

National Institute of Neurological Disorders and Stroke
Biorepository:

BioSpecimen Exchange for Neurological Disorders, BioSEND

**Biospecimen Collection, Processing, and Shipment Manual for
Longitudinal Imaging Biomarkers of Disease Progression in
DLB Study**

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1.0 PURPOSE

The purpose of this manual is to provide collection site staff (PIs, study coordinators, and the sample collection and processing teams) at various study sites with instructions for collection and submission of biological samples. It includes instructions for biospecimen submission to the BioSpecimen Exchange for Neurological Disorders (BioSEND) located at Indiana University.

This manual includes instructions for the collection, processing, aliquoting and shipping of the following samples:

- Serum
- PAXgene™ (for RNA extraction)
- Plasma
- Whole Blood (for DNA extraction)
- CSF

These procedures are relevant to all study personnel responsible for processing blood specimens to be submitted to BioSEND.

2.0 ABBREVIATIONS

BioSEND	BioSpecimen Exchange for Neurological Disorders
CSF	Cerebrospinal Fluid
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
DLB	Dementia with Lewy Body

3.0 BioSEND INFORMATION

3.1 BioSEND Contacts

Tatiana Foroud, PhD, Principal Investigator

Phone: 317-274-2218

Email: tforoud@iu.edu**Scott Kaiser, Project Manager**

Phone: 317-278-0594

Email: sckaiser@iu.edu**General BioSEND Contact Information**

Fax: 317-278-1100

Email: biosend@iu.eduWebsite: www.BioSEND.org**Sample Shipment Mailing Address**

BioSEND

Indiana University School of Medicine

980 W. Walnut Street , R3 C102

Indianapolis, IN 46202-5188

3.2 Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped Monday- Wednesday only. Ambient samples must be shipped Monday-Thursday only.

For packaging and shipment details, please refer to Appendix K (Frozen Shipping Instructions) and Appendix L (Ambient Shipping Instructions).

Check the weather reports and the FedEx.com website to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples. FedEx often reports anticipated weather delays on their website.

3.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday deliveries will not be accepted.**

3.4 Holiday Observations

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
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 Day

Please note that between December 24th and January 2nd (or the first business day after New Year's Day) Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University between December 24th and January 2nd. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 24th by e-mailing biosend@iu.edu, so that arrangements can be made to have staff available to process incoming samples. Frozen specimens collected during this period should be held at your site to ship after the first business day in January.

Please see https://www.biosend.org/holiday_closures.html for additional information.

4.0 BIOSEND SAMPLE REQUIREMENTS

NINDS approves each study for a specific biospecimen collection protocol. Studies and study sites should make every effort to meet their approved biospecimen collection requirements. The expected number of samples from each site that should be returned to BioSEND are listed in [sections 4.1-4.2](#).

If a sample is not obtained at a particular visit, this should be recorded in the notes section of the **Sample Record and Shipment Notification Form (see Appendix I)**. This form is submitted with your sample shipment to BioSEND.

4.1 Protocol Schedule for Biospecimen Submission to BioSEND - DLB

Visit (month)	BL	6M	12M	24M	36M	48M
Ambient Whole Blood sample for DNA 6ml EDTA	1					
Frozen Whole Blood, 6ml EDTA	1	1	1	1	1	1
Whole blood for RNA (PAXGene™ tube, 2.5ml)	2	2	2	2	2	2
Plasma aliquots, 1ml	6	6	6	6	6	6
Serum aliquots, 1ml	6	6	6	6	6	6
CSF aliquots, 1ml	10		10	10	10	10

5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS AND SUPPLIES

Research specimen collection kits as well as clinical lab supplies (except dry ice and equipment listed in Section 5.7) will be provided by BioSEND. These materials include blood tubes, LP trays (when applicable), boxes for plasma/serum/CSF aliquots, as well as partially completed shipping labels to send materials to BioSEND. Barcoded kit labels, collection tube labels, and aliquot tube labels will all be provided by BioSEND. For sites collecting CSF, labels will also be included for the CSF aliquots to be returned to BioSEND. Collection tube labels and aliquot tube labels will be pre-printed with study information specific to the type of sample being drawn. BioSEND will provide a sufficient number of labels only for those specimens that are to be shipped back to the BioSEND repository (See the Protocol Schedule for Biospecimen Submission to BioSEND for your site in [Sections 4.1-4.2](#)); any tubes that will remain at the collection site should be labeled accordingly. Ensure that all tubes are properly labeled during processing and at the time of shipment according to [Section 6.2](#).

5.1 Kit Supply to Study Sites

Only the baseline kit will need to be ordered by the site. From that point forward, all kits will be sent automatically based on a 60-day study-visit window. All subject visits must be scheduled within 30 days (i.e. one month before or after) of the timeframe of the next scheduled visit. For example, if a subject is seen at baseline on April 1, the 12-month visit must occur between March 1 and May 1 of the following calendar year.

Each individual site will be responsible for ordering the baseline kit from BioSEND. We advise sites to proactively confirm kits are on hand ahead of study visits. If a longitudinal visit is scheduled to occur before the start of the 60-day window, **a kit order must be placed in the kit request module.**

Within the kit request module, there is a drop down menu to request kits based on the Principal Investigator at that site. Kits and individual items can be ordered as required through the kit request module.

The link to the kit request module is shown below:

- DLB: <http://kits.iu.edu/biosend/dlb>

Please allow **TWO weeks** for kit orders to be processed and delivered.

5.2 Specimen Collection Kit General Contents

Collection kits contain the following (for each subject) as designated per your protocol and/or NINDS resource development agreement. Kits provide the necessary supplies to collect samples from a given subject. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NINDS/BioSEND Study team to do so. *Please store all kits at room temperature until use.* Note that “supplemental” kits will be provided should you require additional supplies from those contained in the visit specific kits. See the next page for LP Kit contents.

BioSEND Supplies

Available upon request from the online kit request module ([Section 5.1](#))

General Items
Ambient shipping kits
25 cell cryobox
Cryovial tube (2 ml) with clear cap
FedEx return airbill
Shipping container for dry ice shipment (shipping and Styrofoam box)
Plastic biohazard bag
Warning label packet
CSF Items
Needle - Spinal Needle Introducer 20G, 0.90 x 32mm
Needle - Whitacre Needle 24G, 0.55 x 90mm
2 Individually Packaged Sterile 50 ml Conical Tube
Conical centrifuge tubes (15 ml)
Lumbar puncture tray (Sprotte 24G or 22G) (see Lumbar Puncture Tray Components)
Blood Collection Items
PAXgene™ tube (2.5 ml)
Lavender-top EDTA blood collection tube (10 ml)
Purple-top EDTA blood collection tube (6 ml)
Serum (red top) blood collection tube (10 ml)
Blue-top 15mL conical tubes for Homogenize Serum and Plasma

We realize there may be instances where additional supplies are needed; therefore, one supplemental kit will be provided with the initial kit shipment for new studies. Replacement supplemental kits can be requested on the kit request website. In addition, individual supplies can be requested as well.

Quantity	Lumbar Puncture Tray Components
1	Sprotte needle, 24G x 90mm OR Sprotte needle, 22G x 90mm
1	Introducer needle, 1 mm x 30 mm
1	Hypodermic needle, 22G x 1.5"
1	Plastic syringe, (3 ml, luer lock) with 25G x 5/8" needle attached
4	Polypropylene syringe (6 ml, luer lock)
1	Needle stick pad
1	Adhesive bandage
1	Drape, fenestrated, 2 tabs, paper, 18" x 26"
2	Towel, 13.5" x 18"
6	Gauze pad, 2" x 2"
3	Sponge stick applicator
1	Lidocaine 1%, 5 ml
1	Povidone-Iodine Topical Solution, 0.75 oz

5.3 Specimen Collection Kit Contents - DLB

Specimen Collection Supplies	PAXgene (2.5ml)	EDTA (10mL)	Serum (10ml)	EDTA (6mL)	Cryovial (2ml)	LP Tray (24 or 22 gauge)	Conical Tube (15ml)	Conical Tube (50ml)	Conical Tube (50ml, sterile)	Ambient Shipping Kit	Frozen Shipping Kit
Baseline visits	2	2	2	2	24	1	4	1	1	1	1
Annual visits	2	2	2	1	24	1	4	1	1		1
6M visit		2	2	1	13						1

5.4 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquets
- Alcohol Prep Pads
- Gauze Pads
- Bandages
- Butterfly needles and hubs
- Microcentrifuge tube rack
- Test tube rack
- Sharps bin and lid
- Wet ice bucket (for CSF only)
- Wet ice (for CSF only)

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- Centrifuge capable of ≥ 1500 rcf (1500 x g) with refrigeration to 4°C
- -80°C Freezer

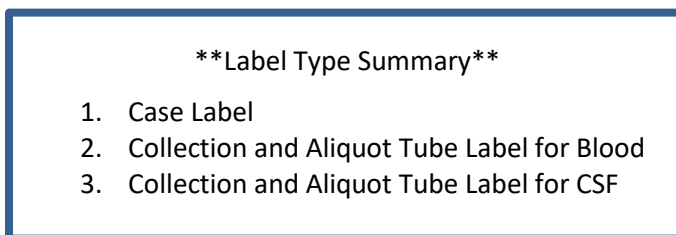
In order to ship specimens, you must provide:

- Dry ice (approximately 30-40 pounds per shipment)

6.0 SPECIMEN LABELS

Labels must be affixed on all collection and aliquot tubes to ensure unique specimen identity. BioSEND provides labels for all samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site. **If labels are provided but the sample is not collected, please return the unused labels to BioSEND when the specimens are shipped to BioSEND for permanent storage.**

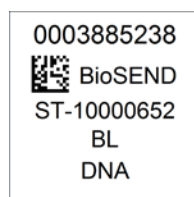
6.1 Types of Labels



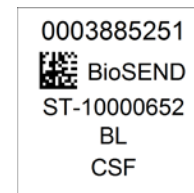
Each kit contains all labels required for the return of biospecimens to BioSEND.



The **Case Labels** do not indicate a specimen type, but are affixed on BioSEND forms and on specific packing materials. See Appendices I-L for further instructions.



The **Collection and Aliquot Tube Labels for Blood** are placed on all blood collection and aliquot tubes. See [Appendices A-F](#) for further instructions.

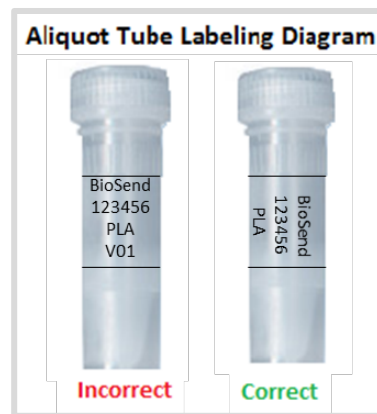


The **Collection and Aliquot Tube Labels for CSF** are placed on all CSF collection and aliquot tubes. See [Appendix G](#) for further instructions.

6.2 Affixing Labels

In order to ensure the label adheres properly and remains on the tube, follow these instructions:

- Place blood collection and aliquot labels on **ALL** collection and aliquot tubes **BEFORE** sample collection, sample processing, or freezing. This will help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- The blood collection and aliquot tube labels contain a 2D barcode on the left hand side of the label. When turned horizontally, the barcode should be closer to the top (cap end) of the tube.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram).



- Take a moment to ensure the label is **completely affixed** to each tube. It may be helpful to roll the tube between your fingers after applying the label.

7.0 SPECIMEN COLLECTION AND PROCESSING PROCEDURES

Consistency in sample collection and processing is essential for biomarker studies. All samples are drawn in the same order and then processed in a uniform fashion. **Please read the instructions before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.**

7.1 Order of Specimen Collection

Blood collection should be performed in the following order:

1. Serum (red top) blood collection for serum
2. PAXgene tube for RNA
3. EDTA (purple top) blood collection for DNA (only at BL)
4. EDTA (lavender top) blood collection for plasma
5. EDTA (purple top) blood collection for Whole Blood (Frozen)

7.2 Blood Collection Protocols

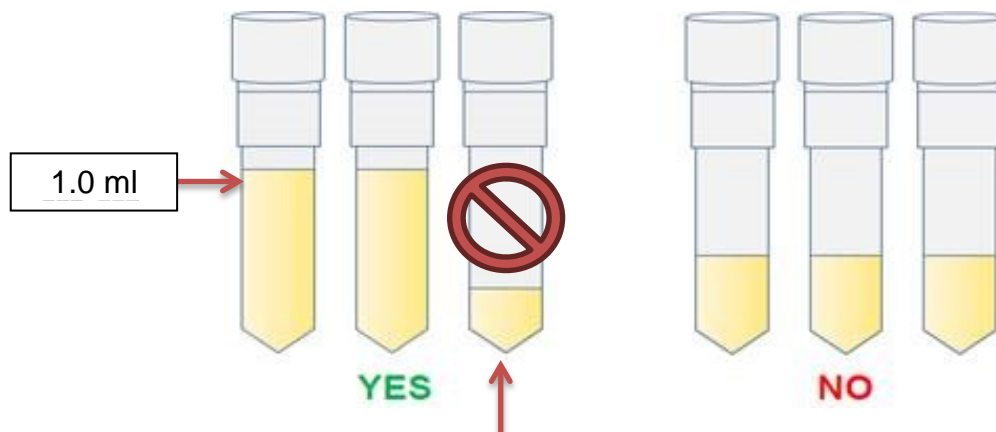
1. Serum (red top) blood collection for serum (**Appendix F**)
2. PAXgene tube for RNA (**Appendix A**)
3. EDTA (purple top) blood collection for DNA (**Appendix E**)
4. EDTA (lavender top) blood collection for plasma (**Appendix B**)
5. EDTA (purple top) blood collection for FROZEN Whole Blood (**Appendix D**)

7.3 Lumbar Puncture Protocol

1. Cerebrospinal Fluid Collection (**Appendix G**)

7.4 Filling Aliquot Tubes (Plasma, Serum, and CSF)

In order to ensure that BioSEND receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample. Each site is supplied with sufficient collection tubes to provide the specimen volume described in the Protocol Schedules for Biospecimen Submission ([see Section 4](#)). Specimens collected in addition to those described in Section 4 are collected at the site's discretion and are not returned to BioSEND.



Please note: It is critical for the integrity of future studies using these samples that study staff **not submit** residual aliquot tubes (anything under 1.0 ml) to BioSEND.

8.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biospecimen shipping. If not available at your University, training and certification is available through the CITI training site (Course titled “Shipping and Transport of Regulated Biological Materials” at <https://www.citiprogram.org/>).

8.1 Sample Record and Shipment Notification Form and CSF Processing Form

All sample shipments to BioSEND must include the BioSEND Blood and/or CSF Processing Form(s). The completed forms are:

- Emailed to BioSEND@iu.edu at the time the samples are being shipped
- And the original document should be Included in the shipment with the samples

8.2 Shipping Instructions

There are two types of shipments (ambient and frozen) that are used to send samples back to BioSEND.

Ambient (room temperature) Shipment (only at baseline visit). Reference Appendix L for ambient shipping instructions.

- Blood sample for DNA (EDTA tube, 6 ml)

Frozen Shipment (baseline and follow-up). Reference Appendix K for frozen shipping instructions.

- Frozen PAXgene Tubes
- Frozen EDTA 6ml Whole Blood
- Frozen 1 ml aliquots of plasma
- Frozen 1 ml aliquots of serum
- Frozen 1 ml aliquots of CSF

*****Important Note*****

Include samples for only one subject per shipping container.

Ambient shipments (purple top EDTA tube) must be received at BioSEND within five days of collection. Up to three purple top EDTA tubes can be shipped in one ambient shipping container.

For frozen shipments, include no more than two packing envelopes per shipping container in order to have room for a sufficient amount of dry ice to keep samples frozen up to 24 hours.

8.3 Shipping Address

All samples are shipped to the BioSEND laboratory:

BioSEND
Indiana University School of Medicine
980 W. Walnut Street, R3 C102
Indianapolis, IN 46202-5188

9.0 Data Queries and Reconciliation

Appendix I must be completed the day that samples are collected to capture information related to sample collection and processing. This form includes information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

The NINDS DMR data collection team will be collaborating with BioSEND to reconcile information captured in the database compared to samples received and logged at BioSEND. Information that appears incorrect in the NINDS DMR database will be queried through the standard system. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication. If applicable, a non-conformance report will be provided to sites.

Data discrepancies with samples shipped and received at BioSEND may result from:

- Missing samples
- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Discrepant information documented on the BioSEND Blood and/or CSF Processing Forms compared to information entered into the NINDS DMR database.
- Samples frozen and stored longer than three months at the site

10.0 APPENDICES

- Appendix A: Whole Blood Collection for Isolation of RNA
- Appendix B: Whole Blood Collection for Isolation of Plasma
- Appendix D: Whole Blood Collection for banking
- Appendix E: Whole Blood Collection for Isolation of DNA
- Appendix F: Whole Blood Collection for Isolation of Serum
- Appendix G: Cerebrospinal Fluid Collection
- Appendix I: Sample Record and Shipment Notification Form
- Appendix K: Frozen Shipping Instructions
- Appendix L: Ambient Shipping Instructions
- Appendix O: Low Fat Diet Menu Suggestions