



Biospecimen Exchange for Neurological Disorders

Risk and Resilience, Clinical presentation, and Biomarker Profiles of Chronic Traumatic Encephalopathy and Related Dementias: The DIAGNOSE CTE Research Project II (DxCTE-II)

BIOSPECIMEN COLLECTION & PROCESSING

Overview

1. Specimen uniformity and quality
2. Site Equipment
3. Procedures
 - Kit Contents and Ordering
 - Sample Labelling
 - Sample Collection and Processing
 - Shipping Samples
 - Non-Conformance
4. Contact Information

Specimen Uniformity and Quality

GENERAL REMINDERS

Specimen Standardization and Quality

Most biomarkers are sensitive to *time* and *temperature*

- Standardization of processing across sites is key
- Specimens must be processed within 2 hours of collection
- Reference the *BioSEND Biomarker Specimen Collection, Processing, and Shipment Manual* as needed
- Do not replace or supplement any kit components without first receiving approval from BioSEND/NINDS

Questions? Email biosend@iu.edu

Site Consumables and Equipment

Sites will need to supply the following items:

- Gloves
- Alcohol wipes
- Butterfly needles
- Tourniquet
- Gauze pads
- Bandages
- Microcentrifuge tube rack
- Sharps bin and lid
- Crushed ice
- Pipettes and pipette tips
- Centrifuge capable of maintaining 4°C
- -80°C Freezer
- Dry ice

Procedures

MAINTAINING SPECIMEN UNIFORMITY AND QUALITY

Biospecimen Collection Protocol

| Collection Tube | Drawn At | Specimen Type | Aliquot Volume | Total Number of Aliquots | Cryovial Cap Color | Shipping Temperature |
|---|----------|---------------|----------------|--------------------------|--------------------|----------------------|
| 4 EDTA (plastic) Blood Collection Tubes, 10ml | BL | Plasma | 1.5ml | 12 | Purple | Frozen |
| | BL | Buffy Coat | ~750ul | 4 | Clear | Frozen |

Kit Contents and Ordering

- All sites will be sent a Supplemental Kit with their first kit shipment
 - Contains extra blood collection tubes and processing supplies
 - May be used to replace items in study visit kits
- Study Visit Kits should be ordered as soon as visits are planned
 - Contains collection, processing, and shipping supplies specific to each visit
 - Includes barcoded labels
 - The supplies/labels in each study visit kit are intended for that visit only

Kit Contents and Ordering – REDCap Survey

<https://redcap.link/dxctellkits>

Order kits online through the Kit Request Module for:

- Blood kits
- Shipping kits
- Extra Supplies

Please provide as much notice as possible when ordering kits and/or supplies.

BioSEND

Biospecimen Exchange for Neurological Disorders

NINDS Biosend DxCTE-II Kit Request System

Blood supplies have expiration dates determined by the manufacturer. *Please limit requests to kits you will use within 2-3 months.*

Please provide your preferred email address to receive notifications about this request.

Study Site

* must provide value

Submit

AAA
⊕ ⊞

Kit Contents and Ordering: Confirm Site Info

DxCTE-II Kit Request Module

| | | |
|---|--|---|
| Study Site <small>* must provide value</small> | Indiana University | Select your site from the drop-down list |
| Indiana University School of Medicine Carolyn Dunifon Dept. of Medical & Molecular Genetics 351 West 10th Street, TK-318 Indianapolis, IN 46202-3002 (317) 274-5751 cdunifon@iu.edu | | Verify contact information and update if needed |
| Is the contact name above correct? <small>* must provide value</small> | <input type="radio"/> Yes <input checked="" type="radio"/> No | reset |
| New Contact Name <small>* must provide value</small> | Claire Wegel | |
| Is the shipping address above correct? <small>* must provide value</small> | <input type="radio"/> Yes <input type="radio"/> No | reset |
| Is the e-mail address above correct? <small>* must provide value</small> | <input type="radio"/> Yes <input type="radio"/> No | reset |
| Is the phone number above correct? <small>* must provide value</small> | <input type="radio"/> Yes <input type="radio"/> No | reset |

Kit Contents and Ordering: Kit Types

DxCTE-II Kit Request Module

- Kits are not specific to a subject or time point. After collection, sites will indicate the subject and time point to which BioSEND should link the samples.
- Standard collection kit for plasma and buffy coat contains supplies for one subject-visit.
- Shipping Kits are ordered independently of Blood Kits
- A single Shipping Kit may be used to send samples for 1-2 cryoboxes

| | |
|--|---|
| Kit Type **Please allow two weeks for shipment** <i>* must provide value</i> | <input checked="" type="radio"/> Blood Collection Kit <input type="radio"/> Shipping Kit <input type="radio"/> Extra Supplies |
| Blood Collection Kit Quantity <i>* must provide value</i> | <input type="text" value="3"/> |
| Comments | <input type="text"/> <small>Expand</small> |

Please specify in comments if you need kits before the standard two week shipment time.

Kit Contents and Ordering: Kit Breakdown

DxCTE-II Kit Request Module

Each Blood Collection Kit contains:

- 4 - EDTA tube, 10ml (plastic)
- 12 - Cryovial (Sarstedt®) with purple cap, 2ml
- 4 - Cryovial (Sarstedt®) with clear cap, 2ml
- 2 - Disposable pipette, 3ml
- 4 - Resealable tube pouch
- 1 - Cryobox, 25 slot
- 1 - Specimen/Case label set-- Kit numbers

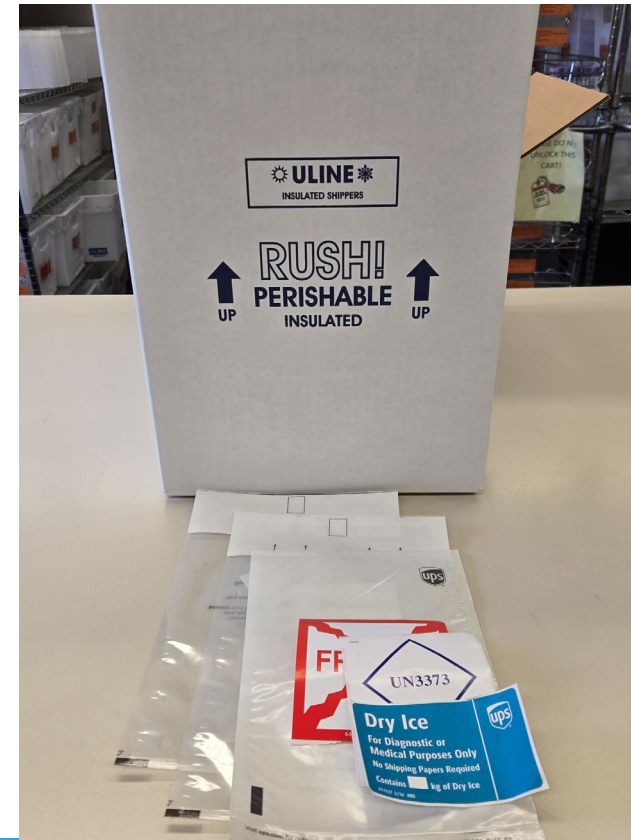
Kit contents of selected kit will appear at the bottom of the page

Kit Contents and Ordering: Kits

Blood Kit (frozen):



Shipping Kit:



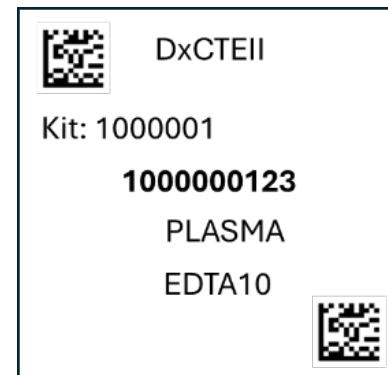
Kit and Supply Ordering

- Click “Submit” to send order to BioSEND; staff will confirm receipt of your order
- Please allow two week turn-around time for kit shipments
- If urgent request needed, please note date needed by in comments and email BioSEND. We cannot guarantee urgent orders, but we will do our best to accommodate.
- BioSEND will send confirmation of shipment and tracking number when supplies ship

Sample Labelling: Example Labels

Labels are provided by Indiana University

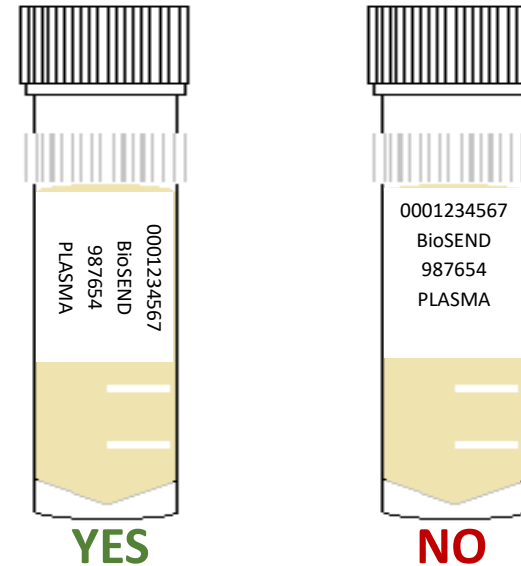
- Please check that all samples are properly labelled to ensure correct identification by IU
- If do not have enough labels to complete a visit, please contact IU *immediately*
- Labelling the tubes during processing prevents sample mix-ups



Sample Labelling: Label Placement

Please...

- Label all collection and aliquot tubes before cooling, collecting, processing, or freezing samples
- Label only 1 subject's tubes at a time to avoid mix-ups
- Wrap the label around the tube horizontally - label position is important for all tube types
- Make sure the label is completely adhered by rolling between your fingers



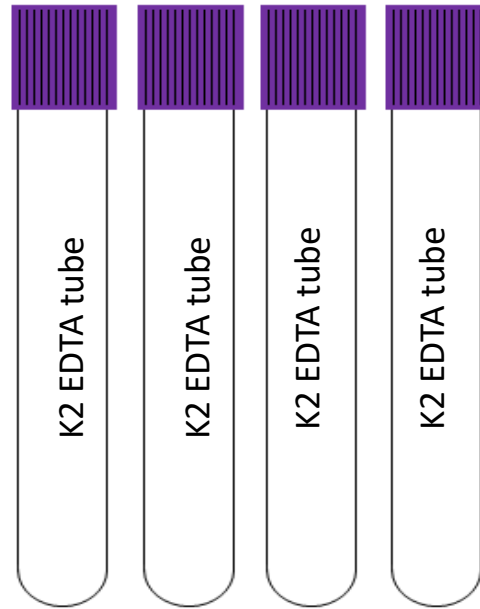
Collection Volumes

Total blood volumes

| Sample Type | Amount |
|---------------------------------------|--------|
| Whole Blood for Plasma and Buffy Coat | 40 ml |

Sample Collection and Processing

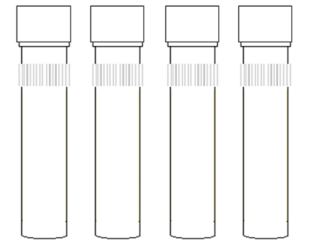
Supplies provided for the collection of plasma and buffy coat



4 x 10ml EDTA
(plastic) collection
tubes

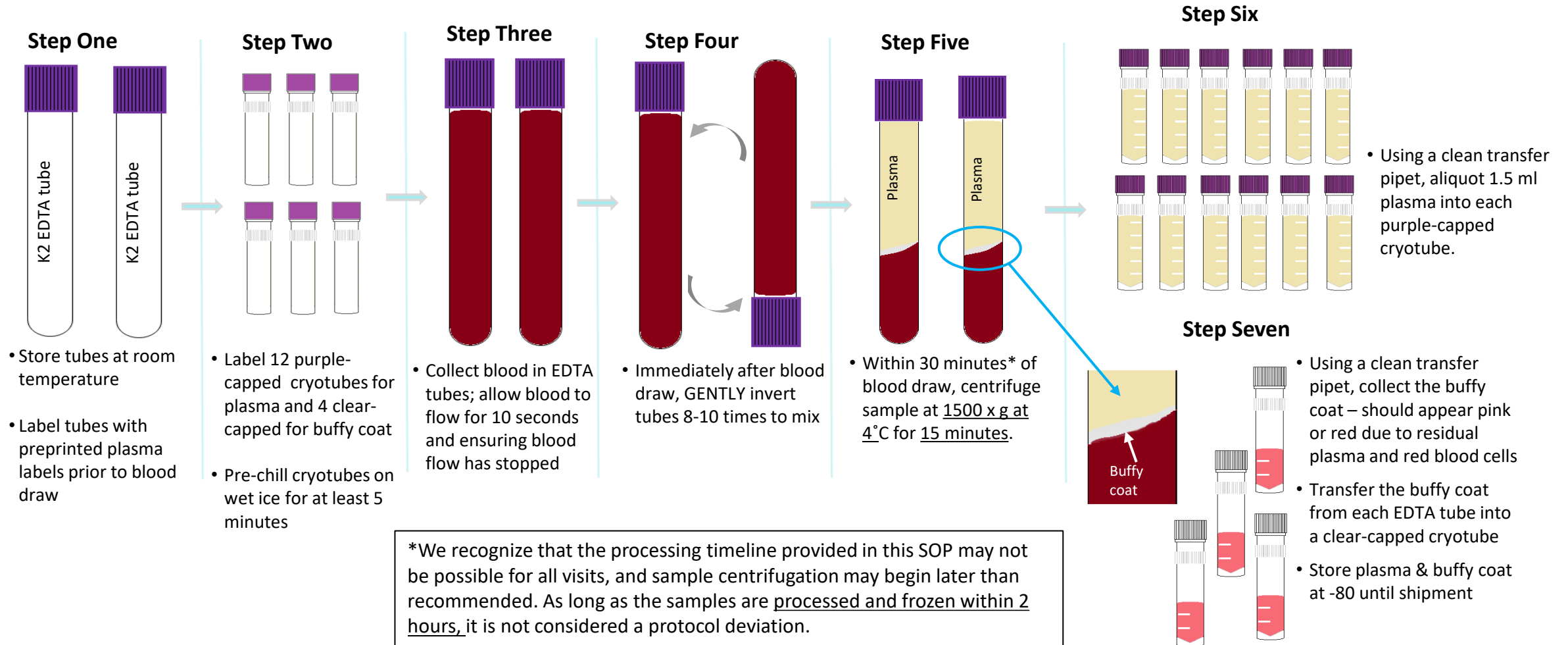


12 x 2ml Cryovials
(purple-capped) for
plasma aliquots



4 x 2ml Cryovials
(clear-capped) for
buffy coat aliquots

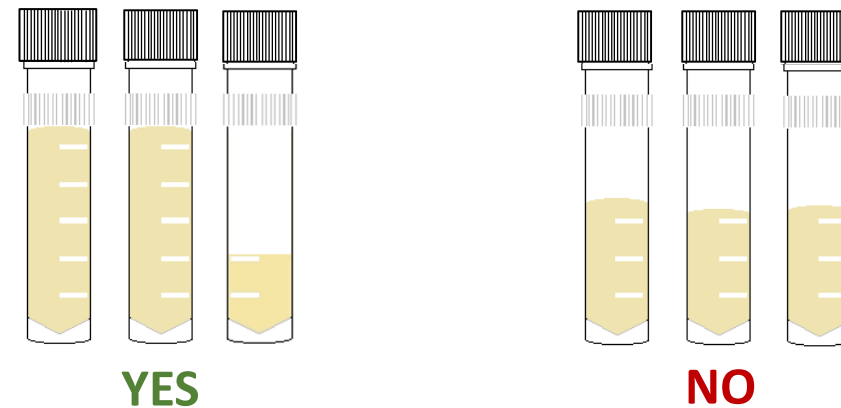
Sample Collection and Processing: Plasma & Buffy Coat



Sample Collection and Processing: Aliquots

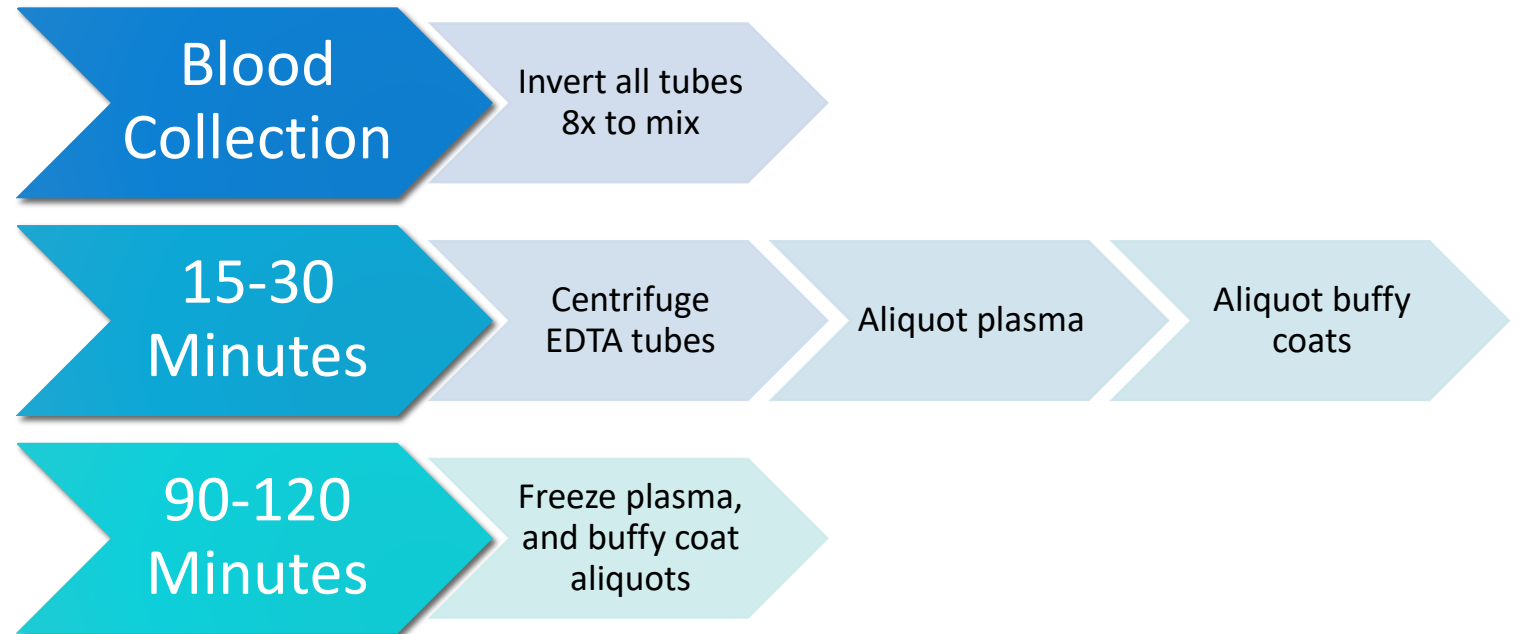
Filling biomarker plasma aliquots:

- Fill as many cryovials as possible to 1.5 ml (plasma)
- Over-filled vials may burst in freezer!
- Ship ALL material to IU, even if final vial is less than standard volume



Sample Collection and Processing: Timeline

Timeline for blood processing



Sample Collection and Processing: Issue #1

Troubleshooting Blood Collection

Issue #1: Tube with little/no vacuum

- Always check expiration date on the tube before beginning blood draw and discard expired tubes
- Store tubes at “room temperature” – extreme temperature can affect vacuum
- Keep extra vacutainer tubes from supplemental kit nearby during blood draw to replace “bad” tubes
- If this is a frequent occurrence, report tube type and lot number to IU.

Sample Collection and Processing: Issue #2

Troubleshooting Blood Collection

Issue #2: Hemolyzed plasma caused by incorrect collection

| Cause: Blood Collection Methods | Corrective Action |
|---|--|
| Improper venipuncture site | Draw from median cubital, basilic, and cephalic veins from antecubital region of arm |
| Prolonged tourniquet use | Tourniquet should be released after no more than 1 min, excessive fist clenching should be avoided |
| Not allowing alcohol to dry on skin before venipuncture | Without touching, allow the venipuncture site to air dry |
| Use of too large/small bore needle resulting in excess force applied to blood | Avoid using too small/large needle. Needle size dependent on the subject's physical characteristics & amount of blood to be drawn. Most commonly used sizes are 19 – 23. |
| Pulling/pushing plunger too fast while drawing/transferring blood | Avoid drawing the syringe plunger too forcefully when collecting blood |
| Ensure all blood collection assemblies are fitted securely, to avoid frothing | |

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing: Issue #2 continued

Troubleshooting Blood Collection

Issue #2: Hemolyzed plasma caused by incorrect processing

| Cause: Sample Processing Methods | Corrective Actions |
|---|---|
| Vigorous mixing/shaking | Gently invert blood collection tube when mixing additive with specimen, follow guidelines in Biologics Manual regarding number of times to invert each type of tube |
| Not allowing serum to clot for recommended time | Serum tubes without clot activator should be allowed to clot for 60 min in a vertical position |
| Exposure to excessive heat or cold | Keep samples at ambient temperature until processing |
| Prolonged contact of serum/plasma with cells | Do not store uncentrifuged samples beyond recommended time |

For more information, visit: http://www.bd.com/vacutainer/pdfs/techtalk/TechTalk_Jan2004_VS7167.pdf

Sample Collection and Processing Form

Specimen Collection and Processing Form

Direct Link for Duke:

<https://redcap.link/DXCTEIISampleForm>

First page captures basic subject and visit information

BioSEND

Returning?

AAA



Biospecimen Exchange for Neurological Disorders

Protocol: Diagnose Chronic Traumatic Encephalopathy Phase II (DxCTE-II)

Please complete the Specimen Collection and Processing Form, below.

Page 1 of 2

Study

DxCTE-II ▼

Study Site

▼

Email address of staff member completing this form

Note: A copy of the completed sample form and the shipping manifest will be sent to this address.

Study ID:

Sex (used for DNA quality control)

▼

Visit

▼

IU Kit Number

Next Page >>

Save & Return Later

Sample Collection and Processing Form

Second page captures processing information

Blood Collection and Processing

Date of venipuncture blood collection  Today M.D.Y

Time of venipuncture blood collection  Now HM
Use 24 Hour clock

2. PLASMA and BUFFY COAT (Purple-top EDTA tubes, 10 mL)

Was blood collected and processed for PLASMA EDTA?

Yes

No

reset

Time of PLASMA EDTA tube centrifugation  Now HM
Use 24 Hour clock

Duration of PLASMA EDTA tube centrifugation
minutes

Rate of PLASMA EDTA tube centrifugation
x g

Temperature of PLASMA EDTA tube centrifugation
degrees Celsius

Total volume of PLASMA EDTA collected
mL

Number of PLASMA EDTA aliquots created for BioSEND
Each aliquot should be 1.5 mL

Number of BUFFY COAT aliquots created for BioSEND

Date PLASMA EDTA and BUFFY COAT were placed in freezer  Today M.D.Y

Time PLASMA EDTA and BUFFY COAT were placed in freezer  Now HM
Use 24 Hour clock.

PLASMA EDTA and BUFFY COAT storage temperature
degrees Celsius

PLASMA EDTA notes

Expand

Sample Collection and Processing Form

PDF form of responses will be emailed to you. Print a copy of the Frozen Shipping Manifest and include with shipment.

DxCTE-II Frozen Shipping Manifest

Please verify/update the information below. When you click the "Submit" button below, a PDF copy of the Frozen Shipping Manifest will be emailed to you for Subject [subj_id].

Please print a copy of that document and include it in the Kit #[kit_num] shipping container.

Study _____

Study Site: _____

Study ID: _____

Visit: _____

IU Kit Number: _____

Date of blood collection: _____

PLASMA EDTA

Number of PLASMA EDTA aliquots shipped: _____

Number of BUFFY COAT aliquots shipped: _____

Shipping Information - Please complete.

Frozen shipments should be sent Monday-Wednesday only. Please check for holiday closures prior to shipping. Contact us at biosend@iu.edu if you are unsure whether or not it is safe to ship.

Date of shipment: _____

Did/will you use the IU UPS interface to generate the shipping label?

- Yes
 No

Which shipping service did you use?

- UPS
 FedEx
 World Courier
 Other

What is the shipment tracking number? _____

Sample Collection and Processing Form

Submission of the Shipping Manifest portion of the form serves as shipment notification to BioSEND

- Must be completed prior to shipment
- If samples are shipped and not received, BioSEND will follow-up with courier. It is recommended that sites also track shipment to ensure safe delivery

Shipping Frozen Samples: Tips

Packing and Shipping Frozen Samples

- All DxCTE-II samples ship frozen
- Ship frozen samples on dry ice
- Frozen samples should be shipped **only** Monday through Wednesday
- Always fill carton to **top** with dry ice
- Do not pack shipment until the day of pickup



Class 9 Dry Ice Label should not be covered with other stickers and must be completed, or UPS will reject/return your package!

Shipping Samples

Packing and Shipping Frozen Samples

Shipper's Declaration not Required.

Dry Ice amount must be in kilograms.

Note: 2 lbs. = 1 kg.

Airwaybills / airmails must have the following:
1. Dry Ice; 9; UN 1845
2. $\frac{\text{Number}}{\text{(Number pkgs)}} \times \frac{\text{wt}}{\text{(wt)}} \text{ Kg}$

Net weight of dry ice in kg

Dry Ice kg.

Your name & address

Shipper's Name and Address

UN 1845

Consignee Name and Address

IU information and address

9

06426 1/01 RRD

The form is a diamond-shaped label with a dashed border. It contains text instructions, a calculation formula, and several input fields. Red arrows point from external text to specific fields: 'Net weight of dry ice in kg' points to the 'kg.' field; 'Your name & address' points to the 'Shipper's Name and Address' field; 'IU information and address' points to the 'Consignee Name and Address' field. The number '9' is printed in the center of the diamond, and 'UN 1845' is printed on the right side. A small reference number '06426 1/01 RRD' is at the bottom left.

Shipping Samples: Frozen

Do not ship more than 4 biohazard bags in a single shipper (equivalent to four subject-visits).

- Allows room for dry ice to keep samples frozen in transit
- Minimizes loss in the rare but unfortunate event of courier issues



Shipping Samples – UPS: <https://kits.iu.edu/UPS>

The screenshot shows the shipping interface for the Indiana University School of Medicine. The header features the IU logo and the text "INDIANA UNIVERSITY SCHOOL OF MEDICINE". Below the header, the text "and Affiliated Biorepositories" is displayed. The interface is divided into two main sections: "Ship From" and "Shipment Information".

Ship From Section:

- Search for address: A search bar with a magnifying glass icon and a "Clear" button.
- Code: A text input field.
- Company: Icahn School of Medicine - Mt. Sinai
- Contact: Kenny Persaud
- Address 1: 1425 Madison (Icahn Building)
- Address 2: Icahn L4 39
- Address 3: (Empty)
- City: New York
- State/Province: NY
- Postal Code: 10029
- Country/Territory: United States

Shipment Information Section:

- Study Group: SSBC (dropdown menu)
- Weight: 20 (input field) LB (dropdown menu)
- Dry Ice Weight: 10 (input field) LB (dropdown menu)
- Description of Return: Biologic Specimens
- Pickup Request: A blue button.

Annotations:

- "Search for address": A dashed box around the search bar with a blue arrow pointing to it.
- "Choose Study": A dashed box around the "Study Group" dropdown with a blue arrow pointing to it.
- "Enter weight": A dashed box around the "Weight" and "Dry Ice Weight" input fields with a blue arrow pointing to them.
- "Schedule Pickup": A dashed box around the "Pickup Request" button with a blue arrow pointing to it.
- "Click 'Ship'": A dashed box around the "Ship" button at the bottom right with a blue arrow pointing to it.

Buttons:

- Clear (blue button)
- Pickup Request (blue button)
- Reset (blue button)
- Ship (blue button)

Shipping Samples via UPS

IU UPS ShipExec Shipping Portal

- Print out UPS air waybill
- Ensure all elements (barcode, return address, etc.) printed clearly
- Fold and insert UPS air waybill into clear plastic sleeve on package

JOHN SMITH
INDIANA UNIVERSITY
410 WEST 10TH STREET
INDIANAPOLIS IN 46202

2 LBS

1 OF 1

RS

SHIP TO:

SCHOOL OF MEDICINE
317-278-2694
INDIANA UNIVERSITY
TK 217
351 W 10TH ST
INDIANAPOLIS IN 46202



IN 461 9-01



UPS NEXT DAY AIR

1

TRACKING #: 1Z 976 R8W 84 3985 8595



BILLING: P/P
DESC: Biological Specimens
RETURN SERVICE

Reference No. 1: 4087277

XOL 20.03.09 NV45 83.0A 12/2019



Shipping Samples: Closures

| Date | Holiday |
|--------------------------------------|-----------------------------|
| January 1 | New Year's Day |
| 3 rd Monday in January | Martin Luther King, Jr Day |
| 4 th Monday in May | Memorial Day |
| June 19 | Juneteenth (observed) |
| July 4 | Independence Day (observed) |
| 1 st Monday in September | Labor Day |
| 4 th Thursday in November | Thanksgiving |
| 4 th Friday in November | Friday after Thanksgiving |
| December 25 | Christmas |

Please also consider weather when shipping. UPS will post service updates on their webpage. Reach out to BioSEND if you are unsure if it is safe to ship.

Non-Conformance

Non-conformance to standard procedures may reduce the utility of the biospecimens:

- Not processing plasma within 2 hours of collection allows for breakdown of certain proteins and small molecules
- Over/under centrifuging changes plasma composition



Non-Conformance Reporting con't

Most common non-conformance issues:

- Shipment notification not sent
- Samples shipped for weekend/holiday delivery
- Sample form incomplete/inaccurate
- Low volume
- Unlabeled or mislabeled tube(s)
- Sample hemolysis



Non-Conformance and Inventory Reporting

Most common non-conformance issues:

- BioSEND will notify sites directly of any issues upon receipt
- BioSEND will email sites a monthly inventory report of all samples received from that site to date
- ***If you are experiencing issues, please reach out to us for help! It is much easier to prevent an issue before sample collection & shipment than trying to fix it after the fact***

BioSEND.org

On the website, you can:

- Access your study's kit request module and sample submission form
- Download the most recent version of the Manual of Procedures
- View a recording of this training
- Find information about holiday closures
- Access shipping resources

Study Resources

KIT REQUEST MODULE

Please follow the below link to access the Kit Request Module. This link will direct you to a REDCap database where study coordinators and staff may request kits, individual supplies, and/or labels. Please allow a total of two weeks for kit requests to be fulfilled.

[Kit Request System](#) →

SPECIMEN COLLECTION AND PROCESSING FORM

Please use the below link to access the collection and processing form for this protocol. This form must be completed prior to shipment. We also ask that all shipments include a physical copy of the shipping manifest portion of the form.

[Specimen Collection and Processing Form](#) →

MANUAL OF PROCEDURES

The below downloadable manual was created specifically for the DxCTEII study. Please feel free to explore the manual through the hyperlinked 'Table of Contents'. Questions concerning any part of the manual may be directed to biosend@iu.edu for further clarification.

[Manual of Procedures](#) ↓

TRAINING SLIDES

These slides correspond to the BioSEND DxCTEII protocol training. Training is available upon request by contacting biosend@iu.edu.

[Training Slides](#) ↓

SAMPLE SHIPPING

BioSEND can receive samples Monday-Friday, excluding holidays. Frozen samples should be shipped M-W. Ambient samples may be shipped on Th.

[Generate UPS airbill or schedule pickup](#) →

[Check holiday closures](#) →

[What do I do for Friday blood draws](#) →

Contacts

Indiana University

General Questions/Shipment Notifications:

biosend@iu.edu

317-278-6158

Request kits:

<https://redcap.link/dxctellkits>