CENETRON

SPONSOR: MASSACHUSETTS GENERAL HOSPITAL
PETAL CLINICAL COORDINATING CENTER

PROTOCOL: **V**itamin D to **I**mprove **O**utcomes by **L**everaging **E**arly **T**reatment

(VIOLET)

Laboratory Manual Version 2.0

11 May 2018

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1.0 CENETRON PERSONNEL AND CONTACT INFORMATION

Medical Director:
Dwight DuBois, MD
Cenetron Diagnostics
2111 West Braker Lane
Suite 300
Austin, Texas 78758

Primary Study Contact:
Ginger McCready
ginger.mccready@cenetron.com
+1 512-439-2000 ext 253
+1 521-717-9253 (Direct)
+1 888-834-6632
+1 512-439-5006 (Fax)

If you have questions pertaining to specimen collection, transport, test results, or ordering supplies, please contact the appropriate department at Cenetron Diagnostics directly:

Clinical Trials	**Kits and Collection Supplies**	**Project Management**
clinicaltrials@cenetron.com	kits@cenetron.com	projectmanagement@cenetron.com
+1 512-439-2000 Option 2	+1 512-439-2000 Option 3	+1 512-439-2000
+1 888-834-6632 Option 2	+1 888-834-6632 Option 3	+1 888-834-6632
+1 512-439-5006 (Fax)	+1 512-439-5000 (Fax)	+1 512-439-5006 (fax)

Please be aware that the hours of service are Monday through Friday, 8:00 am until 5:00 pm Central Time (USA). **If an emergency situation should arise outside of our normal business hours, please call Cenetron Diagnostics at +1 888-520-2045 or +1 512 431 0725 and someone will assist you.**

For protocol-specific questions, please contact the PETAL Clinical Coordinating Center:

Nancy Ringwood, RN
nringwood@mgh.harvard.edu
617.724.9836

Katie Oldmixon, RN
coldmixon@mgh.harvard.edu
617.726.4447

For FedEx please contact:

FedEx: +1 800 GO FEDEX or +1 800.463.3339

2.0 LABORATORY TESTING SCHEDULE

SAMPLE COLLECTION: VIOLET ONLY SUBJECTS

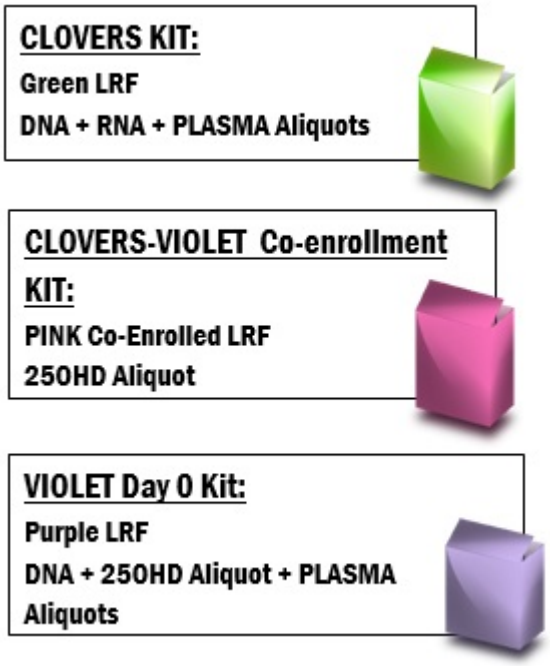
Sample	Day 0 (Baseline)
EDTA Plasma	**X**
Whole Blood DNA	**X**
25 OHD	**X**
Plasma for Vitamin D screening (FastPack)	**X**

SAMPLE COLLECTION: VIOLET-CLOVERS CO-ENROLLED SUBJECTS

Sample	Day 0 (Baseline) CLOVERS Kit	Day 0 Co-Enrollment Kit
EDTA Plasma	**X** *(previously collected)*	
Whole Blood DNA	**X** *(previously collected)*	
25 OHD		**X**
Plasma for Vitamin D testing (FastPack)		**X**

Breakout of Needed Kits by Subject:

Kit Contents by Type:



3.0 RECEIVING SUPPLIES

3.1 Collection Kits and Materials to Site

Cenetron will provide you with the necessary sample collection materials, processing and storage instructions, shipping forms and courier information for VIOLET samples.

Cenetron will provide you with small, medium, and large shippers. Small shippers are recommended for 25-OH D shipments (monthly shipments), while medium and large shippers are recommended for the 6 month shipments. Size of shippers needed is dependent on batched sample quantity.

The use of the supplied materials for VIOLET sampling is mandatory. For any questions related to sampling, handling, storage or shipping please contact Nancy Ringwood at the PETAL CCC.

The following kits will be supplied:

- Day 0
- Co-Enroll: Day 0 – ***Only*** collect for patients who are co-enrolled in the CLOVERS trial and have had a CLOVERS Day 0 Collection

Plasma for Vitamin D testing: When aliquoting plasma retain a portion to use for vitamin D testing with the FastPack device.

Please note: Each kit box contains all the sample collection materials needed for the patient for the timepoint.

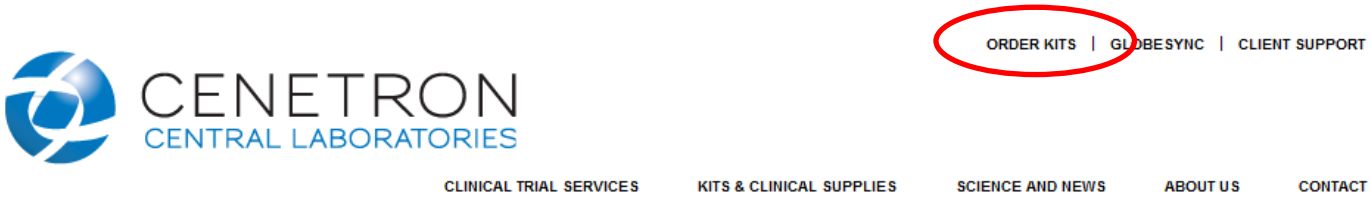
Kits will contain the required prelabeled collection tubes, prelabeled transfer vials, lab requisition, and other required materials. To replace damaged tubes, and to provide substitutes for unexpected events, each site will be provided with bulk supplies of collection tubes and transfer vials. Supplies required for universal precautions during sample collection, must be worn at all times and are the responsibility of the site to provide.

Each individual kit will arrive with labels indicating the expiration date. **Please verify the date before opening the Specimen Collection Kits to maintain the testing integrity of the supplies inside**. If you encounter any expired Specimen Collection Kits, please discard and order more kits as required. Sites should have at minimum sufficient kits to cover enrollment of at least 3 subjects at all times. Should you have any questions concerning the expiration date of the kits, please do not hesitate to contact us.

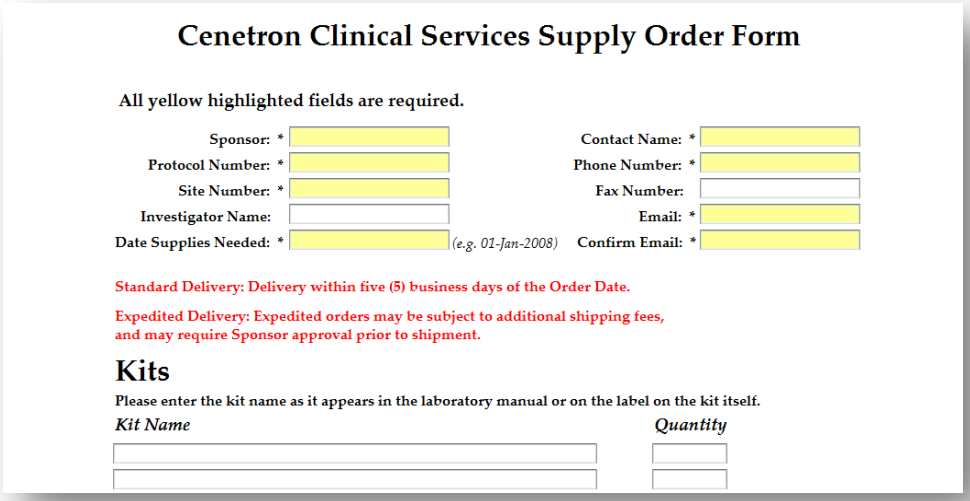
All collection tubes provided by Cenetron must be stored at room temperature (15–25°C).

3.2 Additional Kits and Supplies

For additional kits or supplies, please notify Cenetron Clinical Supplies by completing a Laboratory Supply Request Form and faxing it to Cenetron Diagnostics at 512-439-5000 or by email at kits@cenetron.com. In addition, you may request supplies by visiting the Cenetron website (www.cenetron.com) and clicking the link at the top of the page for "**Order Kits**".



Please ensure all highlighted fields are completed. **A date is required in the "Date Supplies Needed" field. Please do not enter ASAP.** Once the order is submitted, an email will be sent to the email entered indicating receipt of the order. Within one business day, you will also receive a confirmation email with the approximate shipping and delivery date.



Cenetron Clinical Services Supply Order Form

All yellow highlighted fields are required.

Sponsor: * | Contact Name: *
Protocol Number: * | Phone Number: *
Site Number: * | Fax Number:
Investigator Name: | Email: *
Date Supplies Needed: * (e.g. 01-Jan-2008) | Confirm Email: *

Standard Delivery: Delivery within five (5) business days of the Order Date.

Expedited Delivery: Expedited orders may be subject to additional shipping fees, and may require Sponsor approval prior to shipment.

Kits

Please enter the kit name as it appears in the laboratory manual or on the label on the kit itself.

Kit Name | *Quantity*

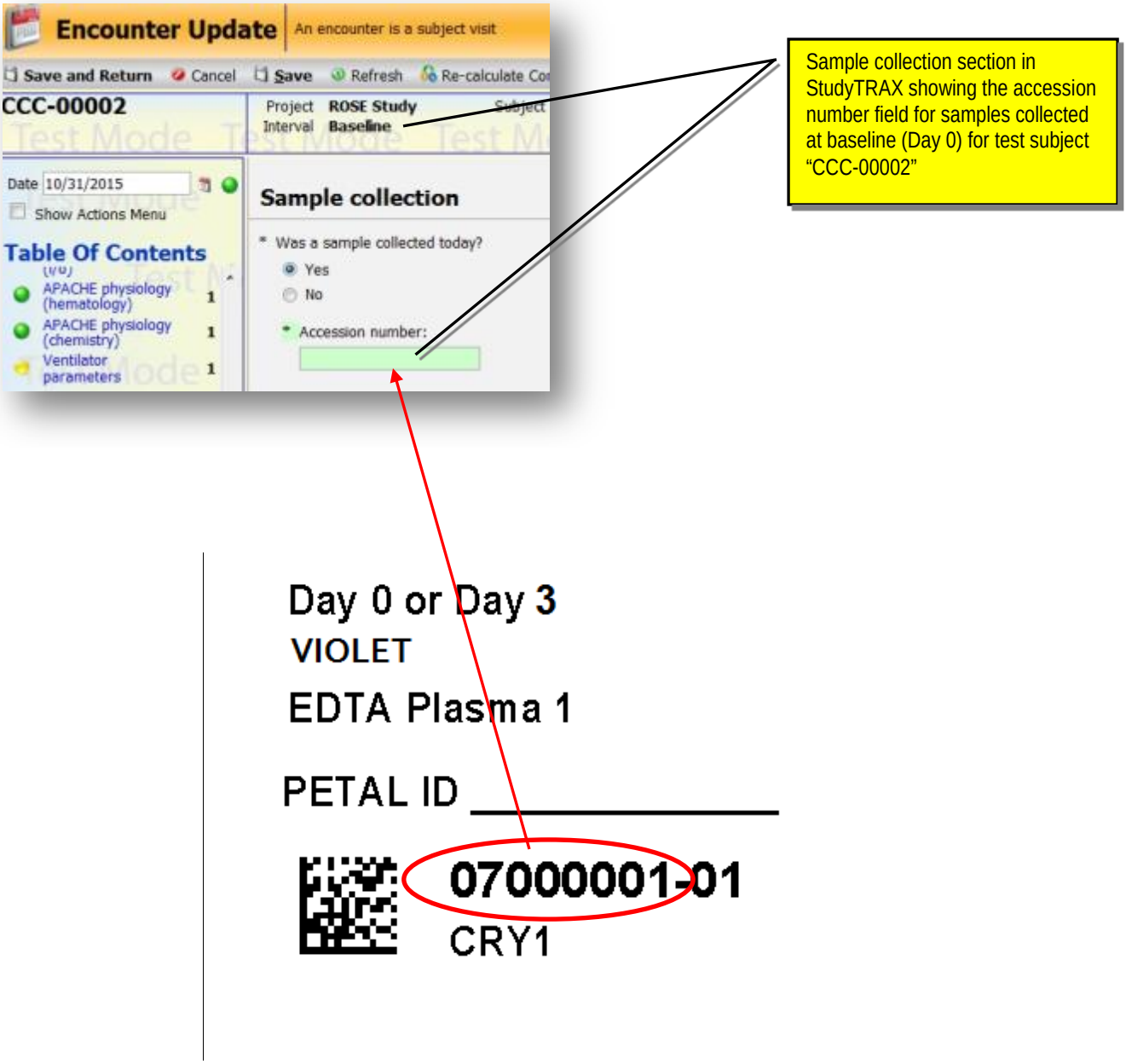
Supply requests will have a two week turnaround time. Exceptions will be made for all emergency requests received prior to 12:00 noon Central Time (USA). All emergency requests received after 12:00 noon will be shipped out the next regular business day.

4.0 COMPLETING THE LABELS

Labels will be provided for all tubes and Laboratory Requisition Forms (LRFs).

THE TUBES AND REQUISITION IN EACH KIT ARE BAR-CODED AND MUST BE USED TOGETHER. DO NOT MIX TUBES AND/OR REQUISITIONS FROM DIFFERENT KITS.

LINKING SAMPLES TO A SUBJECT: In addition to the lab requisition, the 8-digit accession number from the kit used for a sample collection day for a subject will be entered into the eCRF (StudyTRAX). Only include the first 8 digits. **DO NOT INCLUDE THE 2 DIGIT EXTENSION AFTER THE DASH**. Each subject will have one or two accession numbers (one for each collection day, e.g. Days 0 and 3).



5.0 COMPLETING THE LABORATORY REQUISITION FORM (LRF)

A corresponding Laboratory Requisition Form (LRF) must be completed for each patient's sample collection time point (Day 0 and Day 3). In order to ensure proper sample processing, the Laboratory Requisition Form must be filled out completely and correctly using a black or blue ballpoint pen. Please print legibly.

Keep the "Investigator Copy" (pink) of the completed Laboratory Requisition Form for your files, and return the white copy to Cenetron along with the specimen shipment.

Please place the Original (white & purple) copy in the provided Specimen Transportation Bag and seal before placing it into the box with the corresponding specimens.

THE TUBES AND REQUISITION IN EACH KIT ARE BAR-CODED AND MUST BE USED TOGETHER. DO NOT MIX TUBES AND/OR REQUISITIONS FROM DIFFERENT KITS.

Your site is responsible for completing the following information prior to specimen shipment:

- Site: Enter the name of the site (hospital)
- PETAL ID: Enter the Patient's PETAL ID Number (XXX-XXXXX)
- AGE (enter **year of birth** only): 01/JAN/YYYY (e.g. 01/JAN/1981) Month and day will be prefilled with: "01/JAN"
- Gender: Select Male or Female
- Collection Date: DD/MMM/YYYY (e.g. 01/JAN/2009)
- Collection Time: Specimen Collection Time (**indicate in military/24 Hour time – 2:12 pm would be 14:12**)
- Place a check mark next to all samples collected for that visit and note the processing and freezing times in the spaces provided
- Note the number of aliquots (plasma) collected
- Comments: please note any deviations (hemolysis, freezer thaw, etc.) in the comments section
- Study Site Signature: Please sign and date the Laboratory Requisition Form

5.1 Correction of Errors

If an error occurs, please correct in the following way:

- Cross through with a single straight line.
- Write the correct value above or to the side of the error.
- Initial and date the correction.
- **Do not use correction fluid.**

6.0 SPECIMEN COLLECTION AND PROCESSING

6.1 General Guidelines

1. Practice proper universal precautions throughout specimen collection and handling.
2. Obtain the Specimen Collection Kit for the correct visit and time-point.
3. Verify the expiration date on the Specimen Collection Kit. If the expiration date has not expired, open the Specimen Collection Kit.

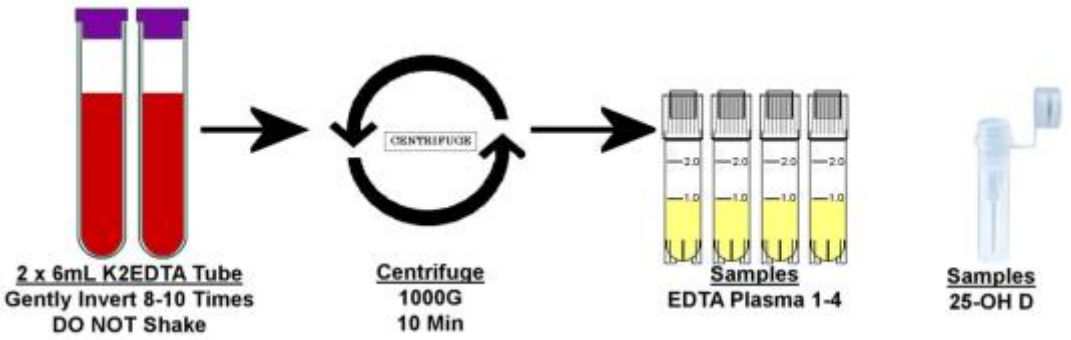
6.2 EDTA Plasma & 25-OH D - *Day 0*

Collection:

1. Completely fill the two provided 6 mL EDTA blood tubes with blood from patient via arterial line, venous line, or by venipuncture. Use largest needle routinely used in your ED or ICU for phlebotomy (up to 18g) for venipuncture and when instilling blood into the EDTA vacutainer to prevent hemolysis of the specimen. Hemolyzed samples should be redrawn if possible.
2. Gently invert the vacutainer 8-10 times to mix. Do NOT shake.
3. Place on ice if anticipated time to processing is greater than 30 minutes.

Processing:

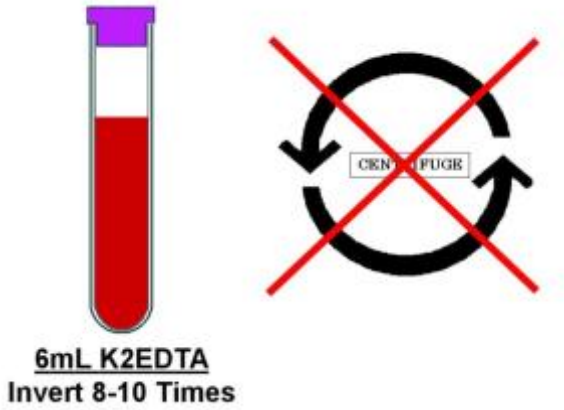
1. Centrifuge for 10 minutes at approximately 1000 G (standard tabletop centrifuge may be used).
2. Aliquoting of plasma:
   - Withdraw plasma (do not remove buffy coat) using a pipette and fill pre-labeled cryovial tubes with plasma.
   - Method for Aliquoting: Fill the 25-OH D vial first with 0.5 ml of plasma then the gray-top aliquots with 1 ml of plasma. This is to ensure that there is enough plasma to fill the 25OHD vial. If you do not have enough plasma to fill all the grey aliquots, fill as many aliquots with 1 ml as volume will allow. If you have extra plasma, you can add additional volume to the gray-top cryovials.
3. Place on ice if anticipated time to freezing is greater than 30 minutes.
4. Store promptly in a -70 or -80˚C freezer.



6.4 DNA Collection – *Day 0 Only*

Collection:

1. Fill the provided 6 mL EDTA blood tube with blood from patient via arterial line, venous line, or by venipuncture. Use largest needle routinely used in your ED or ICU for phlebotomy (up to 18g) for venipuncture and when instilling blood into the EDTA vacutainer to prevent hemolysis of the specimen. Hemolyzed samples should be redrawn if possible.
2. Gently invert the vacutainer 8-10 times to mix. Do NOT shake.
3. Store in a -70 or -80˚C freezer



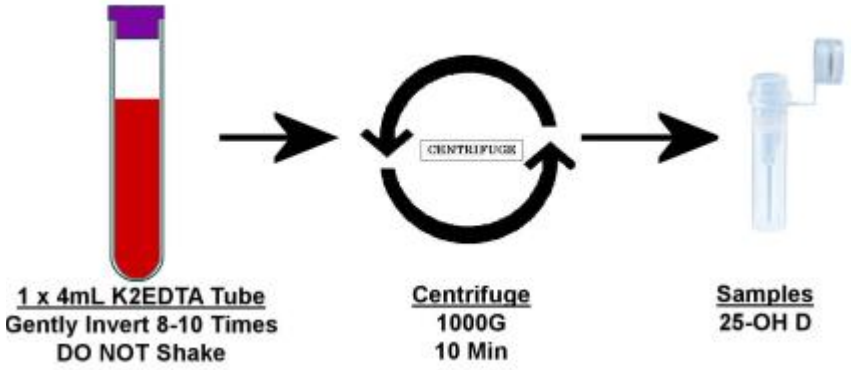
6.5 CLOVERS-VIOLET CO-ENROLLMENT: 25-OH D Only – *Co-Enroll: Day 0 Only*

Collection:

1. Completely fill the provided 4 mL EDTA blood tube with blood from patient via arterial line, venous line, or by venipuncture. Use largest needle routinely used in your ED or ICU for phlebotomy (up to 18g) for venipuncture and when instilling blood into the EDTA vacutainer to prevent hemolysis of the specimen. Hemolyzed samples should be redrawn if possible.
2. Gently invert the vacutainer 8-10 times to mix. Do NOT shake.
3. Place on ice if anticipated time to processing is greater than 30 minutes.

Processing:

5. Centrifuge for 10 minutes at approximately 1000 G (standard tabletop centrifuge may be used).
6. Aliquoting of plasma:
   - Withdraw plasma (do not remove buffy coat) using a pipette and fill pre-labeled cryovial tube with plasma.
   - Method for Aliquoting: Fill the 25-OH D vial with 0.5 ml. Plasma remaining after 25-OH D vial is filled may be properly disposed of.
7. Place 25-OH D on ice if anticipated time to freezing is greater than 30 minutes.
8. Store promptly in a -70 or -80˚C freezer.



7.0 PACKING AND SHIPPING SPECIMENS

<u>Frozen</u> specimens should be shipped Monday through Wednesday only. DO NOT ship frozen samples on Thursday, Friday, or Saturday. <u>Please hold these samples frozen until Monday for shipment.</u>

Holidays observed by FedEx and Cenetron Diagnostics are as listed:

Holiday/Observance
New Year's Day
Memorial Day
Independence Day
Labor Day
Thanksgiving Day
Christmas Day

Please do not ship specimens on a business day prior to a scheduled holiday, follow the observed holidays listed above.

When dealing with frozen specimens, store them at the required temperature as noted in the lab manual until shipment.

7.1 Sample Flow and Shipping Schedule

Site Samples

- EDTA Plasma
- Plasma for 25-OH D
- Whole Blood DNA

→ FedEx → CENETRON

Sample	Shipment frequency	Shipment conditions	Documents to include	Delivery Address
Plasma for 25OHD	Monthly	FROZEN on dry ice	Laboratory Requisition Form (WHITE Copy)	Cenetron Diagnostics 2111 West Braker Lane Suite 300 Austin, TX USA +1 512.439.2000

Sample	Shipment frequency	Shipment conditions	Documents to include	Delivery Address
EDTA Plasma	Every 6 months	FROZEN on dry ice	Laboratory Requisition Form (WHITE Copy)	Cenetron Diagnostics 2111 West Braker Lane Suite 300 Austin, TX USA +1 512.439.2000
Whole Blood DNA				

7.2 Frozen Samples (All Samples)

Please contact FedEx at (800) 463-3339 (800-GO-FEDEX) for US shipments to find out the time you must schedule a same day pickup. Notify the FedEx dispatcher that you require a pick-up and provide him/her with your name, address, and exact location of the box. Also, ask for a pick-up confirmation number to help keep track of when the courier will arrive.

SHIP FROZEN VIA FEDEX TO CENETRON DIAGNOSTICS

Sites will be responsible for supplying dry ice for all frozen shipments to Cenetron.

1. Prior to placing the specimens in the shipping containers (provided by Cenetron), verify that all tubes have been properly identified.

2. Verify that the specimens match those listed on the Laboratory Requisition Form.

3. Be sure to inspect the shipping boxes for accurate labeling information.

4. Remove the vials from the freezer.

5. Place a layer of dry ice at the bottom of the frozen shipper.

6. Ensure that the absorbent sheet is placed in the transport bag with the cryovials. Place the lab requisition into the outer pouch of the bag. It is acceptable to ship specimens from more than one subject together in the box provided each subject's samples are in their own bag.

7. Seal the transport bag and place it into the bottom of the shipping box.

8. Fill the remainder of the box with dry ice (minimum of 8 lbs or ~4 kg). Place the Styrofoam cover on the inner Styrofoam container.

9. Close box flaps and seal the exterior box with tape. **DO NOT TAPE THE STYROFOAM BOX.**

10. Complete the necessary sections on the pre-printed FedEx Air Waybill. Date all forms where necessary and retain the "Sender's Copy" for your files. Place completed air waybill into the clear plastic air bill pouch face up.

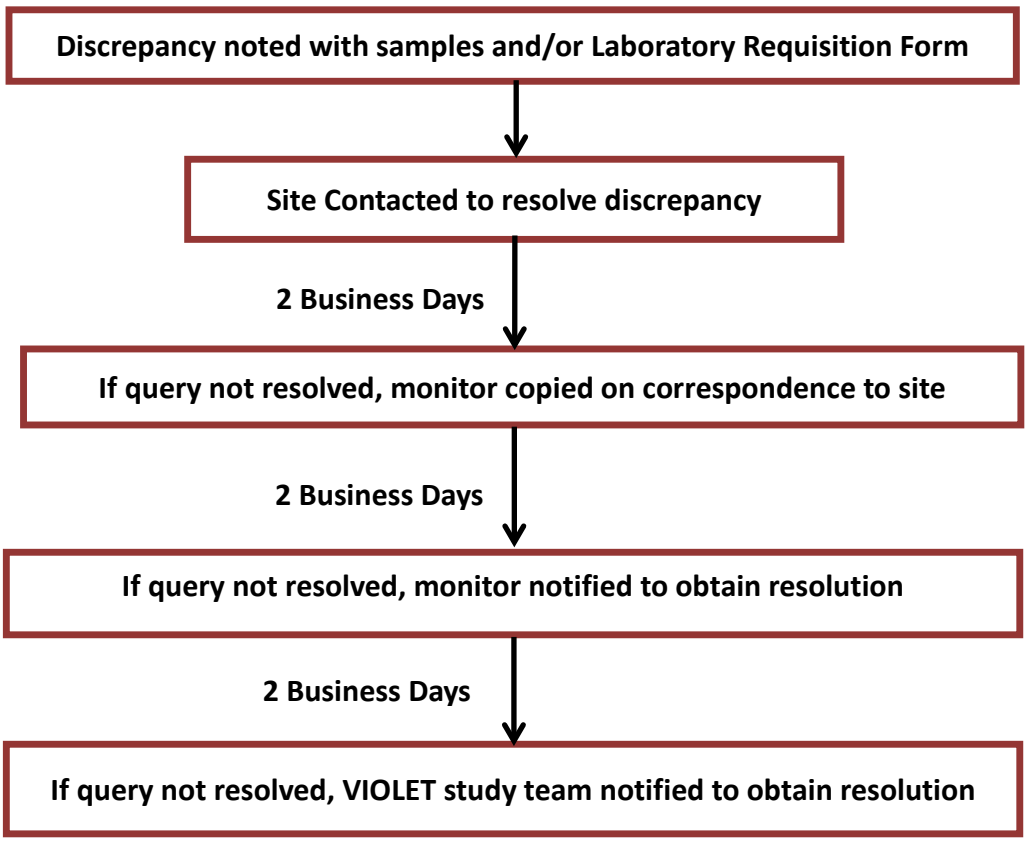
8.0 DATA QUERIES AND RECONCILIATION

8.1 Data Queries

If any discrepancies are noted on the lab requisition and sample labels, Cenetron will contact you directly to resolve the query. Please respond to the query either by phone at +1 512-439-2000 or by email at ClinicalTrials@Cenetron.com.

If an emergency situation should arise outside of our normal business hours, please call Cenetron Diagnostics at +1 888 520 2045 or +1 512 431 0725 and someone will assist you.

Data Query Process



Please note that a failure to respond to data queries in a timely manner could result in delays to samples being shipped for analysis thus resulting in a delay in data being available for study decisions.

9.0 DANGEROUS GOODS TRAINING REQUIREMENTS

Any person who ships dangerous goods (infectious specimens, culture isolates, dry ice) by air must follow certain regulations. The International Air Transport Association (IATA) produces a manual based on the International Civil Aviation Organization (ICAO) Technical Instructions, which outlines the procedures that must be followed to ensure safe transport. Training is an essential part of the process. It is necessary for all individuals involved in the preparation or transport of dangerous goods to be properly trained and tested initially with follow-up training every 24 months. As per the regulations, the investigator site is responsible for ensuring that their staff is properly trained.

Link to IATA endorsed Training Schools
http://www.iata.org/training/pages/endorsed_schools.aspx

10.0 FREQUENTLY ASKED QUESTIONS

Q. I have incorrectly drawn a patient's sample into a tube from another patient's kit, what should I do?
Document what has happened as completely as possible on the Lab Requisition indicating the details currently on the sample label and how the sample should correctly be labelled. Do not correct the details on the label of the incorrectly drawn sample by hand (this sample will be relabelled by Cenetron, as appropriate). Replace the incorrectly used tube from the patient kit using one of the spare tubes provided for that sample type. Spare materials are included in each kit.

Q. I have incorrectly labelled a tube and it has already been sent it to Cenetron, what should I do?
Document what has happened as completely as possible in a file note. Clearly indicate the details currently on the sample and how the sample should correctly be labelled. Send this file note to the PETAL CCC and explain what has happened. Cenetron who will ensure the sample is correctly relabelled. Your site may be contacted by the PETAL CCC to confirm details of the relabelling required.

Q. I have lost a sample label, what should I do?
Use one of the "Extra Labels" provided for that kit, ensuring that you clearly write the sample number and patient number in the spaces provided (using indelible ink). Spare materials are included in each kit.

Q. I have spun my blood sample and have drawn off the plasma/serum, and now have the blood tube left with the red cells in the bottom, should I also send this to Cenetron?
NO, once you have removed the plasma/serum as outlined in the manual instructions, you should discard the blood collection tube following your local site protocol for biohazardous trash.

Q. A sample collection is missed, what shall I do with the pre-labelled tube for this sample collection?
If a sample is missed, please do not send the empty tube to Cenetron.

Q. Can I send more than one patient's samples in a shipment?
Yes, as long as the relevant request form has been completed for all samples. Please separate each patient's samples into a separate specimen bag.

Q. The freezer storing the samples broke down and the samples thawed. What should we do?
Refreeze the samples as soon as possible. The incident should be noted in a note to file for the affected patients and the samples should be sent to Cenetron as normal with a note on the Lab Requisition, indicating the issue. If in doubt, please contact the PETAL Clinical Coordinating Center.

Q. Do we have to use the materials supplied by Cenetron or can we use our own supplies?
Use of the materials supplied by Cenetron is mandatory. The materials have been specifically chosen as they are suitable for processing and sample analysis or because they are compliant with shipping requirements.

Q. How should I dispose of expired Blood Collection Tubes?

Local trash collectors should be consulted to see what the proper method of disposal is for unused, expired blood collection products.

Q. What are the proper numbers of inversions for the various BD Vacutainer® Blood Collection Tubes?

An inversion is one complete turn of the wrist, 180 degrees, and back. Tubes should be inverted according to the following recommendations:

- EDTA plasma tubes – 8-10 inversions

Q. What constitutes a tube inversion?

An inversion is one complete turn of the wrist, 180 degrees, and back. The contents of the tube should have time to touch each side of the tube.

Q. How do I obtain quality certificates and MSDS on BD Blood Collection Products?

You can access most quality certificates (certificate of quality, sterility and conformance) and Material Safety Data Sheets (MSDS) on the BD website, at the following address - catalog.bd.com

Q. I have booked a shipment, but I do not have packaging materials for the shipment. Should Cenetron have sent these to me?

Yes, Cenetron will provide EPS shippers for shipments of frozen samples. Sites are responsible for providing dry ice for the shipments.

Q. I made a mistake when booking the courier, and now samples will be shipped to the wrong laboratory. How can I correct this?

You must phone or email your local courier office to make any changes to your courier booking. You can find their contact details in your Courier Starter pack. The driver cannot make any changes to the shipment destination at the time of pick-up; this can only be corrected by the local courier office in their system. You must also make sure that the samples are sent at the correct temperature.

Q. The sample issue I have is not listed here.....Help!

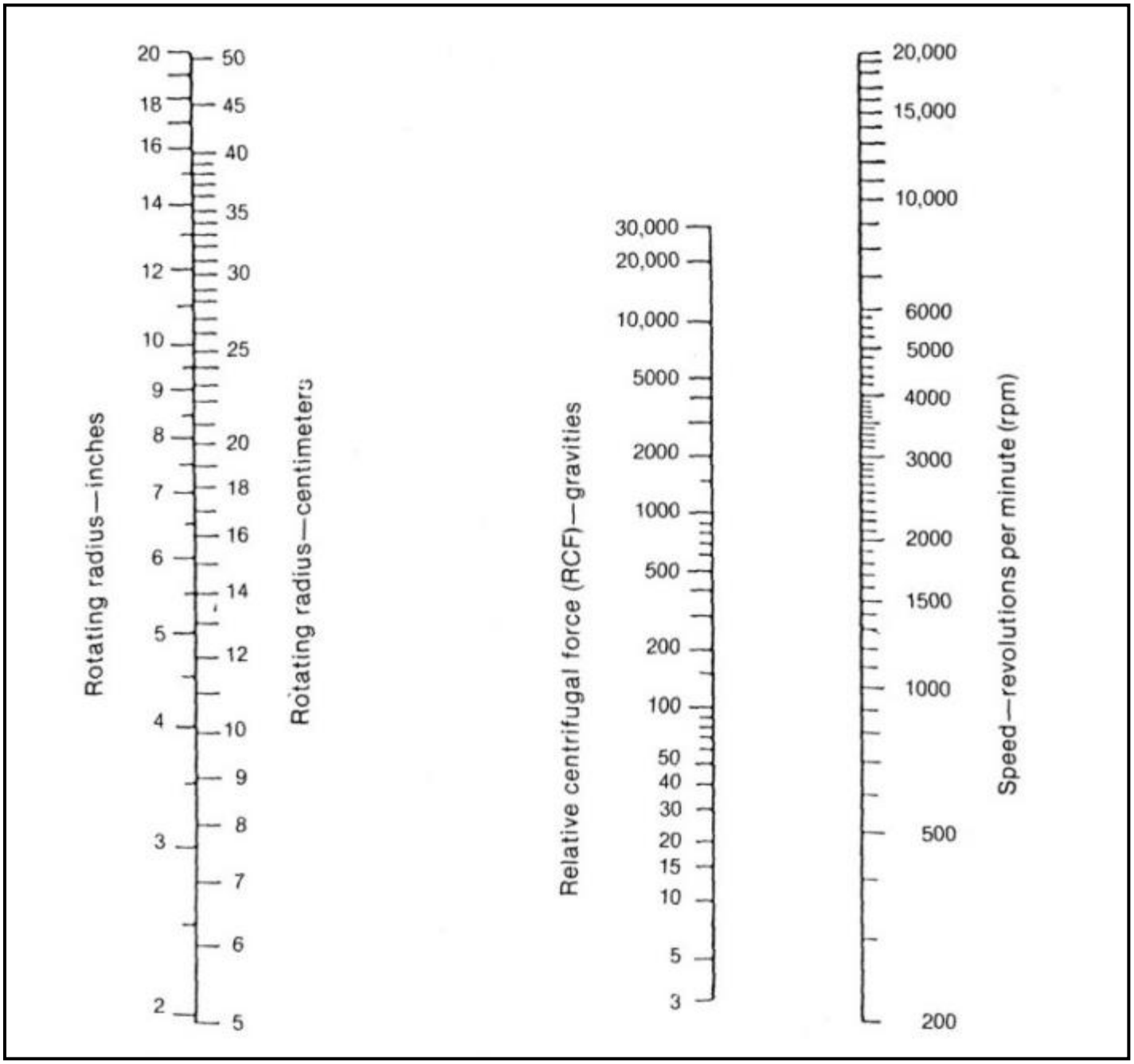
Please contact the PETAL Clinical Coordinating Center for further assistance.

11.0 FORMS AND ATTACHMENTS

11.1 Force to RPM Conversion Nomograph
11.2 Time Conversion Chart
11.3 Laboratory Supply Request Form
11.4 Report Correction Request
11.5 Sample Laboratory Requisitions
11.6 Processing and Shipping Log
11.7 Accreditation and Permits
11.8 BD Vacutainer™ Evacuated Blood Collection System

11.1 Force to RPM Conversion Nomograph

G Force to RPM Conversion Nomograph



11.2 Time Conversion Chart

Time Conversion Chart

TIME IN HOURS		24 HOUR TIME
12:00 AM	----------(MIDNIGHT)----------	00:00
1:00 AM	----------	01:00
2:00 AM	----------	02:00
3:00 AM	----------	03:00
4:00 AM	----------	04:00
5:00 AM	----------	05:00
6:00 AM	----------	06:00
7:00 AM	----------	07:00
8:00 AM	----------	08:00
9:00 AM	----------	09:00
10:00 AM	----------	10:00
11:00 AM	----------	11:00
12:00 PM	----------(NOON)----------	12:00
1:00 PM	----------	13:00
2:00 PM	----------	14:00
3:00 PM	----------	15:00
4:00 PM	----------	16:00
5:00 PM	----------	17:00
6:00 PM	----------	18:00
7:00 PM	----------	19:00
8:00 PM	----------	20:00
9:00 PM	----------	21:00
10:00 PM	----------	22:00
11:00 PM	----------	23:00

11.3 Supply Request Form

Massachusetts General
Protocol *VIOLET* – Supply Request Form

Today's Date: _____

Date Supplies Needed: (exact date needed) ___/___/___ (DD / MMM / YYYY)

Site Number: _____

Contact Name: _____

Investigator Name: _____

Phone Number: _____

Email Address: _____

Collection Kits (Indicate Quantity Needed)

Please indicate quantity required	
Day 0	
Day 3	
Co-Enroll: Day 0*	
EPS Frozen Shipper	

Only for patients co-enrolled in the CLOVERS trial

Additional Supplies (Please indicate supply and quantity needed)

Other (Serum Tubes, Cryovials, Biohazard bag, etc.): _____

UPON COMPLETION FAX TO +1 512-439-5000

11.4 Report Correction Request Form

REPORT CORRECTION REQUEST

If any report is incomplete or contains incorrect information, **please complete ALL of the information in Section 1 as shown on the incorrect report**. The item(s) needing a correction or completion should then be supplied in Section 2. **Only record in Section 2 the information to be changed.** Fax this completed Report Correction Request to Cenetron at (512) 439-5006. A corrected report will be faxed to you as soon as completed.

SECTION 1 – Information as shown on **INCORRECT** report

Site: _____ **Massachusetts General Hospital (PETAL CCC)**
Protocol VIOLET

Subject Initials: _____ _____ _____ Visit: _____

Gender: Male Female

Patient Number (PETAL ID Number): _____

Accession Number: _____

Date of Birth: _01_/___Jan__/_____

Date of Collection: _____/_____/_____ Time of Collection: _____:_____

Other:_____

SECTION 2 – Information to be **CORRECTED**

Site: _____ **Massachusetts General Hospital (PETAL CCC)**
Protocol VIOLET

Subject Initials: _____ _____ _____ Visit: _____

Gender: Male Female

Patient Number (PETAL ID Number): _____

Accession Number: _____

Date of Birth: _01_/___Jan__/_____

Date of Collection: _____/_____/_____ Time of Collection: _____:_____

Other:_____

Study Coordinator Signature:_____ Date:_____

Cenetron Employee Signature:_____ Date:_____

11.5 Sample Laboratory Requisition Forms

CENETRON

2111 West Braker Lane Suite 300
Austin, TX USA 78758
Phone: 888.834.6632 (Toll Free) +1 512.439.2000 (Intl.)
Fax: 512.439.5006

VIOLET: Day 0

Laboratory Requisition Form

SAMPLE INFORMATION

[_____] Hospital Name

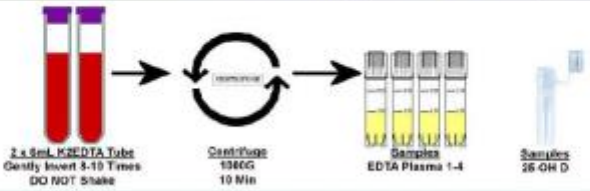
[_____|_____|_____] -- [_____|_____|_____|_____|_____] PETAL ID

Date of Birth **0 1 J A N** [_____|_____|_____|_____] Provide Year Only

Gender: ☐ MALE ☐ FEMALE

Blood Collected on [_____|_____] [_____|_____|_____] [_____|_____|_____|_____] at [_____|_____] : [_____|_____] (24 Hour Clock)
D D M M M Y Y Y Y H H M M

Please Complete ALL Requested Sample Information

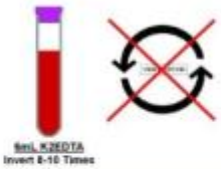


Plasma Sample

# of Aliquots: _____

Processing Time _____:_____ (24-Hr Clock)

Freezing Time _____:_____ (24-Hr Clock)



DNA Sample

Freezing Time

_____:_____
(24-Hr Clock)

COMMENTS AND SIGNATURE

FOR INTERNAL USE ONLY

Laboratory Requisition Completed By:

_____ _____
Signature *Date*

R2812 v.2

SEND THE WHITE COPY OF THIS FORM WITH THE *FROZEN* SAMPLES. RETAIN THE PINK COPY FOR YOUR RECORDS

CENETRON

2111 West Braker Lane Suite 300
Austin, TX USA 78758
Phone: 888.834.6632 (Toll Free) +1 512.439.2000 (Intl.)
Fax: 512.439.5006

VIOLET: Day 3

Laboratory Requisition Form

SAMPLE INFORMATION

[_____] Hospital Name

[_____|_____|_____] -- [_____|_____|_____|_____|_____] PETAL ID

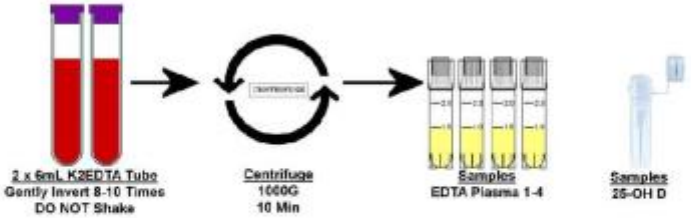
Date of Birth **0 1 J A N** [_____|_____|_____|_____] **Provide Year Only**

Gender: ☐ **MALE** ☐ **FEMALE**

Blood Collected on [_____|_____] [_____|_____|_____] [_____|_____|_____|_____] **at** [_____|_____] : [_____|_____] (24 Hour Clock)
D D M M M Y Y Y Y H H M M

Please Complete ALL Requested Sample Information

# of Aliquots: _____



Processing Time _____:_____ (24-Hr Clock)

Freezing Time _____:_____ (24-Hr Clock)

Plasma Sample

COMMENTS AND SIGNATURE

Laboratory Requisition Completed By:

_____ _____
Signature *Date*

FOR INTERNAL USE ONLY

R28103 v2

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CENETRON

2111 West Braker Lane Suite 300
Austin, TX USA 78758
Phone: 888.834.6632 (Toll Free) +1 512.439.2000 (Intl.)
Fax: 512.439.5006

VIOLET Co-Enrollment: Day 0

Laboratory Requisition Form

SAMPLE INFORMATION

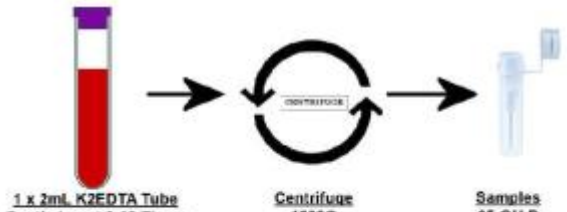
[_____] [_____|_____|_____] -- [_____|_____|_____|_____|_____]
Hospital Name PETAL ID

Date of Birth **0 1 J A N** [_____|_____|_____|_____]
Provide Year Only

Gender: ☐ **MALE** ☐ **FEMALE**

Blood Collected on [__|__] [__|__|__] [__|__|__|__] **at** [__|__] : [__|__] (24 Hour Clock)
D D M M M Y Y Y Y H H M M

Please Complete ALL Requested Sample Information



1 x 2mL K2EDTA Tube
Gently Invert 8-10 Times
DO NOT Shake

Centrifuge
1000G
10 Min

Samples
25-OH D

COMMENTS AND SIGNATURE

FOR INTERNAL USE ONLY

Laboratory Requisition Completed By:

_____ _____
Signature *Date*

R2813 v.1

SEND THE WHITE COPY OF THIS FORM WITH THE *FROZEN* SAMPLES. RETAIN THE PINK COPY FOR YOUR RECORDS

11.6 Permits & Accreditation

CENETRON
DIAGNOSTICS

Accreditation Documents

Enclosed accreditation documents are concurrently valid with the release date of this laboratory manual. If any Cenetron document expires within the duration of the study, please directly contact Cenetron Diagnostics or go to www.cenetron.com/accreditation.html to view and download the updated certificate. Expired documents of affiliate laboratories can be obtained by directly contacting Cenetron.

3. B.L. Pasloske, D. DuBois, D. Brown, M. Winkler. Ribonuclease Resistant RNA Preparation and Utilization United States Patent and Trademark Office. No. 5939262 August 17, 1999.

4. B.L. Pasloske, D. DuBois, D. Brown, M. Winkler. Ribonuclease Resistant RNA Preparation and Utilization United States Patent and Trademark Office. No. 6,214,982 April 10, 2001.

5. B.L. Pasloske, D. DuBois, D. Brown, M. Winkler. Methods of quantifying viral load in an animal with a ribonuclease resistant RNA preparation. No. 6,399,307 June 4, 2002.

6. C.R. WalkerPeach and D.B. DuBois. Method for monitoring nucleic acid assays using synthetic internal controls with reversed sequences. No. 6,395,470 May 28, 2002

Book Chapters and Articles

1. WalkerPeach, C.R., M. Winkler, D.B. DuBois, and B.L. Pasloske. Ribonuclease-resistant RNA controls (Armored RNA) for reverse transcription-PCR, branched DNA, and genotyping assays for hepatitis C virus. Clinical Chemistry 45:2079-2085 (1999).

2. DuBois, D. B. "Standards for PCR Assays" in PCR Applications, Academic Press (1999).

3. DuBois, D.B. and C. George Ray. "Viral Infections of the Lower Respiratory Tract" in Pediatric Pulmonary Disease, Mosby (1999).

4. Pasloske, B.L., C.R. WalkerPeach, R.D. Obermoeller, M. Winkler, and D.B. DuBois. Armored RNA Technology for Production of ribonuclease-resistant viral RNA controls and standards. J. Clinical Microbiology 36:3590-3594 (1998)

5. DuBois, D.B., Gretch, C. dela Rosa, W. Lee, J. Fine, C.R. Blagg, and L. Corey. Quantitation of Hepatitis C Viral RNA in Sera of Hemodialysis Patients: Gender Related Differences in Viral Load. Am. J. Kidney Diseases 24:795-801 (1994).

6. DuBois, D.B., and D.M. Mock. A sequential, solid-phase assay for biotin in physiological fluids that correlates with expected biotin status. Analytical Biochemistry 153:272-278 (1986).

7. Mock, D.M., G. Langford, D.B. DuBois, N. Criscagna, and P. Horowitz. A fluorometric assay for the biotin-avidin interaction based on displacement of the fluorescence probe 2-anilinonaphthalene-6-sulfonic acid. Analytical Biochemistry 151:178-181 (1985).

8. DuBois, D.B., and R.D. Rossen. Use of human antibodies to identify antigens in cultured human tumor cells: Detection of discrete antigen molecules using electroblotting and enzyme-linked antibody probes. Journal of Immunological Methods 63:7-24 (1983).

9. Beck, D.A., R.D. Rossen, D.B. DuBois, and C.O. Felice. Synthesis of antigens, cross-reactive of bovine serum albumin, by cultured neuroblastoma cells. Cancer Research 43:858-863 (1983).

10. Rosenthal, J., R. Katz, D.B. DuBois, A. Morrissey, A. Machicao. Chronic maxillary sinusitis associated with the mushroom Schizophyllum commune in a patient with AIDS. Clin. Infectious Diseases 14(1):46-8 (1992).

11. Madhoun, Z.T., D.B. DuBois, J. Rosenthal, J.C. Findley, and D.C. Aron. Central diabetes insipidus: A complication of herpes simplex type 2 encephalitis in a patient with AIDS. Am. J. Med. 90:658-9 (1991).

CENTERS FOR MEDICARE & MEDICAID SERVICES

CLINICAL LABORATORY IMPROVEMENT AMENDMENTS

CERTIFICATE OF ACCREDITATION

LABORATORY NAME AND ADDRESS

CENETRON DIAGNOSTICS, LTD
2111 WEST BRAKER LANE, BLD 5, SUITE 300
AUSTIN, TX 78758

CLIA ID NUMBER

45D0907734

EFFECTIVE DATE

08/08/2016

LABORATORY DIRECTOR

DWIGHT B DUBOIS M.D.

EXPIRATION DATE

08/07/2018

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C. 263a) as revised by the Clinical Laboratory Improvement Amendments (CLIA), the above named laboratory located at the address shown hereon (and other approved locations) may accept human specimens for the purposes of performing laboratory examinations or procedures.

This certificate shall be valid until the expiration date above, but is subject to revocation, suspension, limitation, or other sanctions for violation of the Act or the regulations promulgated thereunder.

Karen W. Dyer, Acting Director
Division of Laboratory Services
Survey and Certification Group
Center for Clinical Standards and Quality

258 Certs2_080216

If you currently hold a Certificate of Compliance or Certificate of Accreditation, below is a list of the laboratory specialties/subspecialties you are certified to perform and their effective date:

LAB CERTIFICATION (CODE)	EFFECTIVE DATE	LAB CERTIFICATION (CODE)	EFFECTIVE DATE
VIROLOGY (140)	02/10/2016		

FOR MORE INFORMATION ABOUT CLIA, VISIT OUR WEBSITE AT WWW.CMS.GOV/CLIA
OR CONTACT YOUR LOCAL STATE AGENCY. PLEASE SEE THE REVERSE FOR
YOUR STATE AGENCY'S ADDRESS AND PHONE NUMBER.
PLEASE CONTACT YOUR STATE AGENCY FOR ANY CHANGES TO YOUR CURRENT CERTIFICATE.

COLLEGE of AMERICAN PATHOLOGISTS

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

The College of American Pathologists
certifies that the laboratory named below

Cenetron Diagnostics Ltd
Laboratory
Austin, Texas
Dwight DuBois, MD

CAP Number: 6704501
AU-ID: 1191130
CLIA Number: 45D0907734

has met all applicable standards for accreditation and is hereby accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur prior to November 21, 2018 to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Chair, Commission on Laboratory Accreditation

President, College of American Pathologists

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Centers for Disease Control and Prevention
Office of Health and Safety, MS A-46
Atlanta, Georgia 30333
TEL: 404-718-2077; FAX: 404-718-2093; Email: importpermit@cdc.gov

SAFER • HEALTHIER • PEOPLE

Permit to Import Infectious Biological Agents, Infectious Substances, and Vectors

In accordance with 42 CFR Section 71.54 of the Public Health Service Foreign Quarantine Regulators, cited on the bottom of this permit, permission is granted the permittee to import into any port under control of the United States, or to receive by transfer within the United States, the material described in Item 1 below.

PHS PERMIT NO.	**2017-02-051**	
DATES	**ISSUED: Thursday, February 09, 2017**	**EXPIRES: Friday, February 09, 2018**
1. DESCRIPTION OF MATERIAL	**HUMAN BLOOD OR BLOOD PRODUCTS WHICH MAY CONTAIN HUMAN IMMUNODEFICIENCY VIRUS, HEPATITIS C VIRUS, OR HEPATITIS B VIRUS.**	
2. PERMITTEE (NAME, ORGANIZATION, ADDRESS AND CONTACT INFORMATION)	**DWIGHT DUBOIS** **CENETRON DIAGNOSTICS, LLC** **2111 WEST BRAKER LANE, SUITE 300** **AUSTIN, TX 78758**	**TEL: 512-717-9224** **FAX: 512-439-5006**
3. SOURCE OF MATERIAL (NAME, ORGANIZATION, ADDRESS, COUNTRY)	**WORLDWIDE**	
4. TYPE OF PERMIT AND INSTRUCTIONS FOR USE	As the permittee, your facility will be subject to inspection at some time in the future to confirm that the importer's biosafety measures are commensurate with the hazard posed by the items to be imported and the level of risk given its intended use. ☐ Single Importation into the U.S. ☑ Single Transfer Within the U.S. ☑ Multiple Importation into the U.S. ☐ Multiple Transfer Within the U.S. A. Record of each importation shall be maintained on permanent file by permittee. B. Enclosed label(s) must be forwarded to the shipper(s). C. One label shall be affixed to shipping container. Enclosed labels may be photocopied.	
5. CONDITIONS OF ISSUANCE ITEMS APPLICABLE WHEN CHECKED	☐ A. Subsequent distribution, within the U.S., of the material described in this permit is prohibited without prior authorization by the Public Health Service. ☑ B. All material is for laboratory use only - Not for use in the production of biologics for humans or animals. ☑ C. All material is free of tissues, serum and plasma of domestic and wild ruminants, swine and equines. ☐ D. Additional Requirements: ☐ IATA Packaged to preclude escape. ☐ USDA permit may be required (Telephone: 301-851-3300). ☑ E. Work with the agent(s) described shall be restricted to areas and conditions meeting requirements in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories." ☑ F. Packaging must conform to 49 CFR Sections 171-180.	
	6. Signature of Issuing officer Samuel S. Edwin Samuel S. Edwin, Ph.D. Director, Division of Select Agents and Toxins	

CDC 0728 (F 13.40) REV. 4-13

42 CFR 71.54. Permit to Import Biological Agents, Infections Substances, and Vectors
A person may not import into the United States any infectious biological agent, infectious substance, or vector unless: It is accompanied by a permit issued by the Centers for Disease Control and Prevention (CDC). The possession of a permit issued by the CDC does not satisfy permitting requirements placed on materials by the U.S. Department of Agriculture that may pose hazards to agriculture or agricultural production in addition to hazards to human health.

BD Vacutainer™ Evacuated Blood Collection System

BD Vacutainer® Evacuated Blood Collection System

For In Vitro Diagnostic Use.

INTENDED USE

BD Vacutainer® Tubes, Needles and Holders are used together as a system for the collection of venous blood. BD Vacutainer® Tubes are used to transport and process blood for testing serum, plasma or whole blood in the clinical laboratory.

PRODUCT DESCRIPTION

BD Vacutainer® Tubes are evacuated tubes with color-coded (see table below) conventional stoppers or BD Hemogard™ Closures. BD Vacutainer® Plus Tubes are plastic tubes. Both tube types contain additives in varying concentrations dependent upon the amount of vacuum and the required additive to blood ratio for the tube. See each shelf package or case label for specific additive quantity and approximate draw volume. Additive choice depends on the analytic test method. It is specified by the manufacturer of the test reagents and/or instrument on which the test is performed. Tube interiors are sterile. Tube stoppers are lubricated with silicone or glycerine (see individual shelf package or case label) to facilitate stopper insertion.

BD Vacutainer® Tube Closure Color Code Cross Reference

ADDITIVE GROUP/ADDITIVE	CONVENTIONAL CLOSURE	BD HEMOGARD™ CLOSURE
Gel Separation Tubes		
BD SST™ Tubes with Gel and Clot Activator	Red/Grey	Gold
BD PST™ Tubes with Gel and Lithium Heparin[1]	Green/Grey	Light Green
Non-additive Tubes		
Silicone Coated	Red	Red
Uncoated	Red	Pink
No Additive[2]	Cherry Red/Light Grey	Clear
Serum Tubes with Additives		
Thrombin[3]	Yellow/Grey	Orange
Plus Serum with clot activator	Red	Red
Thrombin[3], Soybean Trypsin Inhibitor	Light Blue	Light Blue
Whole Blood/Plasma Tubes		
K_2EDTA or K_3EDTA	Lavender	Lavender / Pink
Citrate/CTAD (Coagulation)	Light Blue	Light Blue or Clear
Citrate (ESR)	Black	Black
Sodium Fluoride/Sodium EDTA (Glucose)	Grey	Grey
Sodium Fluoride/Potassium Oxalate (Glucose)	Grey	Grey
Heparin[1]	Green	Green
Acid Citrate Dextrose (ACD)	Yellow	N/A
Sodium Polyanethol Sulfonate (SPS)	Yellow	N/A
Trace Element Tubes		
Silicone Coated, Heparin[1], EDTA, or with clot activator	N/A	Royal Blue
Lead Tubes		
Heparin[1]	N/A	Tan
K_2EDTA	N/A	Tan

[1]Heparin source is porcine. [2]May only be used as a discard tube or as a secondary specimen collection tube. [3]Thrombin source is bovine.

BD Vacutainer® Serum Tubes

BD Vacutainer® Plus Serum Tubes are coated with silicone and micronized silica particles to accelerate clotting. Particles in the white film on the interior surface activate clotting when tubes are mixed 5 times by inversion. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

A silicone coating on the walls of most serum tubes reduces adherence of red cells to tube walls.

BD Vacutainer® Tubes for Lead and Trace Element Tests

Tubes for lead testing and other trace elements are labeled specifically for these purposes on the shelf package and case label. Use only appropriately labeled tubes for these tests. The tubes for lead and trace element testing have been tested by extraction of the stoppered tube for 4 hours. Atomic Absorption Spectroscopy (AAS) testing yielded results below these concentration limits:

BD Vacutainer® Trace Element Tubes Contamination Upper Limits

Analyte	Glass µg/L	Plus µg/L	Analyte	Glass µg/L	Plus µg/L
Antimony	0.8	-	Lead	2.5	0.2
Arsenic	1.0	0.2	Magnesium*	60	40
Cadmium	0.6	0.1	Manganese	1.5	1.5
Calcium*	400	150	Mercury**	-	2.0
Chromium	0.9	0.5	Selenium	-	0.6
Copper	8.0	5.0	Zinc*	40	40
Iron	60	25			

Water extraction analyzed by *Flame, **Cold Vapor, all others flameless AAS

BD Vacutainer® Tubes for Lead Testing Contamination Upper Limits

Analyte	Glass µg/L	Plus*µg/L
Lead	10	2.5
0.1 Nnitric acid extraction analyzed by flameless AAS		

*Also suitable for routine hematology testing

BD Vacutainer® SST™ Tubes and Transport Tubes

The interior of the tube wall is coated with micronized silica particles to accelerate clotting. A barrier polymer is present at the tube bottom. The density of this material causes it to move upward during centrifugation to the serum-clot interface, where it forms a barrier separating serum from fibrin and cells. Serum may be aspirated directly from the collection tube, eliminating the need for transfer to another container. BD SST™ Transport Tubes contain the same clot activator as BD SST™ Tubes with approximately twice the quantity of barrier. This additional material produces a larger barrier between the serum and cells that is more stable for shipping from a phlebotomy site to a testing site. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

BD Vacutainer® PST™ Tubes

The interior of the tube wall is coated with lithium heparin to inhibit clotting. Heparin activates antithrombins, thus blocking the coagulation cascade and producing a whole blood/plasma sample instead of clotted blood plus serum. A barrier polymer is present at the tube bottom. The density of this material causes it to move upward during centrifugation to the plasma-cell interface, where it forms a barrier separating plasma from cells. Supernatant plasma may be aspirated directly from the collection tube, eliminating the need for manual transfer to another container. Plasma obtained in BD PST™ Tubes should be tested or removed from the tube within 2 hours of collection. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

BD Vacutainer® Tubes for Immunohematology

BD Vacutainer® Plus K_2EDTA and Plus Serum Tubes as well as the BD Vacutainer® Glass Serum and Glass K_2EDTA Tubes may be used for routine immunohematology testing such as red cell grouping, Rh typing and antibody screening. BD Vacutainer® Plus Serum Tubes and Glass Serum Tubes may also be used for red cell phenotyping and DAT testing. Tubes must be filled to capacity (until vacuum is exhausted). Additive tubes (K_2 or K_3) must be inverted 8 to 10 times to assure complete mixing with blood, as erroneous results may occur. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

BD Vacutainer® CTAD Tubes

The CTAD tube is used for the collection and transport of specimens for hemostasis testing. The CTAD solution is a mixture of sodium citrate, theophylline, adenosine and dipyridamole. The purpose of the additive is to anticoagulate the specimen and to minimize in vitro platelet activation. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

BD Vacutainer® Plus Citrate Tubes

The tube component is comprised of two plastic tubes assembled together to maintain the draw volume and liquid additive. The tube contains 0.109M (3.2%) buffered sodium citrate additive. All tube configurations are 'full draw' and utilize BD Hemogard™ closures. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

The product performance has been compared to the 4.5mL glass tube for routine coagulation assays on a variety of donor popula- tions with clinically equivalent results obtained. Note: all studies were performed on donors with hematocrits between 25 and 55%.

BD Vacutainer® Blood Collection Needles

BD Vacutainer® Blood Collection Needles are single-use, double-ended, stainless steel needles. They have a threaded hub that fits into the threads of all BD Vacutainer® Needle Holders. The venipuncture end of the needle has a point specially designed to enter the skin easily during venipuncture. The needle is lubricated with silicone.

BD Vacutainer® Multiple Sample Needles have a rubber sleeve covering the non-patient end of the needle that prevents leakage of blood into the holder during venipuncture. This Product Contains Dry Natural Rubber.

The tubes slide into the holder and are pushed onto the back end of the needle, allowing the vacuum in the tube to draw blood to a predetermined level. The needles are available in 1 and 1-1/2 inch lengths, in 20, 21, and 22 gauge. Needle size and lot number are printed on each individual needle assembly.

LIMITATIONS OF SYSTEM

The quantity of blood drawn varies with altitude, ambient temperature, barometric pressure, tube age, venous pressure, and filling technique. Tubes with draw volume smaller than the apparent dimensions indicated (partial draw tubes), may fill more slowly than tubes of the same size with greater draw volume.

For those tubes subjected to centrifugation to generate plasma or serum for testing, standard processing conditions do not necessarily completely sediment all cells, whether or not barrier gel is present. Accordingly, cell-based metabolism, as well as natural degradation ex vivo affects serum/plasma analyte concentrations/activities beyond acellular changes. It is recommended that testing for glucose, uric acid, and lactate dehydrogenase (LD) be performed as soon after collection and separation as possible. Due to natural degradation, delay in separation of the serum or plasma from the cellular mass or in testing after separation will result in erroneous results for those analytes.

Prior to using CTAD tubes to collect specimens from warfarin patients for PT determinations with citrate sensitive reagents, please contact the BD Technical Services Department at 1-800-631-0174.

BD Vacutainer® SST™ Tubes, PST™ Tubes, and Plus Serum Tubes are not recommended for collection of samples for blood banking procedures. Glass EDTA and glass Serum Tubes are acceptable for blood banking procedures. BD Vacutainer® SST™ Glass Tubes and PST™ Tubes are not recommended for collection of samples for therapeutic drug monitoring (TDM) assays. BD Vacutainer® SST™ Plus Tubes can be used for certain TDM assays.

Please contact BD Technical Services Department at 1-800-631-0174 for details.

Do not use BD Vacutainer® Tubes containing lithium heparin for lithium heparin measurement.

For coagulation tests, if patient hematocrit is above 55%, the final citrate concentration in the specimen should be adjusted.

PRECAUTIONS

1. Storage of glass tubes containing blood at or below 0°C may result in tube breakage.
2. Do not remove conventional rubber stoppers by rolling with thumb. Remove stoppers with a twist and pull motion.
3. Do not use tubes or needles if foreign matter is present.
4. The paper label covering the connection of the needle shields will tear when the needle is opened. Do not use needle if label has been torn before venipuncture.
5. CTAD tubes must be protected from artificial and natural light during storage. Accumulated light exposure in excess of 12 hours can cause additive inactivation.
6. BD Vacutainer® Plus Serum Tubes with clot activator are not to be used as a discard tube for coagulation studies.
7. Separation of serum or plasma from the cells should take place within 2 hours of collection to prevent erroneous test results.

CAUTION:

1. Practice Standard Precautions. Use gloves, gowns, eye protection, other personal protective equipment, and engineering controls to protect from blood splatter, blood leakage, and potential exposure to bloodborne pathogens.
2. All glass has the potential for breakage. Examine all glass for potential damage in transit before use, and take precautionary measures during handling.
3. Handle all biologic samples and blood collection "sharps" (lancets, needles, luer adapters, and blood collection sets) according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury), since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Utilize any built-in used needle protector, if the blood collection device provides one. BD does not recommend recladding used needles. However, the policies and procedures of your facility may differ and must always be followed.
4. Discard all blood collection "sharps" in biohazard containers approved for their disposal.
5. Transferring a sample collected using syringe and needle to a tube is not recommended. Additional manipulation of sharps, such as hollow bore needles, increases the potential for needlestick injury.
6. Transferring samples from syringe to an evacuated tube using a non-sharps device should be performed with caution for the reasons described below. • Depressing the syringe plunger during transfer can create a positive pressure, forcefully displacing the stopper and sample, causing splatter and potential blood exposure. • Using a syringe for blood transfer may also cause over or under filling of tubes, resulting in an incorrect blood-to-additive ratio and potentially incorrect analytic results. • Evacuated tubes are designed to draw the volume indicated. Filling is complete when vacuum no longer continues to draw, though some tubes may partially fill due to plunger resistance when filled from a syringe. The laboratory should be consulted regarding the use of these samples.
7. If blood is collected through an intravenous (I.V.) line, ensure that line has been cleared of I.V. solution before beginning to fill blood collection tubes. This is critical to avoid erroneous laboratory data from I.V. fluid contamination.
8. Overfilling or under filling of tubes will result in an incorrect blood-to-additive ratio and may lead to incorrect analytic results or poor product performance.

STORAGE

Store tubes at 4-25°C (39-77°F), unless otherwise noted on the package label. All liquid preservatives and anticoagulants are clear and colorless, except CTAD which is yellow. Do not use if they are discolored or contain precipitates. Powdered and freeze-dried additives such as heparin and thrombin are white; fluoride and fluoride/oxalate may be pale pink. Do not use if color has changed. Do not use tubes after their expiration date.

SPECIMEN COLLECTION and HANDLING

READ THIS ENTIRE CIRCULAR BEFORE PERFORMING VENIPUNCTURE.

Required Equipment Not Provided for Specimen Collection

1. Practice Standard Precautions. Use gloves, eye protection, coats or gowns, and other appropriate apparel for protection from exposure to bloodborne pathogens or other potentially infectious materials.
2. Any BD Vacutainer® Needle Holders of the standard size may be used with 13 or 16 mm diameter tubes. Use the small (pediatric) needle holder with 10.25 mm diameter tubes. A pediatric tube adapter should be used to modify the standard holder to fit the 10.25 mm diameter tubes.
3. Alcohol swab for cleansing site. If additional tubes requiring sterile collections, such as blood cultures, are filled from the same venipuncture, use tincture of iodine or suitable alternative for cleansing. Follow the laboratory policy for sterile sample collection for site preparation and tube handling instructions. Do not use alcohol based cleansing materials when samples are to be used for blood alcohol testing.
4. Dry sterile gauze.
5. Tourniquet.
6. Needle disposal container for used needle or needle/holder combination.

Required Equipment Not Provided for Specimen Processing

1. Disposable transfer pipets if direct sampling from the instrument is not used or if specimen is stored separately.
2. Centrifuge capable of generating the recommended RCF at the tube bottom. A horizontal centrifuge head is preferred for barrier quality with BD SST™ and BD PST™ Tubes and to obtain platelet poor plasma for coagulation studies.
3. Gloves and other personal protective equipment as necessary for protection from exposure to bloodborne pathogens.

Preparation for Specimen Collection

Be sure the following materials are readily accessible before performing venipuncture:

1. See Required Equipment Not Provided for Specimen Collection above.
2. All necessary tubes, identified for size, draw, and additive.
3. Labels for positive patient identification of samples.

Recommended Order of Draw

1. Tubes for sterile samples
2. Tubes for coagulation studies (e.g., citrate)
3. BD SST™ and Serum Tubes.
4. Tubes with other additives (e.g., heparin, EDTA, fluoride)

BD SST™ Tubes and BD Vacutainer® Plus Serum Tubes contain particulate clot activators and are considered additive tubes. Therefore Plus Serum Tubes are not to be used as discard tubes before drawing citrate tubes for coagulation studies. A glass or BD Vacutainer® Plus discard tube must be used if only citrate tubes are drawn with a Blood Collection Set for venipuncture.

Prevention of Backflow

Since some evacuated blood collection tubes contain chemical additives, it is important to avoid possible backflow from the tube, with the possibility of adverse patient reactions. To guard against backflow, observe the following precautions:

1. Place patient's arm in a downward position.
2. Hold tube with the stopper uppermost.
3. Release tourniquet as soon as blood starts to flow into tube.
4. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

Venipuncture Technique and Specimen Collection General Instructions

WEAR GLOVES DURING VENIPUNCTURE AND WHEN HANDLING BLOOD COLLECTION TUBES TO MINIMIZE EXPOSURE HAZARD.

1. Select tube or tubes appropriate for required specimen. For sterile collections, see the specific instructions noted in the collection device product circular.
2. Assemble needle in holder. Be sure needle is firmly seated to ensure needle does not unthread during use.
3. Gently tap tubes containing additives to dislodge any material that may be adhering to the stopper.
4. Place tube into holder. Note: Do not puncture stopper.
5. Select site for venipuncture.
6. Apply tourniquet. Prepare venipuncture site with an appropriate antiseptic. DO NOT PALPATE VENIPUNCTURE AREA AFTER CLEANSING.
7. Place patient's arm in a downward position.

8. Remove needle shield. Perform venipuncture WITH ARM DOWNWARD AND TUBE STOPPER UPPER-MOST.
9. Center tubes in holder when penetrating the stopper to prevent sidewall penetration and resultant premature vacuum loss. Push tube onto needle puncturing stopper diaphragm.
10. REMOVE TOURNIQUET AS SOON AS BLOOD APPEARS IN TUBE. DO NOT ALLOW CONTENTS OF TUBE TO CONTACT THE STOPPER OR END OF THE NEEDLE DURING PROCEDURE.
    Note: Blood may occasionally leak from the needle sleeve. Practice Standard Precautions to minimize exposure hazard. If no blood flows into tube or if blood ceases to flow before an adequate specimen is collected, the following steps are suggested to complete satisfactory collection:
    a. Push tube forward until tube stopper has been penetrated. If necessary, hold in place to ensure complete vacuum draw.
    b. Confirm correct position of needle cannula in vein.
    C. REMOVE TUBE AND PLACE NEW TUBE ONTO THE HOLDER.
    d. If second tube does not draw, remove needle and discard. Repeat procedure from Step 1.
11. When first tube has filled to its stated volume and blood flow ceases, remove it from holder.
12. Place succeeding tubes in holder, puncturing diaphragm to begin flow. See Recommended Order of Draw.
13. While each successive tube is filling, turn the filled tube upside-down and return it to upright position. This is one complete inversion.

For proper additive performance, invert BD SST™ Tubes, and Plus Serum Tubes 5 times. Invert Citrate or CTAD tubes 3-4 times. Invert all other filled additive tubes 8-10 times. Do not shake. Vigorous mixing may cause foaming or hemolysis. Insufficient mixing or delayed mixing in serum tubes may result in delayed clotting and incorrect test results. In tubes with anticoagulants, inadequate mixing may result in platelet clumping, clotting and/or incorrect test results.

14. As soon as blood stops flowing in the last tube, remove needle from vein, applying pressure to puncture site with dry sterile swab until bleeding stops.
15. Once clotting has occurred, apply bandage if desired.
16. After venipuncture, the top of the stopper may contain residual blood. Take proper precautions when handling tubes to avoid contact with this blood.
17. Dispose of needle and holder per your facility's policy and guidelines.

Clotting Instructions

Allow blood to clot thoroughly before centrifugation. The following table gives the recommended minimum clotting times for specific tube types or additives: BD SST™ Tubes, and Plus Serum Tubes should be inverted five times.

Minimum Clotting Time Recommendations	
PRODUCT	TIME (min)
Serum Tubes (Red or Pink Closures)	60
BD SST™ Tubes	30
Thrombin Tubes	5

Recommended times are based upon an intact clotting process. Patients with abnormal clotting due to disease, or those receiving anticoagulant therapy require more time for complete clot formation. Separation of serum or plasma from cells should take place within 2 hours of collection to prevent erroneous test results according to NCCLS guidelines. See Limitations of System, Precautions, Specimen Collection and Handling Sections.

Centrifugation

Caution: **Do not centrifuge glass tubes at forces above 2200 RCF in a horizontal head (swinging bucket) centrifuge as breakage may occur. Glass tubes may break if centrifuged above 1300 RCF in fixed angle centrifuge heads.**

BD Vacutainer® Plus Tubes will withstand up to 10,000 RCF in a balanced centrifuge. Always use appropriate carriers or inserts. Use of tubes with cracks or chips or excessive centrifugation speed may cause tube breakage, with release of sample, droplets, and an aerosol into the centrifuge bowl. Release of these potentially hazardous materials can be avoided by using specially designed sealed containers in which tubes are held during centrifugation. Centrifuge carriers and inserts should be of the size specific to the tubes used. Use of carriers too large or too small for the tube may result in breakage.

RCF is related to centrifuge speed setting (rpm) using the following equation:

$$rpm = \sqrt{\frac{RCF \times 10^5}{1.12 \times r}}$$

where "r", expressed in cm, is the radial distance from the center of the centrifuge head to the bottom of the tube. The following table gives recommended centrifuge RCF and time.

Centrifugation RCF and Time		
PRODUCT	RCF (g)	TIME (min)
BD SST™ and BD PST™ Tubes (glass)	1000 - 1300	10
BD SST™ Plus and BD PST™ Plus Tubes – 13mm	1100 - 1300	10
BD SST™ Plus and BD PST™ Plus Tubes – 16mm	1000 - 1300	10
BD SST™ Transport Tubes	1100 - 1300	15
All non-gel tubes	≤1300	10
Citrate Tubes*	1500	15

15 minutes for all gel tubes in a fixed angle centrifuge

RCF = Relative Centrifugal Force, g's

*Citrate tubes should be centrifuged at a speed and time to consistently produce platelet-poor plasma (platelet count <10,000/µL) per NCCLS Guidelines.

Ensure that tubes are properly seated in the centrifuge carrier. Incomplete seating could result in separation of the BD Hemogard™ Closure from the tube or extension of the tube above the carrier. Tubes extending above the carrier could catch on centrifuge head, resulting in breakage. Balance tubes to minimize the chance of glass breakage. Match tubes to tubes of the same fill level, glass tubes to glass, tubes with BD Hemogard™ Closure to others with the Closure, gel tubes to gel tubes, BD Vacutainer® Plus Tubes with Plus Tubes, and tube size to tube size.

Always allow centrifuge to come to a complete stop before attempting to remove tubes. When centrifuge head has stopped, open the lid and examine for possible broken tubes. If breakage is indicated, use mechanical device such as forceps or hemostat to remove tubes.

Caution: Do not remove broken tubes by hand.

See centrifuge instruction manual for disinfection instructions.

Barrier Information

The flow properties of the barrier material are temperature-related. Flow may be impeded if chilled before or during centrifugation. To optimize flow and prevent heating during centrifugation, set refrigerated centrifuges to 25°C (77°F). Gel separation tubes should be centrifuged no later than 2 hours after collection.

Tubes should not be re-centrifuged once barrier has formed. Barriers are more stable when tubes are spun in centrifuges with horizontal (swinging bucket) heads than those with fixed angle heads. Note: Some push-down filters may not be compatible with plastic tubes due to the tapered inner diameter of the tube.

Separated serum or plasma is ready for use. The tubes may be placed directly on the instrument carrier or serum/plasma may be pipetted into an analyzer cup. Some instruments can sample directly from a separator tube with the stopper in place. Follow the instrument manufacturer's instructions.

ANALYTIC EQUIVALENCY

Evaluations of BD Vacutainer® Tubes have been performed for an array of analytes over a variety of test methods and time periods. The BD Technical Services Department is available to answer questions regarding these studies. Please contact them to obtain references and technical reports on these evaluations and any other information regarding the use of BD Vacutainer® Tubes with your instrument/reagent system.

BD Technical Services may be reached at 800-631-0174. You may write to BD Diagnostics for information at:

BD Technical Services
BD, Franklin Lakes, NJ 07417
www.bd.com

Whenever changing any manufacturer's blood collection tube type, size or storage condition for a particular laboratory assay, the laboratory personnel should review the tube manufacturer's data and their own data to establish/verify the reference range for a specific instrument/reagent system. Based on such information, the laboratory can then decide if changes are appropriate.

REFERENCES

NCCLS Document H1-A5. Evacuated Tubes and Additives for Blood Specimen Collection; approved standard, 5th ed. Wayne, PA: National Committee for Clinical Laboratory Standards; 2003.

NCCLS Document H3-A5. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; approved standard, 5th ed. Wayne, PA: National Committee for Clinical Laboratory Standards; 2003.

Landt M, Smith CH and Hortin GL. Evaluation of evacuated blood-collection tubes: Effects of three types of polymeric separators on therapeutic drug-monitoring specimens. Clin Chem 1993; 39:1712-1717.

Dasgupta A, Dean R, Saldana S, Kinnamon G and McLawhon RW. Absorption of therapeutic drugs by barrier gels in serum separator blood collection tubes. Am J Clin Path 1994; 101:456-461.

Yaron BP, Loge C and Dale J. Prothrombin time, one tube or two? Am J Clin Path 1996; 105:294-97.

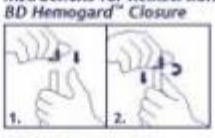
Gottfried, EL and Adachi, MM. Prothrombin time (PT) and activated partial prothrombin time (APTT) can be performed on the first tube. Am J Clin Path 1997; 107:681-683.

NCCLS Document H21-A4. Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays; approved guideline, 4th ed. Wayne, PA: National Committee for Clinical Laboratory Standards; 2003.

Instructions for Removal of BD Hemogard™ Closure

1. Grasp the BD Vacutainer® Tube with one hand, placing the thumb under the BD Hemogard™ Closure. (For added stability, place arm on solid surface.) With the other hand, twist the BD Hemogard™ Closure while simultaneously pushing up with the thumb of the other hand ONLY UNTIL THE TUBE STOPPER IS LOOSENED.
2. Move thumb away before lifting closure. DO NOT use thumb to push closure off tube. ***Caution:*** *Any glass tube has the potential to crack or break. If the tube contains blood, an exposure hazard exists.* To help prevent injury during closure removal, it is important that the thumb used to push upward on the closure be removed from contact with the tube as soon as the BD Hemogard™ Closure is loosened.
3. Lift closure off tube. In the unlikely event of the plastic shield separating from the rubber stopper, DO NOT REASSEMBLE CLOSURE. Carefully remove rubber stopper from tube.

Instructions for Reinsertion of BD Hemogard™ Closure



1. Replace closure over tube.
2. Twist and push down firmly until stopper is fully reseated. Complete reinsertion of the stopper is necessary for the closure to remain securely on the tube during handling.

Symbol Key:

08/04
BD1285

CENETRON
CENTRAL LABORATORIES

SHIPPING INSTRUCTIONS

SHIPPING SAMPLES TO CENETRON

Specimen containers as well as instructions for specimen collection and shipment are provided to each site. Federal regulations [49 CFR 100-185] mandate that sites transporting or shipping hazardous materials be subject to and comply with all provisions of the Federal Hazardous Materials Transportation Law. Additional information concerning these regulations is available from the Department of Transportation by contacting the DOT hotline at 800-467-4922.

The International Air Transport Association (IATA) produces a manual based on the International Civil Aviation Organization (ICAO) Technical Instructions, which outlines the procedures that must be followed to ensure safe transport. Training is an essential part of the process. It is necessary for all individuals involved in the preparation or transport of dangerous goods to be properly trained and tested initially with follow-up training every 24 months. As per the regulations, each site is responsible for ensuring that their staff is properly trained.

GENERAL SHIPPING INSTRUCTIONS (United States & Canadian Sites)

Frozen specimens should only be shipped Monday through Wednesday.

1. VERIFY DELIVERY:

If directed by Cenetron to ship specimens on Friday, check the box for Saturday Delivery on the air waybill and place a Saturday Delivery sticker on the box. If this is not filled out, the package will be held over until Monday and the specimens may thaw, compromising the integrity of the results. Please DO NOT collect or ship specimens on a business day prior to a scheduled holiday to ensure delivery of frozen specimens.

2. VERIFY SPECIMAN LABELS:

Prior to placing the specimens in the shipping containers, verify that all tubes have been properly identified with the following information: Patient's Screening and/or Patient Number, Date and Time of Specimen Collection.

3. VERIFY FORMS:

Verify that the specimens match those listed on the Laboratory Requisition Form.

4. VERIFY SHIPPING INFO:

Be sure to inspect the shipping boxes for accurate labeling information.

5. DRY ICE:

Place a layer of dry ice at the bottom of the frozen shipper.

6. CHECK TRANSPORT BAG:

Ensure that the absorbent sheet is in the transport bag and place cryovials into the transport bag. Place the lab requisition into the outer pouch of the transport bag. It is acceptable to ship specimens from more than one patient together in the transport bag.

7. SEAL TRANSPORT BAG:

Seal the transport bag and place it into the bottom of the shipping box.

8. FILL STYROFOAM BOX:

Fill the remainder of the box with dry ice (minimum of 5 lbs or ~2 kg). Place the Styrofoam cover on the inner Styrofoam container.

9. CLOSE FLAPS:

Close box flaps and seal the exterior box with tape. DO NOT TAPE THE STYROFOAM BOX.

10. COMPLETE SECTIONS:

Complete the necessary sections on the pre-printed FedEx Air Waybill.

11. DATE FORMS:

Date all forms where necessary. Retain the Sender's Copy for your files.

12. PLACE BILL:

Place completed air waybill into the clear plastic air bill pouch face up.

PETAL Studies Packaging & Shipping Tips

Before You Ship

- **Review** the shipping instructions noted in the relevant study lab manual. Lab Manuals are located on each of the Study Pages of the PETAL website under the "Specimen Management" tab.
- **Order** shipping materials in advance! Allow **3-5 days** to receive shipping materials after ordering. Except in rare circumstances there should **not** be a need to order these "overnight". We have a fixed budget for shipping costs; please plan ahead when ordering supplies.
- **Shipper size:**
  - Large shippers hold approximately 200 samples + dry ice
  - Medium shippers hold approximately 100 samples + dry ice
  - Note the size of shipper(s) needed on your order
- **Payment** for sample shipments is covered by the CCC and the shipping form with the account information is included with the shippers.

Packaging Your Shipment

- **Package** samples by **SUBJECT**: Using the provided biohazard bags, put all vials for a subject into one bag along with their lab requisition form. Vials should ideally be separated out by visit day (if possible) within a subject bag.
- **Schedule** shipments for Monday-Wednesday ONLY. Avoid shipping on Thursday or Friday.
- Place a **minimum of 8lbs (~4kg)** of dry ice in the shipper. In summer months additional dry ice may be needed.
- **Air Bill**:
  - Complete the necessary sections on the pre-printed FedEx Air Waybill.
  - Date all forms where necessary and retain the “Sender’s Copy” for your files.
  - Place completed air waybill into the clear plastic air bill pouch face up.

Ship Schedule

- **SHIP** every 6 months: Plasma, Urine, DNA, & RNA (all PETAL studies)
- **SHIP** monthly: VIOLET 25OHD samples
- **DO NOT Ship** samples from VIOLET subjects that are not randomized to the trial. These should be destroyed on site and documented on the VIOLET Screened Sample Destruction Log. See VIOLET PROCESS DOCUMENT: Sample Destruction & Documentation for further info.