
# COPDGene™ Study
# Johns Hopkins Biological Repository
# Biologics Manual

*Version 3.1*

Revised:
June 2008

## Signature Page

**Instructions:** The original version of this manual must be reviewed by all applicable staff members. Staff members must sign the signature sheet after reviewing version 3.1 of the manual. All subsequent revisions and amendments must also be reviewed by applicable staff members and signed and dated accordingly.

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## Section 1. Amendments and Revisions

Note, this version dated, June 2008, shall be considered version 3.1. Any subsequent amendments or revisions to the subsections will be added where appropriate and noted in section 1. Staff members must sign the signature sheet after reviewing version 3.1 of the manual. All subsequent revisions and amendments must also be reviewed by applicable staff members and signed and dated accordingly.

## Section 2. Purpose of COPDGene™ JHU LAB Manual

This manual serves to identify those involved with the COPDGene™ JHU LAB and to clarify standard operating procedures for work on this study. These procedures should be adhered to by all staff members and monitored by quality assurance and quality control activities.

## Section 3. Contact Information

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Elizabeth Regan, MD, PhD

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Frank Sciurba, MD
Carl R. Fuhrman, MD
Denise Filippino RN, BSN, MEd
Joel Weissfeld, MD MPH

***UTHSC at San Antonio***
Antonio Anzueto, MD
Sandra Adams, MD
Paul McCartor
Timothy J. Houlihan, RN
C. Santiago Restrepo, MD

# Emergency contact information

| Site | Contact Person | Office/Lab Phone Number | Cell Phone Number | Pager Number | Pager Number |
|---|---|---|---|---|---|
| | | | | | |
| Baylor | Evelyn Flores | 713-798-2681 | 281-513-5110 | 713-768-2157 | |
| Houston VA | Dorothy Williams | 713-794-7668 | | | |
| Columbia | Adina Lemeshow-Study coordinator | 212-305-9821 | don't know yet | none | none |
| Duke | Kim Hamilton | 919 684 9428 | | 919 970 2157 | |
| HealthPartners, MN | Natalie Woodruff | 952-967-5493 | 612-919-8044 | N/A | N/A |
| Johns Hopkins | Teresa Concordia | 410-550-2449 | | 410-283-5728 | 410-283-8909 |
| | | | | | |
| | | | | | |
| | | | | | |
| National Jewish | Christina Schnell | 303-398-1772 | 720-218-2881 | 303-851-0164 | NA |
| | | | | | |
| Iowa | Angie Delsing | 319-356-1810 | | 2152 | 2152 |
| | | | | | |
| University of MN | Cheryl Stibbe | 612-625-1435 | 612-810-5755 | 612-899-6249 | 612-899-7439 |
| | | | | | |

# Section 4. Biological Specimen Handling

## Section 4a. Labeling and identification

**Purpose:** To ensure all collected samples are labeled accurately and adequately.

**By Whom:** COPDGene™ Site/phlebotomist

**Procedure:**

The COPDGene™ site will use the COPDGene™ information system, as provided by the Data Coordinating Center (DCC), to print bar code labels that will be used to label all specimen collection containers, documents, etc. Example of the label designed by the DCC is below.


These labels will be printed on Avery 5267 8.5 x 11 paper labels, 4 across and 20 down, using a standard office printer. The items labeled with this label will not require a cryogenic, thermal transfer label at the Clinical Centers. Cryogenic, thermal transfer labels will be printed and used at the JHU LAB Lab.

These labels will be used for all blood tubes and specimen transmittal documents for the lab. They can be used for other documentation.

## Section 4b. Blood Draws/Phlebotomy and Storage at Clinical Sites

**Purpose:** To ensure that venipunctures are performed following standard safety guidelines, to ensure that blood samples are collected in the correct order and in accordance with study protocols, and to ensure that samples are stored appropriately at the Clinical Center.

**By Whom:** COPDGene™ Clinical Center/Phlebotomist/nurse/coordinator

**Procedure:**

The following table summarizes draw order and volume for the collection of blood samples.

| Draw Order | Tube Type | Tube Amount |
|---|---|---|
| 1 | EDTA purple top | 10 ml |
| 2 | EDTA purple top | 10 ml |
| 3 | EDTA purple top | 10 ml |
| 4 | SST red-grey top | 10 ml |
| | Total | 40 ml |

Immediately after collecting the blood samples, ensure the barcode labels are on each tube and correspond to the correct study subject

After collecting the blood samples, the SST tube should be left to rest for 30 minutes. Then centrifuge it at 2500 RPM or 1000 to 1300 g for 15 minutes. The centrifuge needs to be properly balanced before operating.

Samples should be refrigerated as soon as possible after they are drawn; samples MUST be refrigerated within 4 hours. All samples should be placed in a sample storage refrigerator. The refrigerator should be kept at +2 to +8 degrees C.

A transmittal form (Appendix 9A) must be completed by the phlebotomist/nurse/coordinator while collecting the blood samples. The form must be shipped with the samples to the JHU LAB lab. The sites should retain a carbon copy of the transmittal form before shipping. A 2-part (original and yellow carbonless copy) will be sent to each site by JHU LAB.

# Section 5. Sample Distribution/Shipping

## Section 5a. IATA Shipping Guidelines

**See appendix** 9D. Complete shipping information is provided as a resource for study personnel and includes other methods beyond the routine COPDGene™ shipping procedures.

## Section 5b. Shipping from COPDGene™ sites to JHU LAB

**Purpose:** To ensure samples are correctly packaged and shipped according to specimen needs, study protocol, and IATA guidelines.

**By Whom:** COPDGene™ Site/Study Coordinators

**General Procedure:**

Coordinators and others involved with shipping samples must be familiar with IATA guidelines outlined in Section 5a.

**Note:** Samples should be shipped as soon as possible. For the most efficient use of resources, samples from three subjects should be placed in one shipping container whenever possible. **Samples should not be stored at the clinical site for more than four days prior to shipping**; to meet this requirement, samples from only a single subject may need to be packed in a single shipping container. Specimens may only be shipped Monday through Thursday to ensure arrival at the JHU LAB on a weekday. Avoid sending shipments that will be expected to arrive at JHU LAB on holidays.

**SHIP TO:**
**Stacey Meyerer**
**Johns Hopkins University**
**Bloomberg School of Public Health**
**615 North Wolfe Street**
**Room W6618**
**Baltimore, MD 21205**
**410-955-7203**

**Detailed procedure:**

1. Refer to the appendix 9D for detailed instructions on assembling and packaging the shippers.

2. Place the specimen transmittal forms for each subject on the top of foam on the inside of the shipping box and seal the box.

3. Complete the preprinted FedEx airway bill with DATE, PRIORITY OVERNIGHT, OTHER PACKAGING and list the number of boxes you will be shipping.

4. Place the airway bill on the box, inside the clear plastic sleeve and call for a FedEx pick up.

5. Enter the shipment tracking number on the COPDGene™ web site, which will generate a shipment notification that will be sent to the lab.

6. Specimens should be shipped on Monday – Thursday only. Notices will be sent via email to coordinators one month prior to holidays to indicate any changes to lab and shipping schedules.

7. Specimens should be shipped when you accumulate 3 subjects. **However, no sites should hold onto samples for more than 4 days.**

8. Once shipments are received in the lab, the received date will be entered on the COPDGene™ web site by the lab staff. This will confirm and resolve the shipment. Problems or delays with the shipments will be handled on a case by case basis with clinical sites as well as logged on the COPDGene™ website.

9. The JHU LAB will return the empty boxes to each site so they can be used for future shipments.

## Section 6.Overview of Processing and Storage at the JHU LAB

| Sample | Label Names | Aliquot # | Aliquot volume | Freezing Temp. |
|---|---|---|---|---|
| Red-gray SST Tubes | Serum | 1 | 1.5 ml | -80º |
| | | 2 | 1.5 ml | |
| | | 3 | 1.5 ml | |
| Purple-top Tube #1 (JHU Lab) | Plasma | 1 | 1.5 ml | -80º |
| | | 2 | 1.5 ml | |
| | | 3 | 1.5 ml | |
| | Buffy Coat | 1 | 0.5 ml | |
| | | 2 | 0.5 ml | |
| Purple-top Tube #2 (Scott Lab) | Whole blood | 1 | 10 ml | Scott lab for DNA extraction |
| Extracted DNA from Scott Lab | DNA | 1 | 0.5 ml (50 ng/ul) | -80º |
| | | 2 | 0.5 ml (50 ng/ul) | |
| | | 3 | DRV | |
| | | 4 | DRV | |
| Purple-top Tube #3 (RBC Lyse) | Plasma | 4 | 1.5 ml | -80º |
| | | 5 | 1.5 ml | |
| | | 6 | 1.5 ml | |
| | WBC | 1 | 0.6 ml | |
| | | 2 | 0.6 ml | |

## Section 6a. Red-Gray Top Tubes

**Purpose:** To ensure all red-gray top blood tubes are processed and stored according to study protocol.

**By Whom:** JHU Lab/Laboratory Technician

**Procedure:**

1. Samples should be entered into Freezerworks.
    1) Enter all sample information into an excel spreadsheet (a template will be located on the Desktop)
    2) Sign on to Freezerworks©.
    3) Import the excel spreadsheet for that day.
    4) Run a query on the date and all samples should appear.
    5) Print labels according to the table below.

| Sample | Label Name | Aliquot # | Aliquot volume |
|---|---|---|---|
| SST Red/gray Tube (10 ml) | Serum | 1 | 1.5 ml |
| | | 2 | 1.5 ml |
| | | 3 | 1.5 ml |

2. Properly label the cryovials using the NUNC™ CryoTube™ vials.

3. Prepare the Biological Safety Cabinet (BSC) for processing:
    1) Place a blue absorbent pad in the working area
    2) Place a sealable bag to dispose of the empty draw tubes.
    3) Restock pipettes
    4) Pour a 1:10 bleach solution in plastic beakers for pipette disposal.

4. Aliquot the sample in 1.5 ml aliquots. If there is extra serum, increase the volume of stored serum up to 1.8 ml.

5. Aliquoted specimens are then placed into the appropriate cryobox and gridded in the appropriate grid sheet.

6. Cryoboxes are placed in the appropriate -80ºC freezer.

## Section 6b. Purple Top Tubes

**Purpose:** To ensure all sample plasmas, buffy coats, and white blood cells are processed and stored in a standardized manner according to study protocol.

**By Whom:** JHU Lab/Laboratory Technician

**Procedure:**

1. Samples should be entered into Freezerworks.
    1) Enter all sample information into an excel spreadsheet (a template will be located on the Desktop)
    2) Sign on to Freezerworks©.
    3) Import the excel spreadsheet for that day, or manually enter the sample information into the database.
    4) Run a query on the date and all samples should appear.
    5) See the table below that lists the sample types, number of labels needed and label name.

| Sample Tube | Sample Type/ Label Name | Aliquot # | Aliquot Volume |
|---|---|---|---|
| Purple Top Tube # 1 (JHU Lab) | Plasma | 1 | 1.5 ml |
| | | 2 | 1.5 ml |
| | | 3 | 1.5 ml |
| | Buffy Coat | 1 | 0.5 ml |
| | | 2 | 0.5 ml |
| Purple-top Tube #2 (Scott Lab) | Whole blood | 1 | Scott lab for DNA extraction |
| Extracted DNA from Scott Lab | DNA | 1 | 0.5 ml (50 ng/ul) |
| | | 2 | 0.5 ml (50 ng/ul) |
| | | 3 | *DRV |
| | | 4 | *DRV |
| Purple-top Tube #3 (RBC Lyse) | Plasma | 4 | 1.5 ml |
| | | 5 | 1.5 ml |
| | | 6 | 1.5 ml |
| | WBC | 1 | 0.6 ml |
| | | 2 | 0.6ml |

* DRV---divide remaining volume

2. Properly label the cryovials:
    a. For all samples 0.5 ml or under, use the NUNC™ CryoTube™ vials (1.0 ml)
    b. For all samples between 0.5 ml and 1.8 ml, use ™ CryoTube™ vials (1.8 ml)

3. Prepare the BSC hood for processing:
    a. Place a blue absorbent pad in the working area
    b. Place a sealable bag to dispose of the empty draw tubes.
    c. Pour a 1:10 bleach solution in plastic beakers for pipette disposal.

4. Centrifuge tube #1 at 2500 rpm for 15 minutes.

5. Aliquot the plasma from the top of the centrifuge tube into 3 x 1.5 ml aliquots.

6. Using a 1 ml pipet, carefully remove the cloudy white layer (buffy coat) above the red cells but below the plasma. Some plasma and red blood cells may be collected. Aliquot in 2 x 0.5 ml aliquots.

7. Grid and store the samples at -80°C.

8. Take purple tube #2 to Blalock 1005 for transport to Alan Scott's CIDR lab at Bayview along with a 2 inch freezer box containing 4 labeled empty vials. Labels #1 and 2 should read 0.5 ml, 50 ng/ul. Labels #3 and 4 will not have a volume on them. The extracted DNA will be returned to JHU lab in these vials.

9. Refer to section 9C of the appendix for DNA extraction protocols. Also refer to section 9F of the appendix for low DNA yield protocol.

10. The RBC lyse protocol in the appendix section 9B should be followed for purple top #3.

## Section 6c. Repository Quality Management

**Purpose:** Ongoing quality control and assurance activities to ensure the quality of the repository and its respective databases.

**By Whom:** JHU Lab

**Quality Control**

**Procedure for Grid sheets:**

1. On a daily basis the repository monitor notes any discrepancies between the sample grid sheets and the database.

2. Every Friday these changes are made in Freezerworks.

**Quality Assurance**

**Procedure:**

1. Every Wednesday from 9-11 am, routine quality control activities will be performed.

2. A schedule of those activities planned will be distributed indicating which samples need to be audited.

3. The audit activities will be performed in teams of two or individually.

4. First, pull the appropriate manifest sheets.

5. Locate relevant sample boxes in freezers using the freezer inventory.

6. Compare sample labels in boxes to sheet.

7. Note discrepancies by crossing out the incorrect information in pen with a single line, initialing and dating. Mark any changes on the comments line.

8. Highlight missing samples on the sheet, but not in the box.

9. If a sample previously thought to be missing is found, enter the letters “TF” where highlighted.

10. Staff members’ initials and date must be written on all sheets when finished.

11. Any manifest sheets requiring correction will be sent to the data house for entry.

12. Manifest sheets will be returned and filed.

Other activities: Corrections sent regularly to the DCC (Friday with rest of data)

## Section 7. Supplies and Ordering

**By Whom:** JHU Lab and Clinic site technicians

| Item Name | Supplier | Catalog Number | Cost/Unit | To be supplied by |
|---|---|---|---|---|
| **Sample Collection Supplies** | | | | |
| Purple top EDTA tubes 10 ml | VWR | BD366643 | case of 10x100 $270.81 | Site |
| Red-Gray SST tubes 10 ml | VWR | BD367985 | case of 10x100 $499.43 | Site |
| Butterfly needles 21 G (Preferred) | VWR | BD367281 | case of 4x50 $249.75 | Site |
| Butterfly needles 23 G | VWR | BD367283 | case of 4x50 $249.75 | Site |
| Butterfly needles 25 G | VWR | BD367285 | case of 4x50 $249.75 | Site |
| Tourniquets (reusable) | VWR | VT367203 | case of 20x25 $186.95 | Site |
| Tube adapters | VWR | VT367290 | case of 10x100 $317.86 | Site |
| Gauze | VWR | 82004-740 | case of 5000 $126.54 | Site |
| Bandage | VWR | 56612-996 | case of 12 x 100 $62.02 | Site |
| Alcohol pads | VWR | 15648-981 | case of 15x200 $91.81 | Site |
| Hamilton Bell™ Vanguard Centrifuge | MarketLab | JL9576 | $359 | Site |
| Tube/Document Labels | AVERY | 5267 | Local pricing | Site |
| **Shipping Materials** | | | | |
| Shipper | Saf-T-Pak | STP 309 | $64.00 | JHU lab |
| Cold Packs | Cold Ice, Inc | CI- 24CA | $7.88 | JHU lab |
| Absorbent material | Saf-T-Pak | STP 152 | case of 250 89.75 | JHU lab |
| Tyvek envelope and inner bag | Saf-T-Pak | STP 710 | case of 50 $133.00 | JHU lab |
| Bubble wrap | Saf-T-Pak | STP 600 | | JHU lab |
| FedEx airbill | FedEx | | | JHU lab |

## Section 8. Good Laboratory Practices

**Purpose:** To train all staff members in good laboratory practice and ensure these are followed in all laboratory activities.

**By Whom:** All laboratory staff members

- Eating, drinking, smoking, applying cosmetics, and handling contact lenses are prohibited in the laboratory working areas.
- Standard precautions should always be followed. Personal protective equipment (gown, gloves, eye protection) should be worn in the laboratory when handling and processing specimens and performing diagnostic testing.
- BSCs (Biological Safety Cabinets) or other physical containment devices should be used for all manipulations that may cause splashes or droplets of infectious materials.
- Mouth pipetting is forbidden.
- Contaminated materials must be disposed of in appropriate biohazard containers.
- Work surfaces must be decontaminated after any spill of potentially dangerous material using a bleach solution. Work surfaces and equipment should be decontaminated after specimens are processed.
- Personnel must wash their hands often – especially after handling infectious materials, before leaving the laboratory working areas, and before eating.
- Personal protective equipment must be removed before leaving the laboratory.

## Section 9. Appendices

A. **Specimen Transmittal/ Blood Collection form**
B. **RBC Lyse protocol**
C. **Laboratory and quality control procedures from the Scott lab for DNA extraction, DNA quantification, and aliquot preparation.**
D. **IATA Shipping guidelines**
E. **Shipper assembling and packaging instructions**
F. **Low DNA yield protocol**
G. **Holiday Calendar**

| ID # | (affix barcode label here on BOTH white and yellow copies of form) |
|---|---|

**COPDGene™**
**Blood Collection Form**

| | |
|---|---|
| Date/time samples were collected | ___ ___ / ___ ___ / 20 ___ ___ (M M / D D / Y Y) ___ ___ : ___ ___ AM PM (circle one) |
| Date/ time SST tube was centrifuged | ___ ___ / ___ ___ / 20 ___ ___ (M M / D D / Y Y) ___ ___ : ___ ___ AM PM (circle one) |
| Date/time specimens were refrigerated | ___ ___ / ___ ___ / 20 ___ ___ (M M / D D / Y Y) ___ ___ : ___ ___ AM PM (circle one) |

| | Blood Tube # | | | |
|---|---|---|---|---|
| | **1** 10ml EDTA | **2** 10ml EDTA | **3** 10ml EDTA | **4** 10ml SST |
| Collected (check if yes) | ☐ | ☐ | ☐ | ☐ |
| QC (check if problems occurred and list below) | ☐ | ☐ | ☐ | ☐ |

**Quality Control**
1) *Enter the date and time Blood Tube #4 (SST) was centrifuged.*
2) *List any comments or problems that occurred during sample collection.*

Signature of Nurse/Phlebotomist: Date:

| | | |
|---|---|---|
| Date samples shipped to JHU Lab | ___ ___ / ___ ___ / 20 ___ ___ (M M / D D / Y Y) | **One copy – include in shipment to JHU Lab**<br>**One copy – retain for site records** |

**To be completed by JHU Lab**

Date and Time of Receipt ___ ___ / ___ ___ / 20 ___ ___ (M M / D D / Y Y) ___ ___ : ___ ___ AM PM (circle one)

| Specimen | | Received | Special Instructions | | QC Comments |
|---|---|---|---|---|---|
| Blood Tube | 1 | ☐ | Sent to Scott/CIDR Lab | ☐ | |
| | 2 | ☐ | Processed for repository | ☐ | |
| | 3 | ☐ | Processed for repository | ☐ | |
| | 4 | ☐ | Processed for repository | ☐ | |

Signature of Technician Date

## B. Purple Top #3 Processing Protocol

ALWAYS WEAR: GLOVES, FACESHIELD, AND LABCOAT WHEN PROCESSING.

1. Make sure to keep sample(s) cold at all times (before and during processing), i.e. 4$^{o}$C.

2. Centrifuge Purple Top vacutainer at 2000RPM at 4$^{o}$C for 10min.

3. Remove Purple Top vacutainer from centrifuge. Note blood has separated into 3 layers:

   - Top = Plasma: Yellow, transparent layer
   - Middle = White Blood Cells: Thin, delicate white layer aka “Buffy Coat”
   - Bottom = Red Blood Cells (RBC): Red, opaque layer

4. Remove rubber stopper with kimwipe. Using plastic transfer pipet, carefully aliquot 1.5 ml of plasma into each of three 1.8ml NUNC tubes. Do not disturb the buffy coat. Remove less than 4.5 ml of plasma if necessary to protect the buffy coat.
   **Be careful not to aspirate the Buffy coat!**

5. Pour remainder of Purple Top vacutainer contents (some excess plasma AND entire Buffy coat and RBC layers) into a 50ml centrifuge tube that has been pre-filled with 30ml RBC Lysis Solution. Mix well with pipet, by aspirating and dispensing mixture. Rinse out the Purple Top vacutainer tube with some of the liquid from the 50 ml centrifuge tube to insure that all of the cells have been removed from the Purple Top tube. Discard Purple Top vacutainer and pipet in Biohazard wastebin.

6. Cap 50ml tube, shake <u>vigorously</u>, and incubate on ice for 15min. At this time grid and store the plasma aliquots in appropriate cryobox at -80°C.

7. After incubation is complete, centrifuge 50ml tube at 2000RPM at 4$^{o}$C for 10min.

8. Once centrifugation is complete, you should see a white pellet at the bottom of the 50ml tube. If you do NOT see a pellet, shake tube vigourously and centrifuge again.

9. Pour supernatant (RBC Lysis Solution mixture) into appropriate Biohazard waste jug/container. Make sure the white pellet does not slip out.

10. Using Pipetman and disposable BD Falcon pipet, add 5ml RBC Lysis Solution to white pellet. *Thoroughly* break up pellet either by continually aspirating and dispensing with pipet OR capping 50ml tube and shaking *vigorously*.
    **Make sure the pellet is completely broken up and mixed well with RBC Lysis Solution.**

11. Incubate 50ml tube on ice for 5min.

12. After incubation, centrifuge at 2000RPM at 4$^{o}$C for 10min.

13. Pour off supernatant. Remove excess supernatant with 1000ul pipettor and filter tip(s).
    **Try to clean the white pellet as best as possible, without disturbing it.**

14. With 1000ul pipettor and filter tip, add 600ul NE Buffer TWICE for total of 1200ul NE Buffer.

15. Use pipettor and filter tip to break up the pellet in the NE buffer so that you have a homogenous solution.

16. Aliquot mixture into 1.8ml NUNC tube.

17. Store WBC aliquots in –80 freezer.

**NE Buffer (pH = 7.4)**
75 mM NaCl (M.W. = 58.44 g/mol)
24 mM EDTA (M.W.=372.2g/mol)
STORE at ROOM TEMPERATURE

**For FINAL VOLUME=500ml Solution**
2.19g NaCl
4.47g EDTA
**For FINAL VOLUME=1500ml Solution:**
6.57g NaCl
13.41g EDTA

## C. Laboratory and quality control procedures from the Scott lab for DNA extraction, DNA quantification, and aliquot preparation.

Genomic DNA is isolated from peripheral blood leukocytes using Puregene Blood Kit chemistry on an Autopure LS automated DNA purification instrument (Qiagen,Valencia, CA). DNA concentrations are determined using a ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE).

### Logging in Samples

Blood, frozen buffy coats, and cell pellets will be transported from the Farzadegan Laboratory to the DNA Extraction Laboratory and processed within two to three days of receipt. **Cell pellets must be kept frozen at all times. If they are received thawed, isolate immediately. See protocol for instructions.**

When a sample comes in it should have clearly labeled tubes and a DNA isolation request form. If this is not the case, verify with the contact person. (This list is located on the wall by the phone). Mark all correspondence on the request form as well as any changes to the form or tubes.

On the request form write the date received in the top left corner in red pen and circle date on the request paper. Check to make sure that label on tube on request form matches that written on the tube. If this is not the case, verify with the contact person.

Make note of the volume or cell count of each sample. Write on the request form.

Log in sample in respective PI log (located in Laura Kasch's Computer, Fragment dropbox). Each PI log is different. Fill in necessary information for each investigator, i.e. Number, Sample ID, Date received, # of tubes, Volume.

If the sample has multiple tubes that need isolation, log in other tubes in the same row; these should not be logged in as separate samples.

File DNA request forms into respective folders. At the end of every month these folders should be cleaned out and filed into the DNA Isolation Logs located on the shelf above the lab bench. It may be necessary to do this more often if the file folders get big. The request forms are filed by investigator, study, and date with the most current date on top.

Store blood in refrigerator until processed. Store cell pellets in freezer.

### DNA Purification Using Autopure

Refer to Gentra Autopure manual for instruction on running samples. When run is complete:

1. Visually check DNA to see if needs more hydration solution. DNA is hydrated in TE (10mM Tris, 1 mM EDTA, pH 7-8). If the DNA pellet appears large, add more hydration solution. **Make sure that you log in any additional amount of hydration solution.**
2. After you log in amounts, lightly vortex each sample, and put into shaker at 37ºC overnight.
3. Bring samples to room temperature.

### Determining Concentration of DNA

1. Turn on the Nanodrop. Launch Nanodrop software application.
2. After the sample has hydrated overnight at 37ºC, check to see if the DNA is in solution. If not, add more hydration solution. **Make sure that you log in any additional amount of hydration solution.**
3. Scan sample into Nanodrop software. Pipet 1.5µl onto Nanodrop platform. Click read button. OD each sample. The sample should range between 0.2 to 1.2 µg/µL. If the sample is above this range add additional hydration solution. Normally 100µL hydration solution will bring the OD down .2µg/µl, i.e. if the OD is 1.8µg/µL you can add 300µL hydration solution. Log in additional hydration solution, vortex and shake at 37ºC and re-OD. Clean off platform with a Kimwipe. Scan in next sample and OD. Repeat process for all samples. Copy and paste OD and 260/280 ratio from Nanodrop report into investigator's DNA log.

Calculate amount of DNA for each of the 4 supplied aliquot tubes that have been prelabeled. Tubes one and two should contain 500µl at 50ng/µl. Usually this will require that the DNA be diluted. Dilute DNA with TE buffer. Tubes three and four should contain the remaining DNA divided equally between the two tubes. Pipet appropriate amounts of DNA and TE into respective tubes checking DNA isolation label against the aliquot tube label. Store the DNA tubes at 4ºC until returned to the Farzadegan laboratory.

4. Turn the Nanondrop off when done for the day.

**DNA Isolation Quality Control**

Daily
1.Check and record temperatures of freezers and refrigerators.
2.Determine 260/280 ratio for all samples processed.
3.Wipe down work areas with sanitizer.
4.Visibly check hydration buffer for contamination.

Weekly
1.Sanitize pipetmen.
2.Replace hydration buffer.

Monthly
1.Check 5 random samples on 1% agarose gel for high molecular weight DNA.
2.Re-OD CEPH controls. OD should be approximately the same. If not contact Beckman for service.

Equipment Maintenance
Check that maintenance schedule for Autopure and spectrophotometer are up to date. Refer to service contracts.

**Agarose Gel Electrophoresis**

Electrophoresis is a technique used to separate nucleic acids that differ in size by passing an electrical current through a matrix or "gel". When nucleic acids, which are negatively charged, are placed in an electric field they migrate toward the positive pole, the anode. Most commonly, the gel is cast in the shape of a thin slab, with wells for loading the sample. The gel is immersed within an electrophoresis buffer that provides ions to carry a current and a buffer to maintain the pH.

The gels we use in the FAF are made of agarose, a polysaccharide extracted from seaweed. Agarose gels have a wide range of separation, but relatively low resolving power. By varying the concentration and type of agarose, DNA fragment from about 100 to 50,000 bp can be separated. Below is a chart showing percentage gels for resolution of fragment in varying size ranges.

**Recommended agarose gel percentages for resolution of linear DNA:**

| Percentage agarose (%) | Size of DNA fragment (bp) |
|---|---|
| 0.5 | 1,000–30,000 |
| 0.7 | 800–12,000 |
| 1.0 | 500–10,000 |
| 1.2 | 400–7,000 |
| 1.5 | 200–3,000 |

| | |
|---|---|
| 2.0 | 50–2,000 |

The DNA is visualized in the gel by addition of ethidium bromide (EtBr). EtBr is a fluorescent compound that intercalates between the DNA bases. When exposed to ultraviolet light it fluoresces a red-orange color.

**Making an Agarose Gel**

What you will need:

- Agarose
- NuSieve
- Weighing paper
- Spatula
- Scale
- TBE buffer
- Flask
- Stir bar
- Hot Plate

Directions

1. Zero weighing paper on scale.
2. Measure out appropriate amount of NuSieve and/or agarose using a spatula. See table below. Note: Do not use the same spatula for both agarose and NuSieve.
3. Place agarose in a 250ml conical flask containing a stir bar.
4. Add appropriate amount of 1x TBE, swirl to mix. See table below.
5. Place flask on stir plate, turn on stirring and heat.
6. Heat agarose to boiling. Check that all agarose has dissolved. Continue heating if has not. Agarose boils over very easily so keep an eye on it – do not walk away.
7. Place on the bench to allow to cool to about 60ºC. The time it takes to cool depends on the volume of the gel being made. A good rule of thumb for cooling is should be able to pick up the flask with bare hands but it should be just too hot to keep holding it. There are two reasons to allow the agarose solution to cool. One, to reduce ethidium bromide vapor in the following step, and two, to not warp the gel casting tray and comb.
8. Wearing gloves, add 1µl ethidium bromide (10mg/ml) per 50ml of volume. Swirl to mix.
9. Slowly pour the cooled agarose solution into the prepared casting tray (pouring slowly reduces air bubbles). Use a disposable tip to push any air bubbles to the side. Insert the comb, checking that it is positioned correctly. Immediately rinse out flask so that residual agarose does not solidify to the flask.
10. Let the gel set for a minimum of 30 minutes, preferably 1 hour. The gel may appear to be set sooner but running DNA into it too soon will give terrible looking results.
11. Remove tape from gel casting tray. Carefully remove comb. Place gel tray in electrophoresis tank.
12. Add 1X TBE to tank so that buffer is 2 to 3mm above gel. Note: The running buffer must be the same concentration as the buffer used to make the gel.
13. Load samples and a size standard.
14. Place cover on tank. Attach the leads and run at 5V/cm (the distance between the electrodes. Running faster may melt your agarose gels if running for a long period of time. If running overnight, run at 0.25 – 0.5 V/cm. Check that there is a current passing through the gel by examining the electrodes. You should see tiny bubble rising from it.
15. Switch off the power supply when the bromophenol blue has migrated ¾ the length of the gel. Unplug the leads, remove the cover, and while wearing gloves transport the gel, using the tray, to the UV light box. Slide the gel off the tray onto the glass surface of the UV box. Cover the gel with the camera skirt. Turn on UV light box.
16. Photograph the gel and save the picture file to appropriate folder. Note: When touching the computer key-board, do not wear gloves that have come in contact with the gel or the electrophoresis buffer, as they contain ethidium bromide.

**Reagent Amounts**

| Gel Box | Percent | Size | # of Combs | TBE | Agarose | NuSieve |
|---|---|---|---|---|---|---|
| Regular Gel | 1% each | 290 | 4 | 290mL | 2.9g | 2.9g |
| Regular Gel | 1% each | 217.5 | 3 | 217.5mL | 2.18g | 2.18g |
| Regular Gel | 1% each | 145 | 2 | 145mL | 1.4g | 1.4g |
| Regular Gel | 1% each | 72.5 | 1 | 72.5mL | .73g | .73g |
| Old Gel box | 1% each | 200 | 2 | 200mL | 2g | 2g |
| Old Gel box | 1% each | 100 | 1 | 100mL | 1g | 1g |
| Small Gel | 1% each | 60 | 1 or 2 | 60mL | .6g | .6g |
| Old Gel box | 0.70% | 200 | 2 | 200mL | 1.4g | 0 |
| Old Gel box | 0.70% | 100 | 1 | 100mL | .7g | 0 |
| Small Gel | 0.70% | 60 | 1 or 2 | 60mL | .4g | 0 |

## D. IATA Shipping guidelines

**Purpose:** To ensure that all packages meet IATA guidelines for safe shipping.

**By Whom:** All staff members involved with shipping samples and reagents.

**Disclaimer:** This information is provided to each site as a quick reference to IATA shipping guidelines. **Not all information provided in this section pertains to COPDGene™ sites** (e.g., dry ice/carbon dioxide)

**Procedure:**

The shipper, not the transport company, is responsible for determining the hazard class and properly packaging and marking the hazard information on the shipment.

### DIAGNOSTIC SPECIMENS

A diagnostic specimen is any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue fluids, and body parts being transported for research, diagnosis, investigational activities, disease treatment or prevention.

Diagnostic specimens transported under the IATA regulations are assigned the UN identification number 3373, and are subject to Packing Instructions 650. Any specimens shipped in dry ice must be labeled with the UN identification number 1845.

Labeling of shipping box:

- All diagnostic specimens must be labeled with a white diamond on point UN 3373 DIAGNOSTIC SPECIMENS label.
- If the shipper contains dry ice, there must be a Class 9 diamond on point label on the face of the shipper, as well as a DRY ICE UN 1845 label that includes the approximate weight (in kgs) of the dry ice included.
- The consignee and the shipper must be identified clearly on the face of the box.

PACKING INSTRUCTION 650

STATE VARIATIONS: DOG-03
OPERATOR VARIATIONS: AF-04, AO-03, AS-08, CO-07, CS-07, FX-09, LA-07, LH-12, OF-03

General Requirements

Diagnostic specimens must be packed in good quality packagings, which must be strong enough to withstand the shocks and loadings normally encountered during transport, including trans-shipment between transport units and warehouses as well as any removal from a pallet or over pack for subsequent manual or mechanical handling. Packagings must be constructed and closed so as to prevent any loss of contents when prepared for transport which might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

Primary receptacles must be packed in secondary packagings 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 substantially impair the protective properties of the cushioning material or of the outer packaging. Packages must be prepared as follows:

**(a) For Liquids:**

- The primary receptacle(s) must be leak proof and must not contain more than 500 ml;

- There must be absorbent material placed between the primary receptacle and the secondary packaging; if several fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated so as to prevent contact between them. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacles and there must be a secondary packaging which must be leak proof.
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa in the range of -40°C to +55°C (-40°F to 130°F).
- The outer packaging must not contain more than 4 L.

**(b) For Solids:**

- The primary receptacle(s) must be sift-proof and must not contain more then 500 g.
- If several fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated so as to prevent contact between them and there must be a secondary packaging which must be leak proof.
- The outer packaging must not contain more than 4 kg.

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.

Each completed package must be capable of successfully passing the drop test described in 6.6.1 except that the height of the drop must not be less than 1.2 m.

Packages must have one side with dimensions of not less than 100 mm x 100 mm (4 in x 4 in) or packages must be in an over pack that has one side with dimensions of not less than 100 mm x 100 mm (4 in x 4 in).

Each package and the "Nature and Quantity of Goods" box of the air waybill must show the text "DIAGNOSTIC SPECIMENS". Each package may also be marked in accordance with 7.1.5.8 to indicate that the shipper has determined that the packaging meets the applicable air transport requirements. The marking must be applied adjacent to the words "Diagnostic Specimens".

A Shipper's Declaration for Dangerous Goods is not required.

Provided diagnostic specimens are packed in accordance with this Packing Instruction, no other requirements of these Regulations apply except for the definition in 3.6.2.1.4 and the reporting of dangerous goods accidents and incidents in 9.6.1. However, where carbon dioxide, solid (dry ice) or liquid nitrogen is used to keep specimens cold, all applicable requirements of these Regulations must be met.

Substances shipped refrigerated or frozen (wet ice, prefrozen packs, Carbon dioxide, solid [dry ice]): Ice Carbon dioxide, solid (dry ice) or other refrigerant must be placed outside the secondary packaging(s) or alternatively in an over pack with one or more completed packages. Interior support must be provided to secure the secondary packaging(s) or packages in the original position after the ice or Carbon dioxide, solid (dry ice) has been dissipated. If ice is used the packaging must be leak-proof. If Carbon dioxide, solid (dry ice) is used the outer packaging must permit the release of carbon-dioxide gas. The primary receptacle must maintain its containment integrity at the temperature of the refrigerant as well as at the temperatures and pressure of air transport to which the receptacle could be subjected if refrigeration were to be lost.

Substances shipped in liquid nitrogen: Plastic capable of withstanding very low temperatures must be used instead of glass receptacles. Secondary packaging must also withstand very low temperatures and in most cases will need to be fitted over individual primary receptacles. If multiple primary receptacles are placed in a single secondary packaging, they must be separated and supported to ensure that contact between them is prevented. Requirements for shipment of liquid nitrogen must also be observed. The primary receptacle must maintain its containment integrity at the temperature of the refrigerant 'used as well as at the temperatures and pressure of air transport to which the receptacle could be subjected if refrigeration were to be lost.

**Dry ice/carbon dioxide, solid**

PACKING INSTRUCTION 904.
STATE VARIATIONS: USG-13
OPERATOR VARIATIONS: HP-02, IC-08, VN-11

Carbon dioxide, solid (dry ice), when offered for transport by air, must be in packaging designed and constructed to permit the release of carbon dioxide gas and to prevent a build-up of pressure that could rupture the packaging.

The net weight of the Carbon dioxide, solid (dry ice) must be marked on the outside of the package.

## E. Shipper assembling and packaging instructions

**Purpose:** To ensure samples are correctly packaged and shipped according to specimen needs, study protocol, and IATA guidelines.

**General Requirements**

Technicians must be familiar with IATA guidelines outlined in Section 9D. The packaging's must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including trans-shipment between transport units and between transport units and warehouses as well as any removal from a pallet or over pack for subsequent manual or mechanical handling. Packaging's must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

The packaging must consist of three components:
a) A primary receptacle; (blood tubes – EDTA and SST)
b) A secondary packaging; (Plastic biohazard bag and Tyvek bag – STP-710 along with the absorbent material – STP-152)
c) A rigid outer packaging. (Styrofoam and the cardboard box – STP-309)

Primary receptacles (blood tubes) must be packed in secondary packaging's (plastic and the tyvex bag) in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings must be secured in outer packagings (Styrofoam and cardboard box) with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

> **Note:** Specimens may only be shipped Monday through Thursday to ensure arrival at the JHU lab on a weekday. This means appointments will be restricted to Monday through Thursday. Exceptions will be dealt with on a case by case basis.

**Step by Step Diagrammatic representation of packaging and shipping COPDGene™ samples using STP-309 shipper**

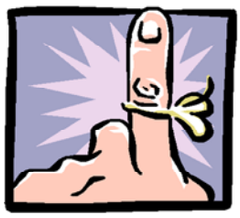
**STEP 1.**


- Items needed for blood shipment are (clockwise from upper left hand corner):
  - Styrofoam insulator box insert and top
  - COPDGene barcode labels
  - Saf-T-Pak Shipping Box STP-309
  - Absorbent Material
  - Blood Transmittal Form
  - 2 **FROZEN** Cold Packs
  - 3 EDTA and 1 SST 10 ml plastic blood tubes with COPDGene™ barcode labels
  - Bubble Wrap Sleeves for Blood Tubes
  - Tyvek Bag
  - Plastic Biohazard Bag

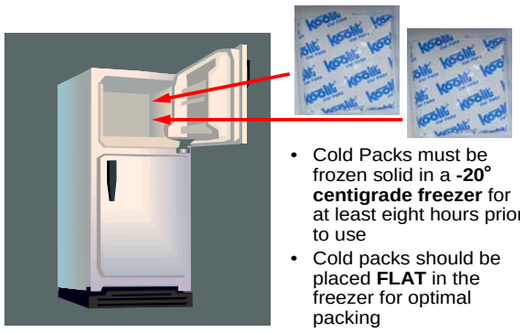
**STEP 2.**

## Before Shipping Blood REMEMBER:

- All blood tubes must have the correct COPDGene™ barcode label
- The SST tube must be centrifuged before shipping



**STEP 3.**



- Cold Packs must be frozen solid in a **-20° centigrade freezer** for at least eight hours prior to use
- Cold packs should be placed **FLAT** in the freezer for optimal packing

**STEP 4.**

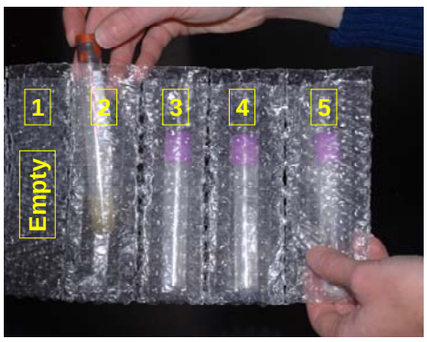
Prepare the Shipping Container:

1. Insert Styrofoam insulator into STP-309 box
2. Put one cold pack at the bottom of the Styrofoam

**STEP 5.**

Insert each blood tube into an individual sleeve in the bubble wrap

**STEP 6.**




Notice that one bubble wrap sleeve can hold 5 tubes. Each bubble wrap should hold one subject's blood tubes—there will be one empty sleeve.

**STEP 7.**

After placing tubes into the bubble wrap sleeves, roll up the bubble wrap and tubes.

**STEP 8.**

Secure the blood tubes in the bubble wrap with a rubber band

**STEP 9.**

Insert the blood tubes in the bubble wrap into the Biohazard bag.

Also insert a loose piece of the absorbent material into the Biohazard bag. It does **not** need to the wrapped around the blood tubes.

**STEP 10.**

## Putting Three Sets of Blood into One Biohazard Bag

- When you put three sets of blood into one shipment, space is very tight.
- To ensure that all materials fit, we suggest that you pack the biohazard bag as shown.


**STEP11.**

- Ensure that all air is removed from the biohazard bag before sealing
- Seal the biohazard bag

**STEP 12.**


Insert the Biohazard bag into the Tyvek bag

**STEP 13.**

- Ensure that all air is removed from the Tyvek bag
- Seal the Tyvek Bag

**STEP 14.**

- Put the sealed Tyvek bag on top of the first frozen cold pack inside the shipping container

**STEP 15.**

Place the second frozen cold pack on top of the sealed Tyvek bag

**STEP 16.**

Place the Styrofoam cover on the container

**STEP 17.**

Place the completed Blood Transmittal Form on top of the Styrofoam container

**STEP 18.**

Place the Styrofoam cover on the container

**STEP 19.**

Place the completed Blood Transmittal Form on top of the Styrofoam container

**STEP 20.**

Place the Styrofoam cover on the container

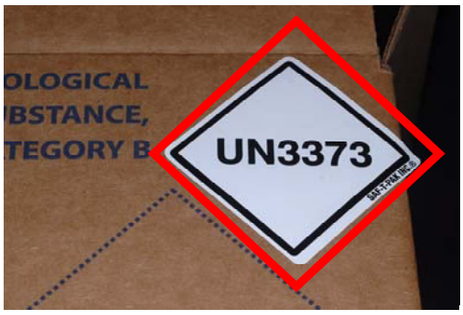
**STEP 21.**

Place the completed Blood Transmittal Form on top of the Styrofoam container

**STEP 22.**

Seal the box with packaging tape

**STEP 23.**



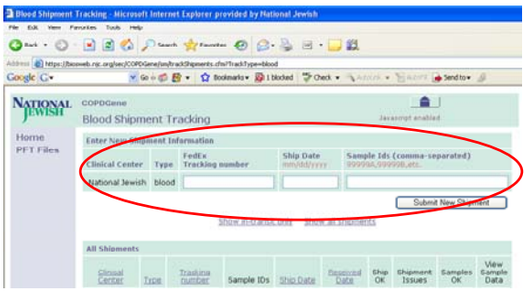

Be sure that the box is compliant with label regulations.

All boxes are required to have the Biological Substances B, UN3373 label.

**STEP 24.**

- Secure pre-filled FedEx label to the top of the box.
- Deposit at FedEx drop-off location

**STEP 25.**




- Record shipment immediately on COPDGene website: https://biosweb.njc.org/sec/COPDGene/sm/trackShipments.cfm?TrackType=blood

## F. Low DNA yield Protocol:

**Purpose:** To ensure at least 50 micrograms of total DNA yield is obtained from every subject whose blood samples are received by the JHU lab.

**By Whom:** JHU lab staff

1. For DNA samples that meet the requirements of a total DNA yield of at least 50 micrograms total DNA yield, two diluted DNA tubes at a concentration of 50 ng/ul and a volume of 500 ul, with remaining DNA divided into two undiluted master tubes.
2. For subjects with less than 50 microgram of DNA yield, the JHU lab staff will send a frozen buffy coat aliquot (first aliquot #1 and if needed aliquot # 2 will be sent) to Alan Scott's CIDR lab for DNA extraction.
3. If the DNA yields from the buffy coat aliquot # 1 and aliquot # 2 remains below 50 micrograms, the JHU lab will send a frozen WBC aliquot (first aliquot #1 and if needed aliquot # 2 will be sent) to Alan Scott's CIDR lab for DNA extraction.
4. If the DNA yields from the WBC aliquot # 1 and aliquot # 2 remains below 50 micrograms, the JHU lab will contact the COPDGene™ Administrative coordinator requesting sites to perform a re-draw or repeat phlebotomy on the subject.
5. Please note that all the aliquots for buffy coat and WBC will be used for DNA extraction before requesting a re-draw from the subject. At this point only plasma aliquots will remain stored in the JHU lab biological repository from the first visit or blood draw of that subject.

## G. Holiday Calendar

| 2008 | |
|---|---|
| New Year's Day | Tuesday, January 1<br>Do not ship on Dec 24 – Jan 2 |
| Martin Luther King's Birthday | Monday, January 21 |
| President's Day*** | Monday, February 18 |
| Memorial Day | Monday, May 26 |
| Independence Day | Friday, July 4<br>Do not ship on July 3 |
| Labor Day | Monday, September 1 |
| Thanksgiving Day | Thursday, November 27<br>Do not ship on November 26 |
| Day after Thanksgiving | Friday, November 28 |
| Holiday Preparation | 1/2 day during December<br>Do not ship Dec 22 – Jan 2 |
| 1/2 Day, Christmas Eve | Wednesday, December 24 |
| Christmas Day | Thursday, December 25 |
| 1/2 Day, New Year's Eve | Wednesday, December 31 |
| **2009** | |
| New Year's Day | Thursday, January 1 |