SUBJECT: **ASTEC BLOOD SPECIMEN PROCESSING**

PURPOSE: To aliquot and process specimens housed in the AsTeC Biospecimen Repository in a manner to ensure proper identification, maintain the integrity of the sample and avoid contamination.

LEVEL: Interdependent
Principal Investigator/designee
Biological Specimen Repository Staff

SUPPLIES:

	CATALOG NUMBER	VENDOR	TELEPHONE NUMBER
Vial storage boxes for 2 ml cryovials	EF6828B	Daigger	847-816-5060
	820011	Thermo Fisher Scientific	866-984-3766
Vial storage boxes for 3 ml cryovials	820013	Thermo Fisher Scientific	866-984-3766
Vial storage boxes for Paxgene tubes	168415	RPI Corp.	847-635-7330
Automatic volumetric pipette	NA	NA	NA
Sterile Cryovial (2.0ml)	1420-9100	USA Scientific	800-522-8477
	T309-2A	BMA	724-935-6840
Sterile Cryovial (3.0ml)	1430-9100	USA Scientific	800-522-8477
	T309-3A	BMA	724-935-6840
Sterile pipette tips	PPT10	Associates of Cape Cod	508-540-3444
	0540311	Fisher Scientific	800-766-7000
Red top serum tubes	366408	BD or Local Vendor	888-237-2762
Cell Preparation Tubes (CPT)	362760	Fisher Scientific	800-766-7000
EDTA tubes	366450	BD or Local Vendor	888-237-2762
PAXgene blood RNA tubes	762165	Preanalytix (VWR)	800-932-5000
RNAlater	AM7022	Ambisome	800-888-8804
Preprinted specimen identification labels	NA	EMMES CORP.	301-251-1161

EQUIPMENT:

Biological safety cabinet
2 – 8 C Refrigerator
-20 C Freezer
-80 C Freezer
Pipettor
Centrifuge
Barcode reader
Computer with internet access

PERSONNEL PROTECTIVE EQUIPMENT:

Laboratory Coat
Disposable latex-free gloves
Thermal protective gloves

RECORDS:

AsTeC Specimen Source Document
AsTeC Case Report Form

COMPUTER PROGRAM:

AsTeC EMMES Case Report Form Database
AsTeC EMMES GlobalTrace™ Specimen Tracking and Inventory System

SPECIMEN: Blood, serum, leukocytes and/or plasma

NOTE 1: TUBES MUST BE COLLECTED IN A SPECIFIC ORDER.
1. CPT → 2. Red top (serum) → 3. Purple top (EDTA) → 4. Paxgene

NOTE 2: ONLY ONE SPECIMEN IS TO BE PROCESSED AND LABELED AT A TIME IN ORDER TO AVOID MISIDENTIFICATION AND CROSS-CONTAMINATION.

NOTE 3: BARCODE LABELS MUST BE PLACED *LENGTHWISE* AS SHOWN IN SECTION 1.4 OF THE GLOBALTRACE USER'S GUIDE.

PROCEDURE 1-CPT (Cell Processing Tube – Blue/Black Top)

A. Inspect container for visual integrity and ensure the tube is labeled with patient name or de-identified patient number upon receipt of the specimen. The container must be free of any breaks or cracks that would compromise the integrity of the specimen. Properly dispose of any specimens exhibiting breaks or crack and complete ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS FORM SOPPM-G 011.02.
B. Store tube upright at room temperature until centrifugation.
C. Centrifuge samples within two hours of collection.
D. Gently invert the tube 8-10 times immediately prior to centrifugation.
E. Place the sample(s) in the centrifuge and ensure the centrifuge is balanced using balancing tubes if necessary. Centrifuge tube holders are to be free swinging once placed on the rotor.
F. Centrifuge specimens at 3000 rpms for 30 minutes at a temperature of 18-25 C. Remove specimens and place in a test tube rack after centrifugation.
G. Label an appropriate number of sterile 2.0 ml cryovials with pre-printed, bar-coded labels (See blood collection chart). Place the corresponding pre-printed bar-code on to the specimen source document (plasma CPT). Refer to LABELING OPERATIONS SOPPM-G 013.02.
H. Aliquot 500 ul samples of plasma(CPT) using DNA free sterile pipette tips into sterile 2 ml sterile cryovials until approximately one half of the plasma has been

removed without disturbing the cell layer (see figure below for appearance of the cell layer under the plasma layer). Do not prepare more than 4 plasma aliquots. Collect cell layer with a 1000 ul pipette and sterile pipette tips being careful not to exceed 2 ml and place into a sterile 2 ml cryovial.

I. Properly dispose of contaminated material into biohazard waste.

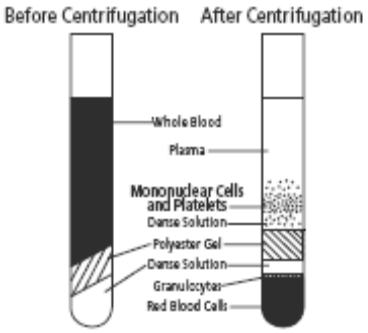
J. Place samples in the appropriate box for storage. Record specimen location information on Specimen Source Document.

K. Notify PI/designee of any protocol deviations. Submit the deviation(s) in a Quality Management Report and document on an Adverse Event Record Form (ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS SOPPM-G 011.02).

L. Quarantine specimens that do meet established criteria for acceptability in an appropriately labeled box at established temperatures and document that the PI/designee was notified.

M. Freeze samples at -80C as soon as possible

Layering of Formed Elements in the
BD Vacutainer™ CPT™ Tube



PROCEDURE 2-SERUM (Red Top)

A. Inspect container for visual integrity and ensure the tube is labeled with patient name or de-identified patient number upon receipt of the specimen. The container must be free of any breaks or cracks that would compromise the integrity of the specimen. Properly dispose of any specimens exhibiting breaks or crack and complete ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS FORM SOPPM-G 011.02.

B. Specimens collected for serum must be completely clotted for a minimum of 60 minutes prior to centrifugation.

C. Centrifuge samples after 60 minutes from the time of collection.

D. Place the clotted peripheral blood samples in the centrifuge and ensure the centrifuge is balanced using balancing tubes if necessary. Centrifuge tube holders are to be free swinging once placed on the rotor.

E. Centrifuge specimens at 3000 rpms for 10-30 minutes at a temperature of 18-25 C. Remove specimens and place in a test tube rack after centrifugation.
F. Remove the Hemogard closure from the blood collection tube.
G. Using a DNA-free sterile pipette tip, aliquot 500 ul of serum into each of the 2.0 ml cryovials. Aspirate all the serum in order to obtain the maximum number of aliquots making sure not to disturb the clot.
H. Label an appropriate number of sterile cryovials with pre-printed, bar-coded labels (See blood collection chart). Place the corresponding pre-printed bar-code on to the specimen source document. Refer to LABELING OPERATIONS SOPPM-G 013.02.
I. Properly dispose of contaminated material into biohazard waste.
J. Place samples in the appropriate box for storage. Record specimen location information on Specimen Source Document.
K. Place cryovials into the -80 C freezer as soon as possible.
L. Notify PI/designee of any protocol deviations. Submit the deviation(s) in a Quality Management Report and document on an Adverse Event Record Form (ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS SOPPM-G 011.02)..
M. Quarantine specimens that do meet established criteria for acceptability in an appropriately labeled box at established temperatures and document that the PI/designee was notified.

PROCEDURE 3-EDTA (Purple Top)

A. Collection of EDTA tubes will consist of three 7 ml tubes for a total collection of 21 ml of whole blood per sampling.
B. Inspect container for visual integrity and ensure the tube is labeled with patient name or de-identified patient number upon receipt of the specimens. The container must be free of any breaks or cracks that would compromise the integrity of the specimen. Properly dispose of any specimens exhibiting breaks or crack and complete ADVERSE EVENTS: ERRORS, ACCIDENTS, and COMPLAINTS AND DEVIATIONS FORM SOPPM-G 011.02.
C. Process EDTA tubes for plasma and whole blood aliquots within 2 hours of collection.
D. Centrifuge one EDTA tube at 3000 rpms for 10-30 minutes at a temperature of 18-25 C. Remove specimens and place in a test tube rack after centrifugation.
E. Remove the closure from the blood collection tube.
F. Using a DNA-free sterile pipette tip, aliquot 500 ul of EDTA plasma into each of the 2.0 ml cryovials. Aspirate all the EDTA plasma in order to obtain the maximum number of aliquots making sure not to disturb the red cell pellet.
G. Label all sterile cryovials with pre-printed, bar-coded labels supplied by EMMES Corporation. Place the corresponding pre-printed bar-code on to the specimen source document (plasma EDTA). Refer to LABELING OPERATIONS SOPPM-G 013.02
H. Properly dispose of contaminated material into biohazard waste

I. Using remaining two EDTA collection tubes, aliquot 500 ul of whole blood into each of two sterile cryovials 3.0 ml size. To each of the cryovials add 1.5 ml RNA later from individual vials (Ambisome Cat #AM7022). Invert tubes several times to mix.

J. Label all sterile cryovials with pre-printed, bar-coded labels supplied by EMMES Corporation. Place the corresponding pre-printed bar-code on to the specimen source document. Refer to LABELING OPERATIONS SOPPM-G 013.02

K. Place RNAlater aliquots in a 2-8C refrigerator overnight and transfer to -80 C freezer for final storage. Note: the final location of the RNAlater aliquots will be recorded upon placement in storage.

L. With remaining EDTA whole blood, aliquot 3 ml of whole blood into each of 3 - 4 sterile cryovials (3.0 ml size) for a total of 13 ml of whole blood.

- If after filling vials with 3ml whole blood less than 1. 5 ml residual specimen remains, divide equally into existing aliquots.
- If greater than 1. 5 ml blood remains, add to additional cryovial (3.0 ml size) and label appropriately.
- Document appropriately on Specimen Source Document.

M. Label all sterile cryovials with pre-printed, bar-coded labels supplied by EMMES Corporation. Place the corresponding pre-printed bar-code on to the specimen source document. Refer to LABELING OPERATIONS SOPPM-G 013.02

N. Place samples in the appropriate box for storage. Record specimen location information on Specimen Source Document. Note: the final location of the RNAlater aliquots will be recorded upon placement in storage.

O. Place RNAlater aliquots in a 2-8C refrigerator overnight and transfer to -80 C freezer for final storage.

P. Place all other aliquots into -80 C freezer for storage.

Q. Notify PI/designee of any protocol deviations. Submit the deviation(s) in a Quality Management Report and document on an Adverse Event Record Form (ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS SOPPM-G 011.02).

R. Quarantine specimens that do meet established criteria for acceptability in an appropriately labeled box at established temperatures and document that the PI/designee was notified.

PROCEDURE 4-PAXgene (Red Top specialty tube)

A. Draw the PAXgene tubes LAST and invert 8-10 times following blood collection.

B. Inspect container for visual integrity and ensure the tube is labeled with patient name or de-identified patient number upon receipt of the specimen. The container must be free of any breaks or cracks that would compromise the integrity of the specimen. Properly dispose of any specimens exhibiting breaks

or crack and complete ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS FORM SOPPM-G 011.02.

C. Label each PAXgene tube with the appropriate barcode label. Place the corresponding pre-printed bar-code on to the specimen source document. Refer to LABELING OPERATIONS SOPPM-G 013.02
D. Freeze PAXgene tubes at -20C overnight.
E. Place samples into the -80 C freezer for long term storage.
F. Record specimen location information on Specimen Source Document
G. Notify PI/designee of any protocol deviations. Submit the deviation(s) in a Quality Management Report and document on an Adverse Event Record Form (ADVERSE EVENTS: ERRORS, ACCIDENTS, COMPLAINTS AND DEVIATIONS SOPPM-G 011.02).
H. Quarantine specimens that do meet established criteria for acceptability in an appropriately labeled box at established temperatures and document that the PI/designee was notified

SUPPORTIVE DATA: N/A

REFERENCES:

1. PreAnalytiX GmbH, 8634 Hombrechtikon, CH©2005 PreAnalytiX GmbH www.PreAnalytiX.com

2. RNAlater product Circular P/N 7020M Revision C Revision Date: March 12, 2008 www.appliedbiosystems.com

ORIGINAL IMPLEMENTATION DATE: _____

APPROVED BY NIH NIAID Project Officer:_____ DATE _____

APPROVED BY PI/designee: _____ DATE _____

APPROVED BY Laboratory Coordinator:_____ DATE _____

Clinical Laboratory Diagnostics for Invasive Aspergillosis

1 Denotes order of tube type to be drawn

Barcode labels must be placed lengthwise

Blue/black CPT

Spin within 2 hr of collection

- Spin at 3000 RPM 30min 18-25C
- Make 2-4 500 ul aliquots from top plasma layer
- Collect whitish cell layer and transfer into cryovial
- Add barcode labels
- Freeze -80C

Red top serum tube

Wait at least one hour

- Spin at 3000 RPM 10-30 min 18-25C
- Make 3-7 500 ul aliquots
- Add barcode labels
- Freeze -80C

X 3

Purple top EDTA tube

Process within 2 hr of collection

- Make **2-4** 3ml aliquots of whole blood
  - Add barcode labels
  - Freeze -80C
- Aliquot 0.5ml Whole blood Into **two** tubes containing RNAlater
  - Add barcode labels
  - Store overnight at 2-8C
  - Freeze -80C
- Spin **ONE** Tube at 3000 RPM 10-30 min 18-25C
  - Make 4-7 500ul Aliquots Of top Plasma layer
  - Add barcode labels
  - Freeze -80C

X 3

Red/clear top PaxGene tube

- Add barcode labels
- Freeze at -20C For 24hours
- Freeze -80C