

National Institute of Neurological Disorders and Stroke  
Biorepository:

**BioSpecimen Exchange for Neurological Disorders, BioSEND**

**Biospecimen Collection, Processing, and Shipment Manual for**

Reducing Disparities in Dementia and VCID Outcomes in a  
Multicultural Rural Population (DVRP)

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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 and shipping of the following samples:

- Whole Blood (for DNA extraction)
- Saliva (for DNA extraction)

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

## **2.0 ABBREVIATIONS**

BioSEND	BioSpecimen Exchange for Neurological Disorders
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association

## 3.0 BioSEND INFORMATION

### 3.1 BioSEND Contacts

**Tatiana Foroud, PhD, Principal Investigator**

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**Claire Wegel, Project Manager**

Phone: 317-278-6158

Email: [cwegel@iu.edu](mailto:cwegel@iu.edu)

**General BioSEND Contact Information**

Fax: 317-278-1100

Email: [biosend@iu.edu](mailto:biosend@iu.edu)

Website: [www.BioSEND.org](http://www.BioSEND.org)

**Sample Shipment Mailing Address**

BioSEND

Indiana University School of Medicine

351 W. 10<sup>th</sup> Street. TK-217

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.

**Ambient samples must be shipped Monday- Thursday only.**

For packaging and shipment details, please refer to Appendix L (Ambient Shipping Instructions for Blood) and Appendix R (Bulk Saliva Shipping Instructions).

Check the weather reports and the UPS.com website to make sure impending weather events (blizzards, hurricanes, etc.) will not impact the shipping or delivery of the samples. UPS® 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 <sup>rd</sup> Monday in January	Martin Luther King, Jr Day
4 <sup>th</sup> Monday in May	Memorial Day
July 4	Independence Day (observed)
1 <sup>st</sup> Monday in September	Labor Day
4 <sup>th</sup> Thursday in November	Thanksgiving
4 <sup>th</sup> Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Please note that between December 24<sup>th</sup> and January 2<sup>nd</sup> (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 2<sup>nd</sup>. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University between December 24<sup>th</sup> and January 2<sup>nd</sup>.

Please see [https://www.biosend.org/holiday\\_closures.html](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 [section 4.1](#).

#### 4.1 Protocol Schedule for Biospecimen Submission to BioSEND: DVRP

Subjects will have DNA collected at one (baseline) time point. Saliva or whole blood will be collected, but not both. Saliva collection is an option for those participants completing a remote visit.

Visit	BL
Saliva for DNA, 4ml	1
OR	
Whole blood for DNA, 6ml	1



## 5.0 SPECIMEN COLLECTION KITS, SHIPPING KITS, AND SUPPLIES

Research specimen collection kits as well as clinical lab supplies (except equipment listed in Section 5.4) will be provided by BioSEND. These materials include blood or saliva tubes, shipping boxes, as well as partially completed shipping labels to send materials to BioSEND. Barcoded kit labels and collection tube labels will all be provided by BioSEND. Collection 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 Section 4.1); any tubes that will remain at the collection site or sent to other repositories should be labeled accordingly. Ensure that all tubes are properly labeled prior to collection and at the time of shipment according to [Section 6.2](#).

### 5.1 Kit Supply to Study Sites

Each individual site will be responsible for ordering kits from BioSEND. We advise sites to proactively confirm kits are on hand ahead of study visits.

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

The link to the kit request module is shown below:

- DVRP: <http://kits.iu.edu/biosend/dvrp>

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. In addition, individual supplies can be requested as well.

### **BioSEND Supplies**

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

<b>General Items</b>
Ambient shipping container
ClinPak
UPS® return waybill
Plastic biohazard bag with absorbent sheet
Shipping labels (Exempt Human Specimen and UN3373 label)
Shipping envelopes (to send saliva kits to participants)
<b>Specimen Collection Items</b>
Purple-top EDTA blood collection tube (plastic, 6 ml)
Oragene® DNA Saliva Collection Kit (OGR-500)

### 5.3 DVRP Kit Contents

DVRP Blood Collection Kit	
Supply	Quantity
EDTA (plastic) tube, 6ml	1
Biohazard bag w/ absorbent sheet	1
Gel Pack	1
UN3373 label	1
IATA List of Contents sheet	1
Waybill	1
ClinPak	1
Ambient shipper	1
Label set (case and specimen labels)	1

DVRP Saliva Collection Kit	
Supply	Quantity
Oragene® DNA Saliva Collection Kit (OGR-500)	1
Biohazard bag w/ absorbent sheet	1
10x13 Envelope	1
Small bubble mailer	1
Label set (case and specimen labels)	1
USPS shipping label	1

DVRP Bulk Saliva Shipping Kit	
Supply	Quantity
Cardboard shipping box, 10x10x10	1
Waybill	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
- Sharps bin and lid

## 6.0 SPECIMEN LABELS

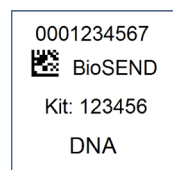
Labels must be affixed on all collection tubes to ensure unique specimen identity. BioSEND provides labels for samples being collected and returned to BioSEND. The site is responsible for providing labels for biospecimens that will be retained at the site.

### 6.1 Types of Labels

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



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

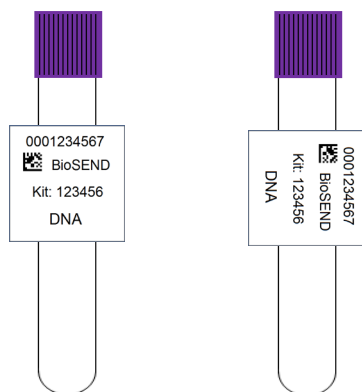


The **Specimen Labels** are placed on all blood and saliva collection tubes.

## 6.2 Affixing Labels

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

- Place blood collection labels on collection tube **BEFORE** sample collection. This will help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- The blood collection 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); see below.



**Incorrect**

**Correct**

- Take a moment to ensure the label is **completely affixed** to tube before draw. 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 should be drawn and handled 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 Collection Tubes for BioSEND

1. EDTA (purple top, 6ml) x 1 for DNA
- OR**
2. Oragene® DNA Saliva Collection Kit (OGR-500) x 1

### 7.2 Specimen Collection Protocols

1. EDTA (purple top) blood collection for DNA (**Appendix E**)
2. Saliva (Oragene) collection for DNA (**Appendix Q**)

## **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**

All sample shipments to BioSEND must include the BioSEND Sample Record and Shipment Notification Form. The completed forms are:

- Emailed to [BioSEND@iu.edu](mailto:BioSEND@iu.edu) at the time the samples are being shipped
- A copy of the Sample Record and Shipment Notification form should be Included in the shipment with the samples

### **8.2 Shipping Instructions**

Reference Appendix L for ambient shipping instructions for whole blood.

Reference Appendix Q for ambient shipping instructions for saliva.



### **8.3 Shipping Address**

All samples are shipped to the BioSEND laboratory:

BioSEND  
Indiana University School of Medicine  
351 W. 10<sup>th</sup> Street. TK-217  
Indianapolis, IN 46202-5188

## **9.0 Data Queries and Reconciliation**

BioSEND will collaborate with the University of Miami to reconcile information captured in the REDCap database compared to samples received and logged at BioSEND. Additional discrepancies that may be unrelated to data entry will be resolved with the Principal Investigator in a separate follow up communication.

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

- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled or mislabeled samples
- Discrepant information reported in the clinical database compared to information on sample form or shipping manifest

## **10.0 APPENDICES**

Appendix E: Whole Blood Collection for DNA

Appendix L: Ambient Shipping Instructions

Appendix O: Low Fat Diet Menu Suggestions

Appendix R: Bulk Saliva Shipping Instructions

## Appendix E – Whole Blood Collection for Isolation of DNA (No Processing)

One 6 ml Purple-Top EDTA Tube is provided by BioSEND for the collection of Whole Blood from which DNA will be extracted. This tube should be shipped to BioSEND at AMBIENT temperature on the day it is drawn; no processing required).



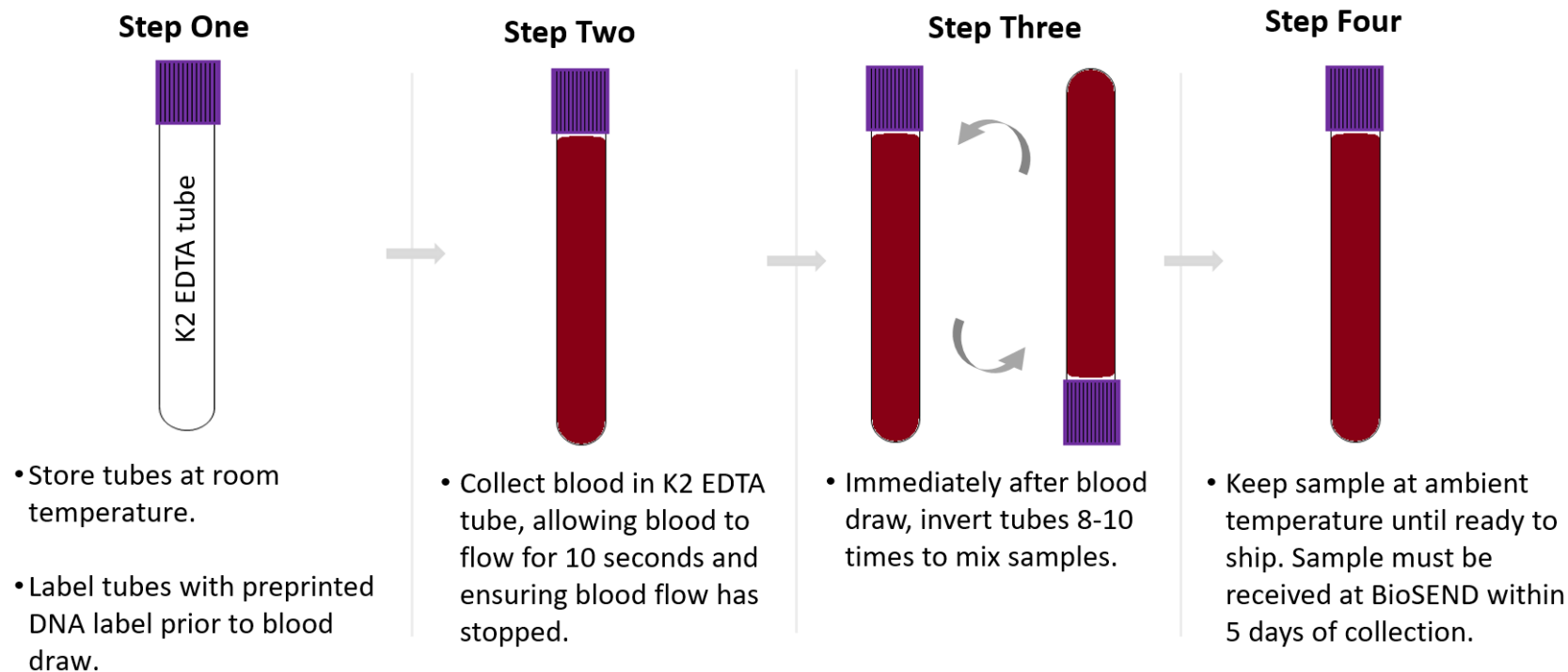
1. **CRITICAL STEP:** Store empty Whole Blood EDTA tubes at room temperature, 64°F - 77°F (18°C to 25°C) before use.
2. Place pre-printed Collection and Aliquot “DNA” label on the 6 ml EDTA tube prior to blood draw.
3. Using a blood collection set and a holder, collect whole blood into the 6 ml purple top whole blood tube using your institution’s recommended procedure for standard venipuncture technique.

0001234567  
 BioSEND  
 Kit: 123456  
 DNA

**The following techniques shall be used to prevent possible backflow:**

- a. Place donor's arm in a downward position.
  - b. Hold tube in a vertical position, below the donor’s arm during blood collection.
  - c. Release tourniquet as soon as blood starts to flow into tube.
  - d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.
4. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.
  5. Complete the **Sample Record and Shipment Notification form for blood (See Appendix L).**
  6. Ship the whole blood tube for DNA extraction to BioSEND according to **Appendix L – Ambient Shipping Instructions.**

## DNA Preparation – 6 ml K2 EDTA (Purple Top) Tube



## Appendix L – Ambient Shipping Instructions for Blood

### **IMPORTANT!**

**AMBIENT SAMPLES MUST BE SHIPPED MONDAY THROUGH THURSDAY ONLY.**

**If a blood draw occurs on a Friday, please wait until the following Monday to ship. Ambient blood for DNA must be received at BioSEND within 5 days of collection.**

**Please be aware of holidays and inclement weather, and plan your shipments accordingly.**

Ambient whole blood tube shipments are Category B UN3373 and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest edition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

### **IATA Packing and Labeling Guidelines**

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging (plastic canister or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovials or blood collection tubes) and the secondary packaging. The absorbent material should be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible persons (shipper and recipient)
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373

**BioSEND Packaging and Shipment Instructions – Ambient Shipments**

1. Place refrigerant pack in the freezer 24 hours before shipment.
2. Contact UPS® to confirm service is available and schedule package to be picked up.
3. **Record the UPS® tracking number (found at the top of the UPS® airbill) onto the DVRP Blood Sample Form (below).**
4. Make a copy of the DVRP Blood Sample Form.
5. Place filled and labeled EDTA tube in the biohazard bag with the absorbent material.
6. Remove as much air as possible from the biohazard bag, and seal the bag according to the directions on the bag. Place Case Label on outside of biohazard bag.



7. Place the specimen into the Styrofoam cooler, and then place the refrigerant pack on top of the specimen.
8. Place a copy of the Sample Record and Shipment Notification form and a completed IATA List of Contents sheet (provided in kit) on top of the cooler and close the cardboard box. Please do NOT tape the cardboard box closed.
9. Close shipping box and place within a UPS® Clinical Pak. **Seal the UPS® Clinical Pak.**
10. Affix prefilled UPS® return airbill to the sealed UPS® Clinical Pak.
11. Specimens should be sent to the address below via **UPS® Priority Overnight**. Ambient shipments of blood for DNA should be sent Monday through Thursday.

BioSEND  
IU School of Medicine  
351 W. 10<sup>th</sup> Street, TK-217  
Indianapolis, IN 46202

12. **Notify BioSEND by email ([biosend@iu.edu](mailto:biosend@iu.edu)) that a shipment has been sent and attach the completed DVRP sample form (below) to your email. Do not ship until you've contacted and notified BioSEND staff about the shipment in advance.**

13. Use UPS® tracking to ensure the delivery occurs as scheduled and is received by BioSEND.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by BioSEND for each sample type. Investigators and clinical coordinators for each project are responsible for ensuring that the requested amounts of each fluid are collected to the best of their ability.



## ***DVRP Blood Sample Form***

Please complete this form and return it to Indiana University with the corresponding blood sample. Ambient samples should be shipped **Monday – Thursdays only**. Please notify BioSEND ahead of shipment by emailing a copy of this form to [biosend@iu.edu](mailto:biosend@iu.edu).

To: Claire Wegel		Fax: 317-278-1100		Phone: 317-278-6158	
Email: <a href="mailto:biosend@iu.edu">biosend@iu.edu</a>		Tracking #: _____			
From: _____		Site: _____			
Phone: _____		Fax: _____			
Email: _____		Date: _____			
Please fill in the fields below for each sample in the shipment.					
Subject ID	Sex	Kit Number	Date Collected	Date of Visit (if different)	Notes

Notes:

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If you have any questions, please contact us at 1-317-278-6158 or [biosend@iu.edu](mailto:biosend@iu.edu).

## Appendix O – Low Fat Diet Menu Suggestions

### Foods to avoid prior to blood collection:

**Avoid:** *All fats and nuts such as:*

- Butter
- Cream
- Bacon fat
- Lard
- All oils
- All margarine
- All nuts
- Peanut butter
- Coconut
- Whole seeds such as pumpkin and sunflower

**Avoid:** *All milk and dairy products such as:*

- All whole milk products
- All cheese
- All products containing cheese
- Sour cream
- All ice cream
- Milk chocolate

**Avoid:** *High fat prepared foods and foods naturally high in fat:*

All red meats or meats containing fat  
such as pork and:

- Fatty meats such as:
  - Luncheon meats
  - Organ meats
  - Bacon
- Fatty fish such as:
  - Salmon
  - Mackerel

• Salad dressing and mayonnaise	• Buttered, au gratin, creamed, or fried vegetables
• Fried foods	• Gravies and sauces
• Fried snacks such as: <ul style="list-style-type: none"> <li>➤ Chips</li> <li>➤ Crackers</li> <li>➤ French Fries</li> </ul>	• Baked goods and frosting

# Appendix Q - Saliva Collection For DNA

Do not eat, drink, smoke, or chew gum for 30 minutes prior to giving your sample.

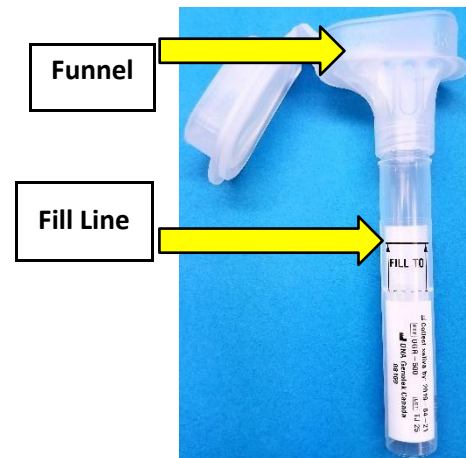
Note: Do not rinse your mouth prior to giving your sample. Most people take between 2 and 5 minutes to deliver a saliva sample following steps 1 through 7 below. Before spitting, relax and rub your cheeks gently for 30 seconds to help create saliva.

To review a video of the saliva collection procedure, please visit:

<http://www.dnagenotek.com/ROW/support/ciOG500.html>

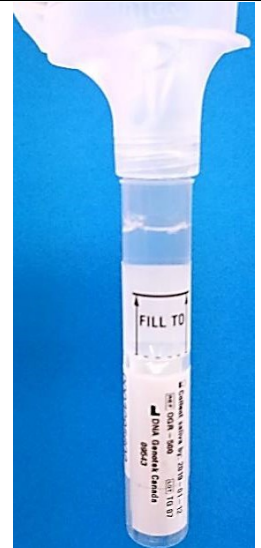
## STEP 1

- Do NOT remove the plastic film from the lid of the container.
- Spit directly into the funnel at the top of the tube until the amount of liquid saliva (not including bubbles) reaches the fill line shown in picture #1.
- **The saliva tube has a false bottom, so you will only need to provide 2 milliliters (less than ½ teaspoon) of saliva to reach the fill line.**
- Do NOT fill above the line.



## STEP 2

- Once the saliva level reaches the fill line, hold the tube upright with one hand.
- Close the lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click.
- The liquid in the lid will be released into the tube to mix with the saliva. Make sure that the lid is closed tightly.



## STEP 3

- Hold the tube upright.
- Unscrew the funnel from the tube.
- Pick up the small cap for the tube.
- Use the small cap to close the tube tightly.



# DVRP Saliva Collection Instructions

## STEP 4

- Shake the capped tube for 5 seconds.
- Discard or recycle the funnel.
- Place sample in provided specimen bag for shipment back to the University of Miami.



## STEP 5

- Peel off blue plastic liner at the top of the specimen bag to expose the adhesive.
- Seal bag by pressing down across the top of the bag.



## STEP 6

- Put your specimen (in the provided specimen bag) into this shipping envelope.



## STEP 7

- Peel off the white paper at the top of the envelope to expose the adhesive, fold this flap down, and press firmly to seal envelope.
- Send the envelope via U.S. Mail as soon as possible after sample collection. The envelope may be placed in your mailbox with any outgoing mail or mailed through any USPS drop box or post office location.



**Questions? Please contact a study coordinator via email [mxr2310@med.miami.edu](mailto:mxr2310@med.miami.edu).**

**Intended Use:** This product is designed for the safe collection of human saliva samples.

**Contents:** The funnel lid contains 2 mL of Oragene•DNA liquid. The solution should be clear and colorless.

**Warnings:** Do not ingest the Oragene•DNA liquid. Wash with water if the Oragene•DNA liquid comes in contact with eyes or skin. Small Cap, choking hazard.

**Storage:** Store at room temperature 15-30°C (59-86°F).

## Appendix R – Bulk Saliva Shipping Instructions

### **IMPORTANT!**

**AMBIENT SAMPLES MUST BE SHIPPED MONDAY THROUGH THURSDAY ONLY!**

**Please be aware of holidays and inclement weather, and plan your shipments accordingly.**

Saliva specimens being shipped to the BioSEND Biorepository should be considered as Exempt Human Specimens and as such must be packaged and compliant with IATA Packing Instructions. *See the Latest Edition of the IATA Regulations for complete documentation.*

### **IATA Packing and Labeling Guidelines**

- The primary receptacle (saliva tube) must be leak proof and in total must not contain more than 1 liter of fluid.
- The secondary packaging (biohazard bag) must be leak proof and, if multiple saliva tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent saliva tubes.
- Absorbent material must be placed between the primary receptacle (within the biohazard bag) and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens included in shipment must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - Sender's name and address
  - Recipient's name and address
  - Responsible Person
  - The words "Exempt Human Specimen"

## BioSEND Packaging and Shipment Instructions – Bulk Saliva Shipments

1. Contact UPS® to confirm service is available and schedule package to be picked up.
  - Please ship samples to:  
BioSEND  
IU School of Medicine  
351 W. 10th Street, TK-217  
Indianapolis, IN 46202
2. Notify BioSEND of shipment by emailing: [biosend@iu.edu](mailto:biosend@iu.edu) In your email, please include:
  - DVRP Saliva Sample Form (see following page) for all specimens included in shipment
  - The tracking number of the shipment
3. Ensure all labeled saliva samples are appropriately packaged as described below:
  - a. Verify that the appropriate BioSEND case label is adhered to each bubble mailer and that no PHI is written on the mailer.
  - b. Record the subject information and assigned kit numbers on the Saliva Sample form.
  - c. Place a corresponding kit label on each bubble mailer.
  - d. Place the bubble mailers into the provided 10x10x10 return box with a physical copy of the completed Saliva Sample Form.
  - e. Seal the shipping container and apply the provided UPS® return label.

In addition to tracking and reconciliation of samples, the condition and amount of samples received are tracked by BioSEND for each sample type. Investigators and clinical coordinators for each project are responsible for ensuring that the requested amounts of each fluid are collected to the best of their ability.

## ***DVRP Saliva Sample Form***

Please complete this form and return it to Indiana University with the corresponding saliva sample. Ambient samples should be shipped **Monday – Thursdays only**. Please notify BioSEND ahead of shipment by emailing a copy of this form to [biosend@iu.edu](mailto:biosend@iu.edu).

Email: <a href="mailto:biosend@iu.edu">biosend@iu.edu</a>		Tracking #: _____			
From: _____		Site: _____			
Phone: _____		Fax: _____			
Email: _____		Date: _____			
Please fill in the fields below for each sample in the shipment.					
Subject ID	Sex	Kit Number	Date Collected	Date of Visit (if different)	Notes

Notes: \_\_\_\_\_

If you have any questions, please contact us at 1-317-278-6158 or [biosend@iu.edu](mailto:biosend@iu.edu).