

Chapter 4

Central Laboratory Procedures

Table of Contents

- A. Donor Specimen Processing and Aliquoting**
Revised 7/10/95
 - I. Purpose
 - II. Intended Use and Test Limitations
 - III. Materials, Reagents and Equipment
 - IV. Procedure

- B. Processing, Aliquoting, and Freezing Phlebotomy Specimens**
Revised 8/1/96
 - I. Purpose
 - II. Specimen Collection and Requirements
 - III. Materials, Reagents and Equipment
 - IV. Procedure

**Viral Activation by Transfusion Study
Irwin Memorial Blood Centers
270 Masonic Avenue, San Francisco, CA 94118-4496**

A. Donor Specimen Processing & Aliquotting

I. PURPOSE

To provide Transfusion Services with instructions on how to process and aliquot specimens from components issued for transfusion for VAT Study subjects.

II. INTENDED USE AND TEST LIMITATIONS OF DONOR SPECIMENS

- A. Donor specimens will be tested for the following parameters:
1. CMV antibody on all units,
 2. Residual leukocyte concentration on filtered components only, and
 3. Determination of HLA type of selected donor units for purposes of analysis of survival kinetics of donor WBC in recipients.
- B. No other testing will be performed on donor blood samples.

III. MATERIALS, REAGENTS AND EQUIPMENT

- A. Provided by the Transfusion Service:
1. **VATS Form 42** "Donor/Specimen Processing Information Form"
 2. **VATS Form W2** "Segment Worksheet"
 3. Donor segment(s), containing **whole blood**, from original collection bag
 4. Residual blood (filtered/non-filtered) from transfer bag tubing
 5. Tube rack (Sarstedt #93.1428)
 6. Transfer pipet (calibrated in 0.5 ml increments)
 7. Freezer ($\leq -40^{\circ}\text{C}$)
- B. Provided by the Central Lab
1. 1.5-ml polypropylene tubes with caps
 2. VATS accession numbers (barcoded labels for tubes)
ie. ##-###-T1, ##-###-T2, and ##-###-B0

3. Prelabeled VATS inventory boxes; 3" height, 8 x 8 cells
ie. Donor Boxes = ##-D-###
4. "VATS Box Inventory" form for Donor Boxes
5. 10-ml tubes with caps

IV. PROCEDURE

A. Aliquots from the Transfused Component:

1. The Transfusion Service will fill in the following information on **VATS Form 42** "Donor/Specimen Processing Information Form":
 - A1 Subject ID
 - A3 Today's Date
 - B2 Blood Unit ID
 - B5 Filtered? (If yes, indicate date filtered, if known.)
 - D1 Date Unit Issued
 - D2 Component Type
 - D3 Product Weight, at issue
 - D4 Initials of Technician
2. Adhere VATS barcode labels (##-###-T1 and ##-###-T2) to two 1.5 ml polypropylene tubes.
3. Drain residual blood (filtered/non-filtered) from the transfer bag tubing into an empty 10-ml tube prelabeled with the corresponding Blood Unit ID.
4. Transfer two 0.5 ml aliquots from the 10-ml tube into the prelabeled VATS tubes.
5. Affix the duplicate barcode labels (##-###-T1 and ##-###-T2) to sequential empty slots on the "VATS Box Inventory" form. Fill the slots from top to bottom, left to right. You may want to leave an empty space for the segment tube (##-###-B0).
6. Place aliquots in corresponding locations in the VATS inventory box, and freeze at $\leq -40^{\circ}\text{C}$.
7. Fill in the following information on **VATS Form 42** "Donor/Specimen Processing Information Form":
 - E1 (0.5 ml) Aliquot (T1)
 - E2 (0.5 ml) Aliquot (T2)
 - E3 Date Frozen
 - E4 Initials of Technician

B. Donor Segments from Original Collection Bag

1. For non-filtered units and units filtered on site immediately prior to issue:
 - a. Retrieve two whole blood segments (minimum total volume of one-ml **whole blood**) attached to the original "packed RBCs" bag.

- b. Fill in top portion of the **VATS Form W2** "Segment Worksheet", Unit ID Number, Filtration Date (write **N/A** if not filtered), Segment Required = **N**, and Segment Retrieved = **N/A**.
 - c. Forward the form to the respective Blood Center to obtain Collection Date and Sex of Donor.
 - d. Proceed to step III.B.3.
2. For pre-filtered units and cases where whole blood segments are not readily retrievable from the Transfusion Service:
 - a. Fill in top portion of the **VATS Form W2** "Segment Worksheet", Unit ID Number, and Segment Required = **Y**.
 - b. Forward the form to the respective Blood Center to obtain Collection Date, Filtration Date, Sex of Donor and to retrieve whole blood segment(s) (minimum total volume of one-ml **whole blood**) from the original collection bag.
3. Upon receipt of segments:
 - a. Tape a VATS barcode label(s) (**##-###-B0**) to the segment(s) and place it/them into a 10.0-ml storage tube which is labeled with the corresponding VATS barcode label.
 - b. Affix another corresponding barcode label (**##-###-B0**) on the empty slot, for the segment(s) tube, on the "VATS Box Inventory" form.
 - c. Place aliquots in corresponding locations in the VATS inventory box, and freeze at $\leq -40^{\circ}\text{C}$.
4. Fill in the following information on **VATS Form 42** "Donor/Specimen Processing Information Form":

B1	Initials of Technician
B3	Date Unit Collected
B4	Sex of Donor
B5	Filtered? (If "yes", indicate date filtered, if not already filled in previously.)
B6	Donor unit segment(s) obtained?
C1	(1.0 ml) Segment (B0)
C2	Date Frozen
C3	Initials of Technician
5. File the "VATS Box Inventory" form until the box is ready to be shipped to the Central Lab.

**Viral Activation by Transfusion Study
Irwin Memorial Blood Centers
270 Masonic Avenue, San Francisco, CA 94118-4496**

B. Processing, Aliquotting, and Freezing Phlebotomy Specimens

I. PURPOSE

To provide instructions for Clinical Site Laboratories on how to accession, aliquot, and freeze specimens collected from VAT Study Subjects.

II. SPECIMEN COLLECTION AND REQUIREMENTS

- A. Tubes should be clearly labeled with Subject ID number, collection date/time, and phlebotomist's initials.
- B. Tubes should be filled to maximum capacity. Any problems during phlebotomy should be documented on the VATS "Phlebotomy Form".
- C. Accessioned specimen aliquots should be **frozen within four hours post-phlebotomy**.

III. MATERIALS, REAGENTS AND EQUIPMENT

- A. Provided by Clinical Coordinator/Phlebotomist
 - 1. One 7-ml ACD (yellow-top)
 - 2. One 7-ml EDTA/pyrogen-free (Terumo lavender-top)
 - 3. One 7-ml clot (red-top)
 - 4. **VATS Form 41** "Phlebotomy Form"
- B. Provided by the Central Lab
 - 1. 1.5-ml polypropylene tubes with caps
 - 2. VATS accession numbers (barcoded labels for aliquots)
ie. ##-###-A1
 - 3. Prelabeled VATS inventory boxes; 2" height, 9 x 9 cells
ie. Working Box = ##-**W**-###, Repository Box = ##-**R**-###
 - 4. "VATS Box Inventory" form for Working Box
 - 5. "VATS Box Inventory" form for Repository Box
- C. Provided by the Clinical Site Lab
 - 1. Tube rack (Sarstedt #93.1428)
 - 2. Transfer pipets (calibrated in 0.5 ml increments)

3. Centrifuge
4. Dimethylsulfoxide (DMSO), eg. Sigma (D8418) with purity $\geq 99.9\%$ and no DNase and/RNase detected.
5. Micropipette (ie. Pipetman)
6. Pipette tips
7. Freezer ($\leq -40^{\circ}\text{C}$)

IV. PROCEDURE

A. The Clinical Coordinator/Phlebotomist will submit the following to the Clinical Site Lab:

1. Three blood specimens (ACD, EDTA, & clot tubes).
2. **VATS Form 41** "Phlebotomy Form" (retain pink copy and forward original & yellow copies).

- A1. Subject ID
- A2. Visit Number
- A3. Subject Initials
- B1. Problem with Phlebotomy
- B2. Date of Phlebotomy
- B3. Time of Phlebotomy
- B4. Initials of Phlebotomist

B. Upon receipt of specimens by the Clinical Site Lab:

1. Record the following on the "Phlebotomy Form"

- C1. Date Received
- C2. Time Received

Note: Specimens should be aliquoted and frozen within four hours post-phlebotomy.

2. Confirm that the identification information on the specimen label correlates to the information recorded on the "Phlebotomy Form". If there are discrepancies, inform the phlebotomist and resolve problem.

C. Assign a VATS clinical specimen ID number (##-###) and record it on the "Phlebotomy Form":

C3. VAT clinical specimen ID Number ___ - ___

D. Apply corresponding VATS aliquot labels to 1.5-ml tubes and place tubes on rack.

1. Six tubes for ACD specimen
2. Seven tubes for EDTA specimen
3. Three tubes for clot specimen

Note: If any of the vacutainer tubes contain less than 7 ml blood, refer to Table I "Algorithm for Preparation of Aliquots" to prioritize which tubes should be aliquoted.

- E. Invert ACD vacutainer tube at least 10 times to ensure that blood is well mixed and aliquot the following volumes into pre-labeled tubes:
1. ###-###-A1 = 1.5 ml whole blood
 2. ###-###-A2 = 1.5 ml whole blood
- F. Invert EDTA vacutainer tube at least 10 times to ensure that blood is well mixed and aliquot the following volumes to the pre-labeled tubes:
1. ###-###-E1 = 0.5 ml whole blood
 2. ###-###-E2 = 0.5 ml whole blood
 3. ###-###-E3 = 1.0 ml whole blood + 0.1 ml DMSO
 4. ###-###-E4 = 1.0 ml whole blood + 0.1 ml DMSO
- G. Centrifuge all three vacutainer tubes (ACD, EDTA, and clot) at 2000 rpm for at least 10 minutes.
- H. Using a different transfer pipet for each vacutainer tube, aliquot the following:
1. ACD tube
 - a. ###-###-A3 = 0.5 ml plasma
 - b. ###-###-A4 = 0.5 ml plasma
 - c. ###-###-A5 = 0.5 ml plasma
 2. EDTA tube
 - a. ###-###-E5 = 0.5 ml plasma
 - b. ###-###-E6 = 0.5 ml plasma
 - c. ###-###-E7 = 0.5 ml plasma
 3. Clot tube
 - a. ###-###-S1 = 1.0 ml serum
 - b. ###-###-S2 = 1.0 ml serum
 - c. ###-###-S3 = 1.0 ml serum
- I. Aliquot 1.0 ml packed RBCs from the the **ACD** tube into the "###-###-A6" tube and add 0.1 ml DMSO.
- ###-###-A6 = 1.0 ml pRBCs + 0.1 ml DMSO
- J. Record volume in each tube on the "Phlebotomy Form" (Section C4. a.- p.), if different from default volume in parentheses. Enter "0.0" if an aliquot is not obtained.
- K. Log aliquots onto the appropriate "working" and "repository" "VATS Box Inventory" forms by adhering duplicate barcode labels to next available slot (starting from top to bottom, left to right).
1. **VATS Working Box = ##-W-###**
 - a. ###-###-A1 Record location on Phlebotomy Form (C5)
 - b. ###-###-A3
 - c. ###-###-A4

- d. ##-###-A6
- e. ##-###-E1
- f. ##-###-E3
- g. ##-###-E5
- h. ##-###-S1
- i. ##-###-S2

2. **VATS Repository Box = ##-R-###**

- a. ##-###-A2 Record location on Phlebotomy Form (C6)
- b. ##-###-A5
- c. ##-###-E2
- d. ##-###-E4
- e. ##-###-E6
- f. ##-###-E7
- g. ##-###-S3

- L. Place aliquots in the corresponding locations in the VATS freezer boxes and freeze at $\leq -40^{\circ}\text{C}$.

Note: When placing aliquots into the "working" and "repository" VATS inventory boxes, do not place aliquots from the same bleed into separate boxes; rather, start a new inventory box. Each "working" and "repository" box should contain aliquots from the same bleed, and each box can accommodate aliquots from ten subjects' visits.

- M. Record the following information on the "Phlebotomy Form":
- C7. Date aliquots placed in freezer
 - C8. Time aliquots frozen
 - C9. Initials of Technician
- N. Send original copy of the "Phlebotomy Form" to NERI and retain the yellow copy for recordkeeping.
- O. File the "VATS Box Inventory" forms until aliquots are shipped to the Central Lab.