Biospecimen Number | Deidentified Biospecimen ID | Date Received | Time Received (24H) | Initials |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

Equipment

| Equipment Name | Equipment ID | Calibration Due Date |
|---|---|---|
| BSC | | |
| Centrifuge | | |
| Pipette | | |
| ☐ N/A Pipette | | |
| ☐ N/A Pipette | | |
| ☐ N/A 2-8°C Refrigerator | | |
| ☐ N/A -80°C Freezer | | |

Consumables

| Consumable Name | Catalog Number | Lot Number | Expiration Date |
|---|---|---|---|
| ☐ N/A 5 mL Sterile Tubes | | | |
| ☐ N/A | | | |


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Processing Steps

| (√) | Process Step | |
|---|---|---|
| | Clot blood biospecimen for 30-60 mins at RT, upright. | |
| | Clot Start Time | Clot End Time |
| | | |
| ☐ N/A Step | If biospecimen is not processed immediately, store at 4°C for up to 4 hours. | |
| | 4°C Storage Start Time | 4°C Storage End Time |
| | | |
| | Centrifuge blood biospecimen for 20 mins, 1300 x g, 20-25°C | |
| | Centrifuge time: _____mins. | |
| | Carefully collect the top serum layer. | |
| | Pool serum from single research participant. | |
| | Invert pooled serum 10 times. | |
| | Aliquot serum into labeled 5 mL sterile tubes. | |
| | Place aliquots into labeled box. | |
| | Store aliquots at -80°C. | |

| Biospecimen Number | Date/Time (24H) Blood Biospecimen Collected | Date/Time (24H) Serum Aliquots Stored at -80°C | Initials |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

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Serum Biospecimen Aliquot

| Biospecimen Number | Number of Aliquots | Aliquot Volume (mL) | Example Biospecimen Aliquot Label |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |

Comments: ☐ N/A

| Performed by/date: | |
|---|---|
| Reviewed by/date: | |

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## Attachment 2: VIC_LAB_002.02, Serum Biospecimen Collection Form

| Frederick National Laboratory for Cancer Research<br>sponsored by the National Cancer Institute | | Vaccine, Immunity and Cancer Program<br>Standard Operating Procedure<br>Form | |
|---|---|---|---|
| **Form Title:** Serum Biospecimen Collection Form | | | |
| **Document ID: VIC_LAB_002.02** | | Version: | 2.0 |
| Associated SOP: VIC_LAB_002 | | Effective Date: | |
| Supersedes: | 1.0 | **Page 1 of 1** | |

| | | | | | |
|---|---|---|---|---|---|
| Deidentified Biospecimen ID: | | | | | |
| Biospecimen Group: | | ☐ Positive ☐ Negative ☐ Serosurveillance | | | |
| Section I. Vacutainer Collection Tube | | | | | |
| Catalog No.: | | | | | |
| Lot No.: | | ☐ N/A | | | |
| Exp. Date: | | | | | |
| Section II. Blood Biospecimen Collection | | | | | |
| Name of Clinic/Company: | | | | | |
| Date: | | Time: (24 Hr) | | Initials: | |

Reviewed by/date: _____


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## Attachment 3: Vial Label and Box / Rack Label

Vial Label

**Aliquot ID**
**Biospecimen Type**
**Volume**

| | |
|---|---|
| Barcode: | Barcode linked to Biospecimen Aliquot ID |
| Line 1: | Deidentified Biospecimen Aliquot ID |
| Line 2: | Biospecimen Type (Serum) |
| Line 3: | Volume (mL) |

Example Label:

**A1_123456_123_1**
**Human Serum**
**4.5 mL**

Box Label

**Study:** ?????? / ??????
**Biospecimen Type:** ?????
**Date:** DDMMMYY
**Shipping ID:** XXXXXXX
**Box** ? **of** ?

| | |
|---|---|
| Line 1: | SeroNet |
| Line 2: | Biospecimen Type (Human Serum) |
| Line 3: | Date in DDMMMYY format |
| Line 4: | Shipping ID |
| Line 5: | Box Number |

Example Label:

**Study:** SeroNet
**Sample Type:** Human Serum
**Date:** 01Jan20
**Shipping ID: XXXXXXX**
**Box** 1 **of** 10

Box Label Placement:

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