

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

LRRK2 Cohort Consortium

Biologics Laboratory Manual

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Summary of Study Goals

Welcome and thank you for participating in the Michael J. Fox Foundation for Parkinson's Research ("MJFF") LRRK2 Cohort Consortium. MJFF established the Consortium to bring together multiple investigators focused on studies of individuals carrying mutations in the Parkinson's disease-associated gene LRRK2 (leucine-rich repeat kinase 2). LRRK2 represents one of the most common genetic forms of PD and is being actively pursued as a therapeutic target among drug makers. MJFF has launched a number of parallel efforts to accelerate and facilitate our genetic, clinical and biological understanding of LRRK2.

A critical part of this effort is the creation of a Biosample Collection to store valuable samples from individuals with LRRK2 mutations and to provide a resource for future research. MJFF is supporting participating sites to collect a minimal set of samples. Samples will be shipped and stored at the main MJFF-supported biorepository at Coriell Institute for Medical Research ("Coriell") in Camden, New Jersey, USA. Some investigators, due to local laws, will store MJFF biosamples at a local repository approved by MJFF and the site investigators. (Please confirm with your site investigator how your samples are to be stored.) The biosamples will be linked to a set of standard clinical data being stored in a common database at the University of Rochester Clinical Trials Coordinator Center ("CTCC") in Rochester, New York, USA. Both clinical data and biological samples will ultimately be made available to the Consortium and other researchers interested in studying further the link between LRRK2 and Parkinson's disease.

The standard protocols and procedures found in this manual are modeled after those established by the Parkinson's Progression Markers Initiative ("PPMI"), a major effort being funded and coordinated by the MJFF to identify biomarkers of Parkinson's disease progression. It is important that each site carefully follow the procedures to ensure that all samples collected both for the LRRK2 Cohort Consortium and PPMI can be easily compared. Success in this effort will provide invaluable insight into the causes and eventual cure for this devastating disease.

Quality control of sample collection will be a critical aspect of this effort. Along these lines, each site should carefully ensure the following:

1. Maintain a copy of each Sample Record Summary and Shipment Notification Form (Appendix B) with your site study materials to document all samples collected and shipped to Coriell or your local repository.
2. Ensure that all information on the Sample Record Summary and Shipment Notification Form are complete and legible.
3. Ensure that the time from sample collection to freezing for serum/plasma/RNA/CSF meets protocol requirements.
4. Ensure consistency in the time and temperature of centrifugation.
5. Ensure that volumes collected meet protocol requirements.

Laboratory Contacts

Study Support

Alison Scutti, Senior Project Manager, Coriell

Phone #: 856-757-9756

International # (00 +1) 856-757-9756

After Hours Phone #: 609-709-1373

International After Hours Phone #: (00 +1) 609-709-1373

Study Support E-mail: LRRK2@coriell.org

Study Support Fax #: 856-966-5067

International Fax #: (00 +1) 856-966-5067

Mailing Address

Coriell Institute for Medical Research
403 Haddon Avenue Camden, NJ 08103

Laboratory Information

Hours of Operation

Coriell operates from 9 AM to 5 PM US Eastern Time, Monday through Friday.

Holiday Schedules

Please note that courier services may observe a different set of holidays. Please be sure to verify with your courier's schedule prior to any holiday.

FedEx Customer Service

Germany: 1-803-123-800

Spain: 902-100-871

USA: 800-463-3339

DHL Customer Service

Norway: 810-01-345

Spain: 902-122-424

For US sites, frozen samples should be shipped **Monday – Wednesday**.

For International sites, frozen samples must be shipped on **Monday or Tuesday**

Weekend/holiday delivery must be arranged in advance with Coriell.

2011 Coriell Holiday Observations – United States

| Date | Holiday |
|----------------|-------------------------------------|
| 30 May | Memorial Day (observed) |
| 4 July | Independence Day (observed) |
| 5 Sep | Labor Day |
| 24-25 November | Thanksgiving/Day after Thanksgiving |
| 26 December | Christmas (observed) |
| 2 January 2012 | New Year's Day (observed) |

Research Samples to be Collected

The following samples will be collected as part of the MJFF LRRK2 Cohort Consortium:

| Sample Type | Amount |
|--------------------------------|---------------|
| Whole Blood for RNA | 2.5 ml |
| Whole Blood for Plasma | 10 ml |
| Whole Blood for Serum | 10 ml |
| Whole Blood | 6 ml |
| Urine | 10 ml |
| CSF (not all sites collecting) | 10 ml |

Sites may also be collecting samples in addition to the MJFF minimal samples list for storage and use by the site investigators. Please refer to your site's protocol and Schedule of Activities for information about type and timing of sample collection.

Blood samples for DNA are not required as part of the minimal MJFF sample requirements but may be part of your study site's own protocol requirements. Blood for DNA samples should be collected using your institution's recommended procedure for standard venipuncture technique, processing and storage.

Specimen Collection Kits and Supplies

Research specimen collection kits will be provided to you by Coriell with most of the materials needed for the blood, urine and CSF collection, as well as shipping supplies and return air waybills to send materials back to the biospecimen repository at Coriell. Sites that have been approved by MJFF to send biospecimens to a local or other repository (not Coriell) will need to generate their own shipping supplies and air waybills appropriate for the specific local repository.

Labels for collection and aliquot tubes will be provided to you by the CTCC and will be pre-printed with study information including a LRRK2 sample label ID number specific to the type of sample being drawn. In addition, if you are collecting the unique CTCC nine digit ID number (obtained from the CTCC website) please enter this on the Sample Record Summary and Shipment Notification Form (Appendix B). Ensure that all tubes are properly labeled during processing and at the time of shipment.

Coriell – Specimen Collection Kit Contents

Collection kits contain the following and provide the necessary supplies to collect samples from one (1) subject. Note that “supplemental” kits will be provided to sites should you require additional supplies from those contained in the study kits. LRRK2 kit components have been carefully selected to suit the needs of this project. Do not replace or supplement any of the tubes or kit components provided by Coriell with your own supplies unless you have received approval from MJFF/Coriell to do so. You may obtain an additional supply of any individual kit components by placing an order through Queue – see section ‘Coriell Initial and Resupply’ for instructions. For sites collecting CSF, please see the ‘Lumbar Puncture Supplies’ section for additional kit contents.

| Kit Component | Qty | | | |
|--|---------------|--------|-------|--------|
| | North America | Europe | China | Israel |
| EDTA (purple top) blood collection tube, glass (10 ml) | 1 | 1 | 1 | 1 |
| Serum determination (red top) blood collection tube, glass (10 ml) | 1 | 1 | 1 | 1 |
| EDTA (purple top) whole blood collection tube, plastic (6 ml) | 1 | 1 | 1 | 1 |
| PAXgene™ blood collection tube (8.5 ml) | 1 | 1 | 1 | 1 |
| Urine collection cup | 1 | 1 | 1 | 1 |
| Conical bottom centrifuge tubes, sterile (15 ml) | 2 | 3 | 2 | 3 |
| Siliconized polypropylene microcentrifuge tubes, sterile (2 ml) | 6 | 11 | 6 | 11 |

| | | | | |
|---|---|---|---|---|
| Microcentrifuge tube box | 1 | 1 | 1 | 1 |
| Bubble wrap pouch | 1 | 1 | 1 | 1 |
| Plastic Biohazard bag | 1 | 1 | 1 | 1 |
| Tyvek envelope | 1 | 1 | - | - |
| Warning label packet | 1 | 1 | - | - |
| Return Air Waybill (pre-filled) | 1 | 1 | - | - |
| Shipping instruction sheet | 1 | 1 | - | - |
| Lumbar puncture tray (to be supplied in a separate box) | - | 1 | - | 1 |

Supplemental Supplies to Sites:

| Quantity | Kit Component |
|----------|--|
| 1 | Pack of 10 individually wrapped pencil-point spinal needles, 24G x 90mm (EU & Israel sites only) |
| 1 | Pack of 10 introducer needles, 1mm x 30mm (EU & Israel sites only) |
| 6 | Serum determination (red top) blood collection tubes, glass (10 ml) |
| 6 | EDTA (purple top) blood collection tube, glass (10 ml) |
| 6 | EDTA (purple top) blood collection tube, plastic (6 ml) |
| 6 | PAXgene™ blood collection tube (8.5 ml) |
| 15 | Polypropylene microcentrifuge tubes, sterile (2 ml) |
| 4 | Conical bottom centrifuge tubes, sterile (15 ml) |

Each Site Will Need To Provide:

- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles (blood collection vacutainers will not work with other collection systems)
- Microcentrifuge tube rack
- Crushed Ice
- Gloves
- Sharps bin and lid
- Dry ice (please see the 'Packaging Instructions' section for details on quantity of dry ice needed per shipment)
- Pipettes and pipette tips
- Lidocaine (European sites only)

Coriell – Initial Supply

Each site will be initially supplied with sufficient sample collection kits along with supplemental supplies and extra lumbar puncture trays (if appropriate). Subsequent kits and/or individual kit components should be ordered from Coriell as needed using the designated website and login information provided.

Coriell – Resupply

Be sure to check your supplies and order additional materials before you run out so you are prepared for both scheduled and unanticipated visits. Please allow two weeks for kit orders to be processed and delivered.

Each individual site will be responsible for ordering additional kits from Coriell after the initial supply has been sent. *Queue* is Coriell's online database that you will use for routine interaction with the biorepository, such as ordering collection kits. Once a site is activated by MJFF, the site coordinator/s will be provided with a username and password to access the database.

- Log in to *Queue* with your username and password at:
<https://queue.coriell.org/g/>
- Under Contracts link in the upper left-hand corner of the screen, select LRRK2 Project Management
- A menu will appear; click on the Kit Request link
- Use the drop-down menu on the Kit Request page to select where the supplies should be shipped. If the shipping address(es) on the list is not the one you need, contact LRRK2@coriell.org
- Enter any special requests/remarks in the Request field
- Individual kit components can be ordered instead of complete kits (such as extra blood tubes) by entering your request in the Request field
- Click the Submit button

Site Required Equipment

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

- 4°C Refrigerated and Room Temperature Centrifuge
- -80°C Freezer
- Access to dry ice

Sample Quality Checks

In addition to tracking and reconciliation of samples, the condition and amount of samples received will be tracked by Coriell. Sites are responsible to ensure the requested amounts of each fluid are collected to the best of their ability. If a sample is not obtained for a subject, this should be recorded on the appropriate data form and a reason should be provided.

Blood Collection and Processing Procedures

It is strongly advised that the research blood samples are collected between 8-10am in a fasted state

In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the specific collection, processing and shipment procedures detailed in the following pages.

(i.e., minimum of 8 hours since last meal/food intake) to ensure the quality of samples for future analyses. If fasting is not possible, then subjects should be strongly advised to eat a low lipid diet as provided (see Appendix P for low fat menu suggestions). Please make sure to record time of last meal on the Laboratory Procedures data form (Appendix D).

Order of Blood Draws

Tubes should be filled in the following order:

- 1 × 2.5 ml PAXgene™
- 1 × 10 ml Plasma EDTA Purple Top
- 1 × 6 ml EDTA Purple Top for Whole Blood
- 1 × 10 ml Serum determination Red Top

SPECIFIC INSTRUCTIONS FOR COLLECTION AND PROCESSING OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES. See Appendices H-M that may be used for the processing of all lab samples.

Labeling Samples

In order to ensure the label adheres properly and remains on the tube, **please follow these instructions when labeling the research sample tubes with the pre-printed labels (see Appendix N diagram):**

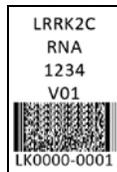
- Place labels on **ALL** collection and aliquot tubes **BEFORE** any cooling of tubes, sample collection or sample processing/freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Place label **horizontal** on the tube (wrapped around sideways if tube is upright) and **just below the ridges** of the aliquot tubes. There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.
- Take a moment to ensure the label is **completely adhered** to each tube. It may be help to roll the tube between your fingers after applying the label.

The Sample Label ID Number is the key number by which the sample and data will be tracked.

PAXgene™ RNA

See training videos for blood collection (<http://www.preanalytix.com/videos/rna-tube-collection-video/>) and freezing (<http://www.preanalytix.com/videos/rna-tube-freezing-video/>). **Note:** Although the PaxGene tube video recommends use of a 'discard tube', this is not a required step in the protocol. If your site wishes to use a discard tube, you must provide your own tube as this is not included in the kit from Coriell

1. Place pre-printed "RNA" label on the PAXgene RNA tube prior to blood draw (see 'Labeling Samples' section of the manual for details on label placement on the tube).



2. **CRITICAL STEP:** Store PAXgene™ Blood RNA Tubes at room temperature 64°F - 77°F (18°C to 25°C) before use.
3. **CRITICAL STEP:** The PAXgene™ Blood RNA Tubes should be the first tubes drawn in the phlebotomy procedure (before plasma, serum, whole blood, etc.).
4. Using a blood collection set and a holder, collect blood into the PAXgene™ Blood RNA Tube using your institution's recommended procedure for standard venipuncture technique.

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.
5. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5ml of blood into the tube. Record time of draw on the Laboratory Procedures data form (Appendix D).
 6. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8 – 10 times.
 7. **CRITICAL STEP:** Incubate the PAXgene™ Blood RNA Tube UPRIGHT at room temperature (18°C to 25°C) for 24 hours.

If blood is drawn on a Friday and you are unable to return on Saturday to place tubes in the freezer, transfer the tubes as late as possible before leaving on Friday. Samples must sit at room temperature for a minimum of 2 hours.

8. After **24 hours** at room temperature, transfer the PAXgene tube to -80°C (minus eighty) freezer. **Keep the PAXgene™ Blood RNA Tube at -80°C until you ship on dry ice.** Record date and time when samples are placed in freezer as well as the storage temperature on the Laboratory Procedures data form (Appendix D).

Plasma EDTA 10 ml Purple Top

1. Place pre-printed "Plasma" label on the 10ml EDTA tube prior to blood draw (see 'Labelling Samples' section of the manual for details on label placement on the tube).

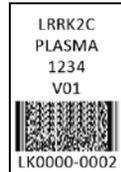


2. **CRITICAL STEP:** Store empty Plasma Separation Tube at room temperature 64°F - 77°F (18°C to 25°C) before use.
3. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tube** using your institution's recommended procedure for standard venipuncture technique.

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. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube. Record the time of plasma collection on the Laboratory Procedures form (Appendix D).
 5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.
 6. Within 30 minutes of plasma collection, centrifuge tube (with appropriate balanced tube) at 4°C for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.**
 - Equivalent rpm for spin at 1500 x g = _____
 - While centrifuging record the start time, rate, duration and temperature of centrifugation on the Laboratory Procedures data form (Appendix D).

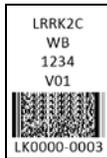
7. Place pre-printed "Plasma" labels on three of the 2-ml microcentrifuge tubes prior to cooling or filling tubes.



8. Using a clean transfer pipette (micropipette preferred), transfer at least 1.5 ml aliquots of blood plasma (top layer) into each labeled aliquot tube. The EDTA tube should yield, on average, 4.5 ml of blood plasma for a total of 2-3 aliquot tubes per subject. Please fill each aliquot to the maximum capacity before attempting to fill an additional aliquot. Record the total volume of plasma aliquotted and number of tubes on the Laboratory Procedures form (Appendix D).
9. Discard the used EDTA purple top tube according to site guidelines for disposing of biomedical waste.
10. Freeze and store samples immediately at -80°C Freezer. Record the time that aliquots were placed into freezer and storage temperature on the Laboratory Procedures data form (Appendix D).

Whole Blood 6 ml EDTA Purple Top

1. Place pre-printed "Whole Blood" label on the **6 ml EDTA tube** prior to blood draw (see 'Labeling Samples' section of the manual for details on label placement on the tube).



2. **CRITICAL STEP:** Store empty Whole Blood EDTA tubes at room temperature 640F - 770F (18°C to 25°C) before use.
3. Using a blood collection set and a holder, collect whole blood into **6 ml purple top whole blood tube** using your institution's recommended procedure for standard venipuncture technique. Record time of the Whole blood draw on the Laboratory Procedures form (Appendix D).

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. Invert the tube gently 3 times.
 5. Transfer the tube immediately to **-80°C Freezer**.

Serum Determination Red Top

1. Place pre-printed "Serum" label on the 10ml Serum tube prior to blood draw (see 'Labelling Samples' section of the manual for details on label placement on the tube).

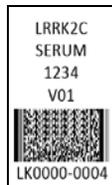


2. **CRITICAL STEP:** Store empty Serum Determination Tube at room temperature 64°F - 77°F (18°C to 25°C) before use.
3. Using a blood collection set and a holder, collect blood into the **serum determination (red top) tube** using your institution's recommended procedure for standard venipuncture technique.

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. Allow at least 10 seconds for a complete blood draw to take place in the tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube. Record time of draw on Laboratory Procedures data form (Appendix D).
 5. **CRITICAL STEP:** Immediately after blood collection, gently invert/mix (180 degree turns) the serum determination tube 8 – 10 times.
 6. **CRITICAL STEP:** Allow blood to clot at room temperature for at least 15 minutes.
 7. Within 30 minutes of serum collection (after 15 minutes of clotting at room temperature), centrifuge balanced tubes at 4°C for 15 minutes at 1500 x g. It is critical that the tubes be centrifuged at the appropriate speed to ensure proper serum separation.
 - Equivalent rpm for spin at 1500 x g = _____
 - While centrifuging record the start time, rate, duration and temperature of centrifuge on the Laboratory Procedures data form (Appendix D).

Place pre-printed "Serum" labels on three of the 2-ml microcentrifuge tubes prior to cooling or filling tubes.



8. Using a clean transfer pipette, transfer approximately 1.5 ml aliquots of serum into each labeled aliquot tube. The serum tube should yield, on average, 4.5 ml of blood plasma for a total of 2-3 aliquot tubes per subject. Please fill each aliquot to the maximum capacity before attempting to fill an additional aliquot. Record the total volume of serum aliquoted and number of tubes on the Laboratory Procedures form (Appendix D).
9. Discard the used red top tube according to site guidelines for disposing of biomedical waste.
10. Within 60 minutes of serum collection, freeze and store samples immediately in a -80°C Freezer. Record the time that aliquots were placed into freezer and storage temperature on the Laboratory Procedures data form (Appendix D).

Additional Blood Draws

Your site may be collecting additional blood above and beyond that requested as part of the MJFF minimal bio-sample requirements. MJFF samples should be collected first and should be separate from any additional blood draws you perform.

Urine Collection and Processing Procedures

1. Write the Sample Label Identification Number matching the pre-printed label on the side of one urine collection cup prior to urine collection. Ask study subject to collect urine specimen in the collection cup. Urine should be collected midstream and all attempts made to collect this sample in as sterile a manner as possible. Record the date and time of urine collection on the Laboratory Procedures form (Appendix D).
2. Pour about 10ml of the urine specimen into one 15-ml centrifuge tube and cap with the screw-cap. Ensure tube has a pre-printed "Urine" label (see 'Labeling Samples' section of the manual for details on label placement on the tube).



3. Within 30 minutes of collection, centrifuge the tube at 4°C. Spin for 15 minutes to remove sediment and cells at 2500 x g.
 - Equivalent rpm for spin at 2500 x g = _____
 - While centrifuging record the start time, rate, duration and temperature of centrifuge on the Laboratory Procedures data form (Appendix D)
4. Place pre-printed "Urine" label on a fresh 15 ml conical tube (orange top). Using a clean transfer pipette carefully transfer urine from the 15 ml centrifuge tube into the new 15 ml conical tube labeled "Urine", then firmly cap with the orange screw cap.



5. After the urine aliquot has been transferred to the conical tube and capped, place the labeled tube upright in dry ice and **allow the urine sample to completely freeze**.
6. Within 60 minutes of urine collection, freeze and store samples immediately in -80°C Freezer. Record time urine sample placed in freezer on the Laboratory Procedures data form (Appendix D).

Cerebrospinal Fluid Collection

It is recommended that CSF is collected in the morning between 8 am – 10 am, preferably fasted. Record time of last meal on the Lumbar Puncture data form (Appendix E).

Lumbar Puncture Supplies

The lumbar puncture tray contains the following items which will be used to perform lumbar puncture. If lidocaine is included in the kit, please check the expiration dates. Supplies for collection and shipment of CSF are sent in a separate kit to sites from Coriell.

Lumbar Puncture Tray:

| Quantity | Kit Component |
|----------|---|
| 1 | Pencil-point spinal needle, 24G x 90mm |
| 1 | Introducer needle, 1mm x 30mm |
| 1 | Hypodermic needle, 22G x 1.5" |
| 1 | Plastic syringe (3 ml, leur lock) with 25G x 5/8" needle attached |
| 4 | Polypropylene syringe (6 ml, leur 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 |

†US trays only

LP Kits - Initial and Resupply

Sites that have been approved to collect CSF will be initially supplied with sample kits. Subsequent trays should be ordered from Coriell when needed (see 'Coriell – Resupply' Section for instructions to access Queue).

Setting up for the LP

On an overbed table, remove the contents of the LP kit from outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the LP is seated, and begins examining the subject.

Feel the outside of the LP kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the LP and begin unwrapping the kit.

Touch only the outside of the paper wrapper. When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside. If you touch any part of the inside of the paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.

Maintaining the sterile field

Keep in mind that there is usually a lot of staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile, and keeping people away from it, and reminding people to be careful around it. If anybody touches the inside of the paper wrapper or any part of the contents of the kit, throw the kit away and start over. If you are in doubt as to whether someone touched the kit, throw it away and start over. Also, you are the monitor for whether the person performing the LP has broken sterility – usually by touching something not sterile with a sterile gloved hand. Feel free to be the boss of people if need be. Be assertive.

Tips for clinicians performing lumbar puncture

Optimizing patient comfort and minimizing risk of adverse events.

- Talk the patient through the procedure - no surprises.
- Use of a 24g atraumatic pencil-point (or Sprotte) spinal needle and careful technique are optimal for reducing post-LP headache risk. A needle such as Spinocan, 22g or 24g may also be used.
- Use adequate local anesthesia. Use the 25g 1.5" needle and inject lidocaine to raise a skin wheal. Then inject lidocaine using the pattern of a square - first the center and then to all 4 corners. If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24g pencil-point spinal needle).
- Increasing fluid intake immediately after LP is helpful.
- Be sure to give post-LP care instructions verbally to subject.

Post-LP Care Instructions

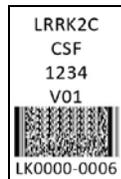
- Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity or any other strenuous activities) for 24 hours after the LP.
- Advise the subject to continue with increased fluid intake.
- Mild to Moderate headache after a lumbar puncture:
 - Mild to moderate headache following lumbar puncture usually resolves within 3-4 days.
 - Treatment of Mild to Moderate headache includes:
 - Limiting physical activity as much as possible
 - Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee (which has some diuretic activity).
 - Tylenol should be used for symptomatic relief. If a subject cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or equivalent could be considered.

- Severe headache after a lumbar puncture:
 - If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus and/or visual disturbances, the subject should contact the site study staff for further instruction per standard clinical care.

Detailed Lumbar Puncture Procedure

Remember, CSF is processed at Room Temperature [64°F - 77°F (18°C to 25°C)]

1. Place pre-printed "CSF" label on the collection and aliquot tubes prior to cooling or filling. Prepare at least 5 aliquot tubes based on the collection of 10 mls of CSF.



2. Place aliquot tubes on ice prior to procedure (but after labeling) so they are pre-cooled.
3. Perform lumbar puncture using the atraumatic technique.
4. Collect CSF into syringes and transfer to 15 ml conical polypropylene tube at room temperature. Mix gently by inverting 3-4 times. Record time (i.e., once collection is complete) and volume of draw on the Lumbar Puncture data form (Appendix E).
5. Within 15 minutes of collection, spin the CSF sample down at 2000 x g for 10 minutes at **Room Temperature [64°F - 77°F (18°C to 25°C)]**
 - a. Equivalent rpm for spin at 2000 x g = _____
 - b. While centrifuging record the time of centrifuge start, rate and temperature on the Lumbar Puncture data form (Appendix E).
6. Pipette (micropipette preferred) at least 1.5 ml of supernatant directly into pre-cooled polypropylene CSF collection aliquot tubes. This will yield, on average, 5 aliquot tubes per subject. Please fill each aliquot to the maximum capacity before attempting to fill an additional aliquot. Record the time the sample was aliquoted, the total volume of CSF aliquoted and number of tubes on the Lumbar Puncture form (Appendix E).
7. Within 60 minutes of CSF collection, freeze aliquots immediately on dry ice and store at -80°C or ship on dry ice in shipping container. Record the time samples were placed into freezer and storage temperature, as well as remainder of the Lumbar Puncture data form (Appendix E).

Shipping and Tracking Instructions

Remember to complete the Sample Record Summary and Shipment Notification Form (Appendix B). Include a copy of the form with your shipment AND notify Coriell IN ADVANCE to confirm the shipment.

For US sites, frozen samples should be shipped **Monday – Wednesday**.

BE AWARE OF HOLIDAYS!!!

See shipping instructions for US and International sites in Appendix F. Please keep the following in mind as you prepare the sample shipments:

1. Complete the Sample Record Summary and Shipment Notification form. Only one specimen type per row should be listed. Multiple subjects and sample types may be included on this form. Use multiple forms as needed.
2. Once completed, package the samples in the return box and include a copy of the Sample Record Summary and Shipment Notification form in the package. **Please ensure package has sufficient dry ice to keep package cold for about one week (as a minimum 40lbs/18kg for each provided shipping container from Europe, and approximately 25lbs/11.5kg from the US). In order to allow sufficient dry ice, do not include more than two subject visits worth of samples per return box.**
3. Provide copy of Shipment Notification to Coriell via email (LRRK2@coriell.org) or fax (856-966-5067; International: 00 +1 856-966-5067) to give advance notice that you are sending samples. Ensure tracking number is indicated.
4. Ship package via appropriate carrier service for your site.

Data Queries and Reconciliation

The Laboratory Procedures (Appendix D) and Lumbar Puncture (Appendix E) data forms must be completed on the day that samples are collected. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses. Electronic PDF copies of these forms should be emailed to CTCC (LRRK2CLAB@chet.rochester.edu) ideally on the day the subject visit is completed or no later than 48 hours later.

The Clinical Trials Coordination Center (CTCC) will be collaborating with Coriell to reconcile information submitted to CTCC with samples received and logged at Coriell. Information that appears incorrect in the CTCC database will be queried. Additional discrepancies that may be unrelated to data entry will be resolved with sites in a separate follow up communication.

Data queries or discrepancies with samples shipped versus received at Coriell may result from:

- Missing samples at Coriell
- Incorrect samples collected and shipped to Coriell
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Sample Record Summary and Shipment Notification Form and logged at Coriell compared to information entered into the CTCC database.

Appendices

| | |
|-------------|--|
| Appendix A: | Rate of Centrifugation Worksheet |
| Appendix B: | Sample Record Summary and Shipment Notification Form |
| Appendix C: | CTCC Unique ID Number |
| Appendix D: | Laboratory Procedures Form |
| Appendix E: | Lumbar Puncture Form |
| Appendix F: | U.S. Sites – Coriell Detailed Domestic Shipping Instructions |
| Appendix G: | USDA Customs Declaration Form |
| Appendix H: | PAXgene™ RNA Processing Diagram |
| Appendix I: | Plasma EDTA Processing Diagram |
| Appendix J: | Whole Blood EDTA Processing Diagram |
| Appendix K: | Serum Processing Diagram |
| Appendix L: | CSF Processing Diagram |
| Appendix M: | Urine Sample Processing Diagram |
| Appendix N: | Aliquot Tube Labeling Diagram |
| Appendix O: | Summary of Shipment Process |
| Appendix P: | Low Fat Diet Menu Suggestions |
| Appendix Q: | Non-Conformance Report |

Appendix A: Rate of Centrifugation Worksheet

Please complete and return this form (by email or fax) to the LRRK2 Clinical Cohort Coordinator Matthew Read if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your LRRK2 Biologics Manual.

Submitter Information

Name:

Site Number:

Submitter Email:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type:

Fixed Angle Rotor

Swing Bucket Rotor

Radius of Rotation (mm): _____

Determine centrifuge's radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Comments:

Fax this form to:

Matthew Read

+44 (0) 1256 316 552

Or

Email to mread@prnservices.co.uk

**It is very important to this study that all samples be processed correctly.
Please call with any questions at + 44 (0) 1256 316 591.**

Appendix B: Sample Record Summary and Shipment Notification Form

Site Number:

Principal Investigator:

Coordinator:

Telephone:

Email:

Date Sample(s) Shipped:

Instructions: US sites should ship Monday – Wednesday ONLY! International sites should ship Monday-Tuesday ONLY! This form must be completed for shipment of all research samples. Notify Coriell (email, fax or phone) in advance of shipment using contact information below. Place a copy of this form in the shipment box and file a copy of the completed form with your site’s study information. Site will be contacted should there be issues with samples noted upon receipt or shipment did not include this form.

Please be sure to list the Sample Label ID Number that corresponds to the pre-printed labels. List only one “Specimen Type” per row.

| Completed by Submitter/Site | | | | | | | Completed by Repository | |
|-----------------------------|---|--|-----------------------------|------------|--------|--------------|-------------------------|----------------------|
| Sample Label ID Number | Subject Unique CTCC Number (if collected) | Specimen Type (RNA, Whole Blood, Urine, CSF, Plasma, or Serum) | Visit Type (V01, V02, etc.) | # of Tubes | Gender | Date of Draw | Repository ID Number | Notation of problems |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| Total number of tubes: | | | | | | | | |

Courier (check one):

FedEx

DHL

Other (specify):

Coriell Contact Information:

LRRK2@coriell.org

Fax: (00 +1) 856-966-5067

Ph: (00 +1) 856-757-9756

Please email a copy of this form
To Rochester Data Centre:

LRRK2CLAB@chet.rochester.edu

Tracking Number:

Version: 1.2

FINAL: January 2012

29

**MJFF LRRK2 COHORT CONSORTIUM (LRRK2C)
LUMBAR PUNCTURE**

| | | | | | | | | | | | | | | | |
|------------|---|---|--|--|--|--|--|--|--|--|--|--|----------|---|--|
| 1 | 3 | 5 | | | | | | | | | | | 0 | 4 | |
| SUBJECT ID | | | | | | | | | | | | | VISIT NO | | |

22. Was a fluoroscopy performed? (0 = No, 1 = Yes) 22.

22a. Date of fluoroscopy: 22a.
MM DD YYYY

23. Was a lumbar spine film performed? (0 = No, 1 = Yes) 23.

23a. Date of spine film: 23a.
MM DD YYYY

24. Comments:

Complete and email to: LRRK2CLAB@chet.rochester.edu

Appendix F: U.S. Sites – Coriell Detailed Domestic Shipping Instructions



LRRK2 Cohort Consortium Shipping Instructions / Domestic

BIOLOGICAL SAMPLE COLLECTION, PACKAGING AND SHIPMENT TO CORIELL: LRRK2 COHORT CONSORTIUM US SITES

SUBJECT VISIT KIT

- Frozen 2ml aliquots of serum & plasma
- Frozen 2ml aliquots of CSF (designated sites only)
- Frozen 15ml tube of urine
- Frozen PAXgene tube
- Frozen 6 ml plastic EDTA tube

IMPORTANT!

FROZEN SAMPLES MAY BE SHIPPED MONDAY-WEDNESDAY ONLY!
Only ONE set of samples may be shipped in a single package.

1. Contact FedEx to confirm service is available and schedule package to be picked up.
2. If your site is located outside of the US, print the Customs Declaration Template (see LRRK2 Biologics Manual Appendix G) on your institution's letterhead and complete all fields.
3. Notify the Coriell Institute of shipment by emailing lrrk2@coriell.org (preferred) or faxing (856-966-5067) a copy of the completed Sample Record Summary and Shipment Notification Form.
4. Place all frozen 2ml aliquots in the provided cardboard cryobox. Label the outside of the cryobox with the same subject ID and visit number as the pre-printed labels on the sample tubes.
5. Place the cryobox in a clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.
6. Insert frozen EDTA, PAXgene and urine tubes into the provided bubble wrap pouch.
7. Place bubble-wrapped tubes into the 2nd clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.
8. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:





LRRK2 Cohort Consortium Shipping Instructions / Domestic

9. Fill the remaining space in the shipping carton with dry ice, ensuring ice surrounds both envelopes and reaches the top of the carton, as shown below:



10. Place the completed Sample Record Summary and Shipment Notification Form in the package, close the Styrofoam carton, and close and seal the outer cardboard shipping carton.
11. Apply all provided warning labels and the FedEx air bill to outside of package. The net weight in Kg of the dry ice must be recorded on both the class 9 dry ice label and on the air waybill; otherwise FedEx is likely to return the shipment.
12. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

Appendix G: USDA Customs Declaration Sheet

Detailed description of material:

Frozen human blood specimens: Plastic vials or glass tubes (please circle)
of vials/tubes _____
of ml per vial/tube ____

Frozen human plasma specimens: Plastic vials or glass tubes (please circle)
of vials/tubes _____
of ml per vial/tube ____

Frozen human serum specimens: Plastic vials or glass tubes (please circle)
of vials/tubes _____
of ml per vial/tube ____

Frozen human urine specimens: Plastic vials or glass tubes (please circle)
of vials/tubes _____
of ml per vial/tube ____

Frozen human spinal fluid (CSF) specimens: Plastic vials or glass tubes (please circle)
of vials/tubes _____
of ml per vial/tube ____

Human material containing no animal material.

Material not obtained from humans that have been exposed to any livestock or poultry disease agent exotic to the United States.

This material is not of tissue culture origin.

Biological Substance, Category B UN3373

NON-INFECTIOUS

For research only

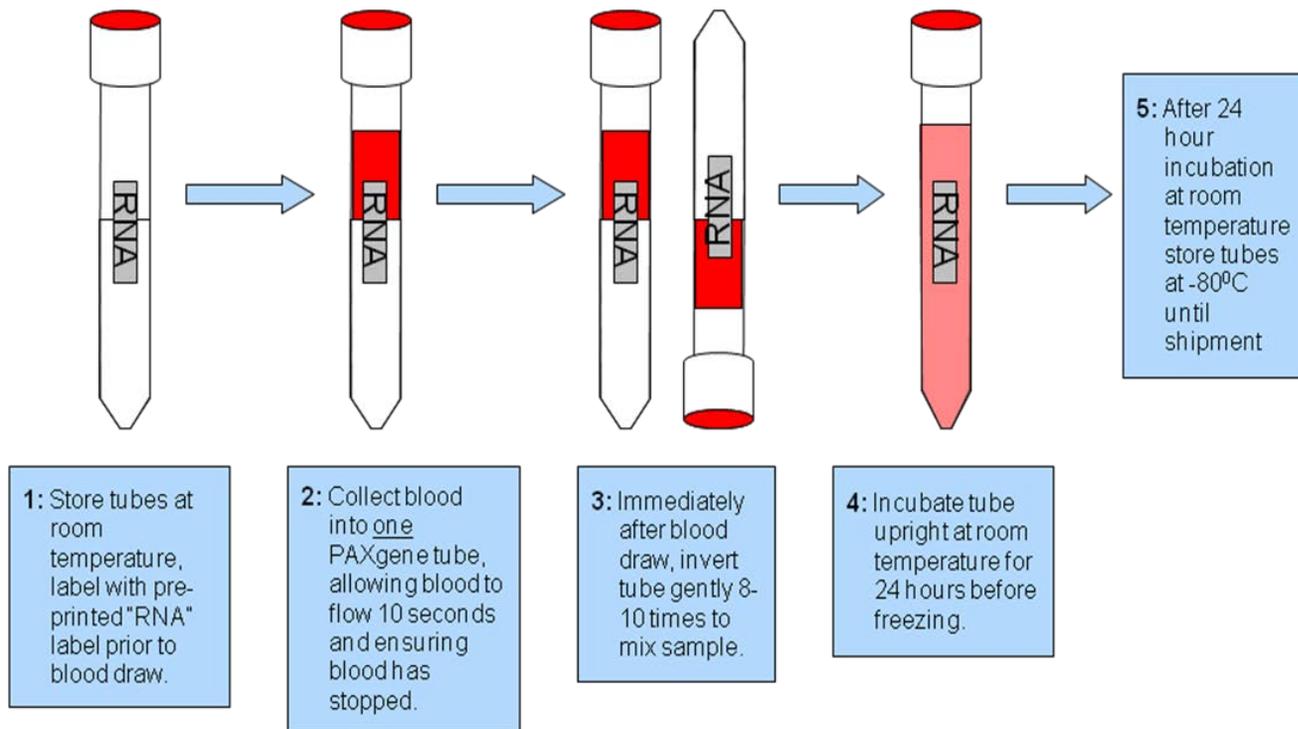
For in vitro use only

Not for plant or animal use

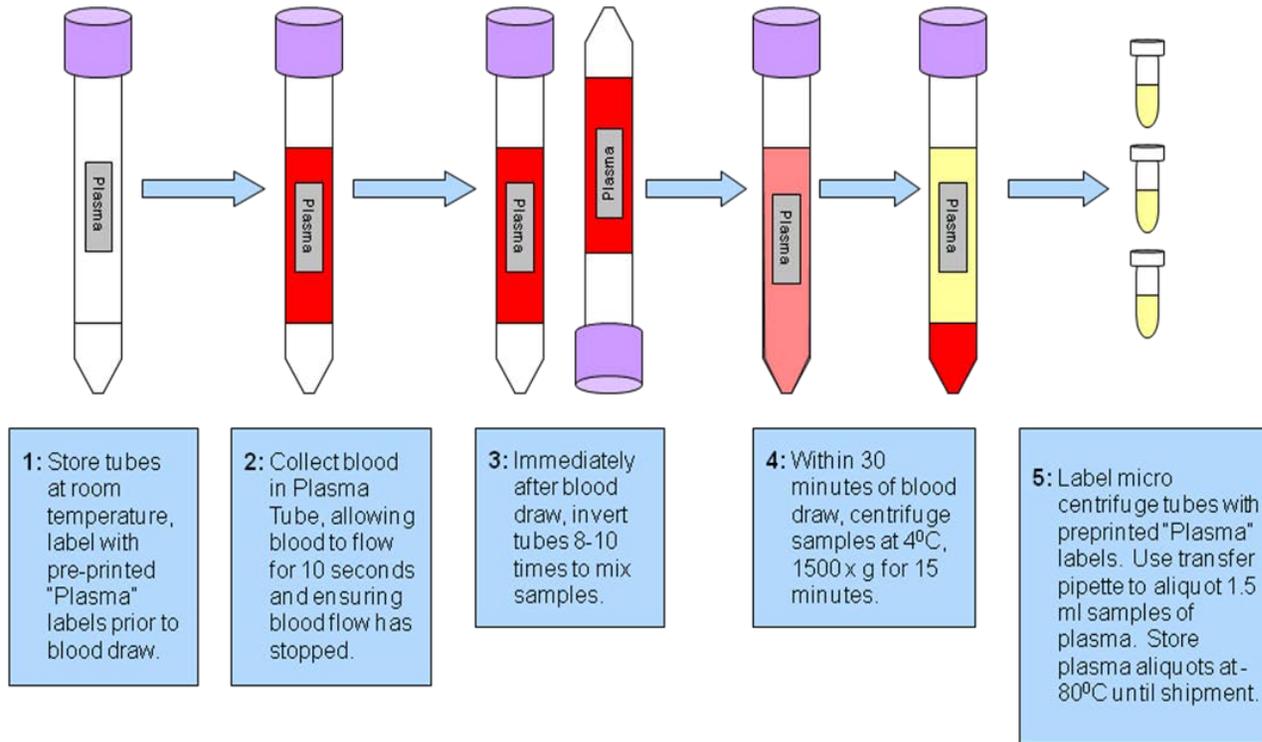
No commercial value

Signature:

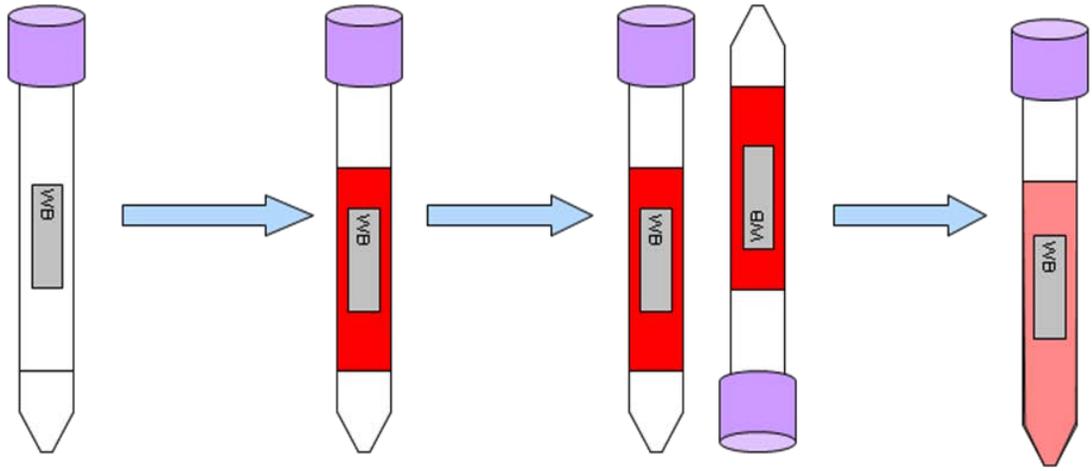
Appendix H: PAXgene™ RNA Processing Diagram



Appendix I: Plasma EDTA Processing Diagram (10ml purple top tube)



Appendix J: Whole Blood EDTA Processing Diagram (6ml purple top tube)



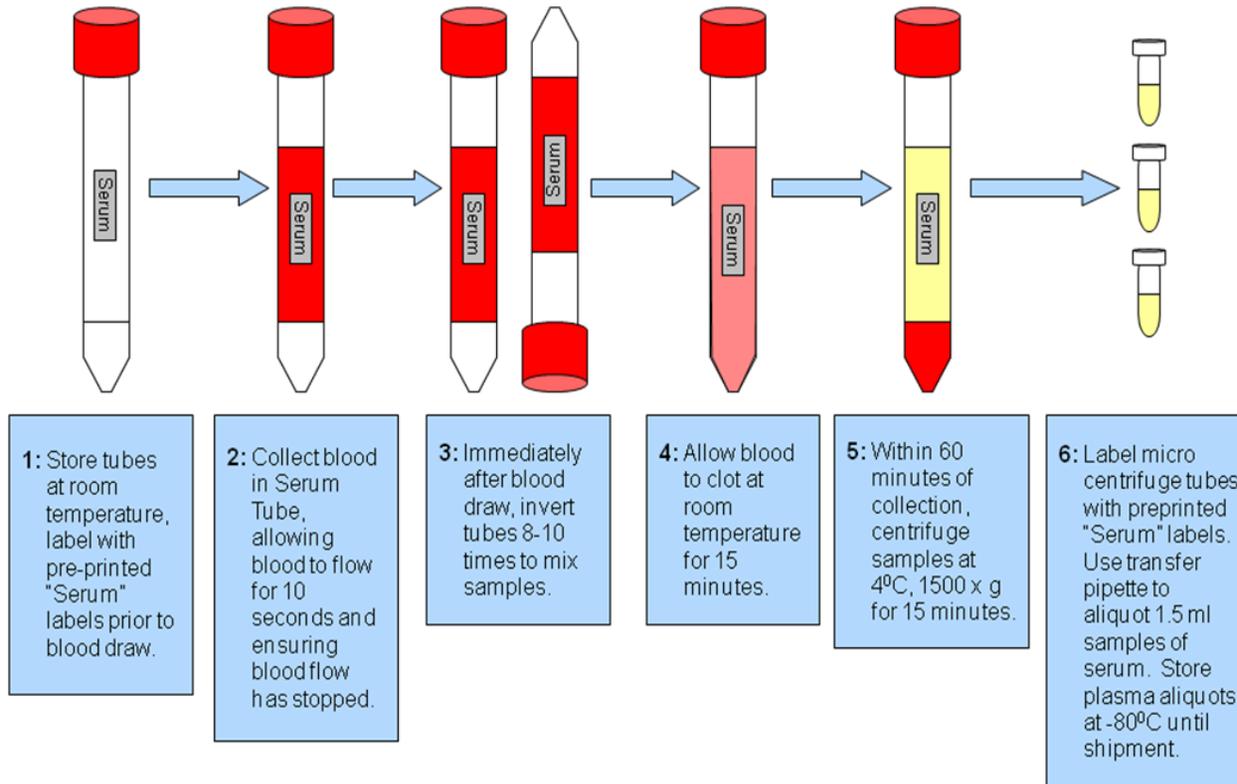
1: Store tube at room temperature, label with pre-printed "WB" label prior to blood draw.

2: Collect blood, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

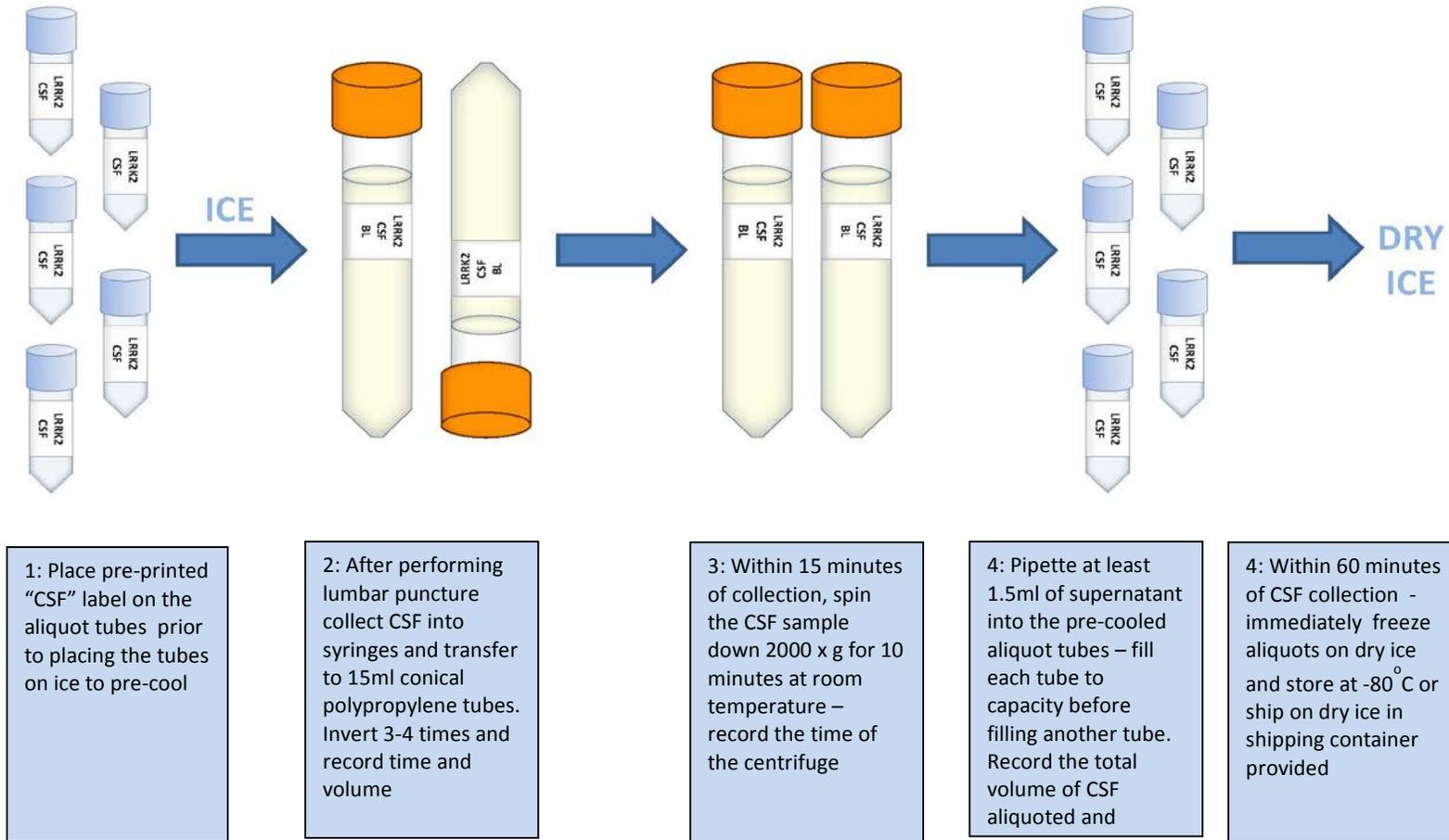
3: Immediately after blood draw, invert tube 3 times to mix sample.

4: Immediately after inversion, freeze the sample in a -80 freezer until ready to ship.

Appendix K: Serum Processing Diagram



Appendix L: CSF Processing Diagram



1: Place pre-printed "CSF" label on the aliquot tubes prior to placing the tubes on ice to pre-cool

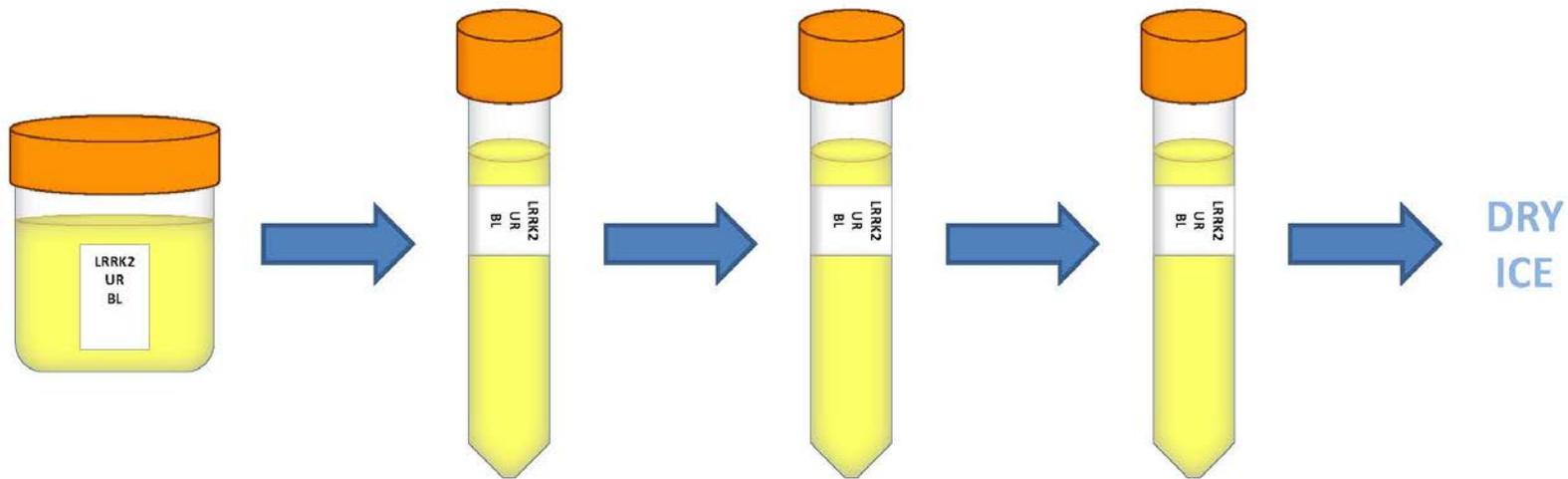
2: After performing lumbar puncture collect CSF into syringes and transfer to 15ml conical polypropylene tubes. Invert 3-4 times and record time and volume

3: Within 15 minutes of collection, spin the CSF sample down 2000 x g for 10 minutes at room temperature – record the time of the centrifuge

4: Pipette at least 1.5ml of supernatant into the pre-cooled aliquot tubes – fill each tube to capacity before filling another tube. Record the total volume of CSF aliquoted and

4: Within 60 minutes of CSF collection - immediately freeze aliquots on dry ice and store at -80°C or ship on dry ice in shipping container provided

Appendix M: Urine Sample Processing Diagram



1: Write the Subject Sample Identification Number matching the pre-printed label. On the urine collection cup. Record the date and time of collection.

2: Pour approximately 10ml of the urine specimen into one 15ml centrifuge tube and ensure a pre-printed label is attached.

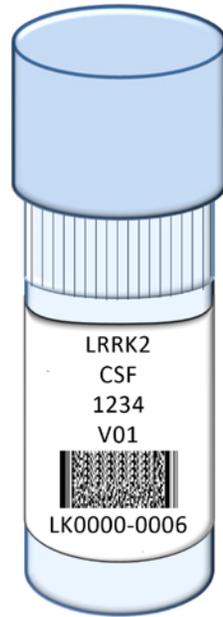
3: Within 30 minutes of collection, spin the urine sample down 2500 x g for 15 minutes at 4°C—record the temperature and time of the centrifugation

4: Place a pre-printed label on a fresh 15ml conical tube. Using a clean transfer pipette, transfer 15ml of urine from the centrifuge tube and secure with the orange screw cap

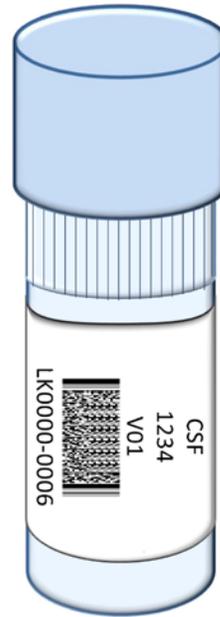
4: Place the urine aliquot in dry ice and allow the sample to completely freeze. Within 60 minutes of urine collection freeze and store samples in a -80°C freezer, recording the time the sample was placed in the freezer.

Appendix N: Aliquot Tube Labeling Diagram

Comment [MR1]: Can we label format to this diagram,

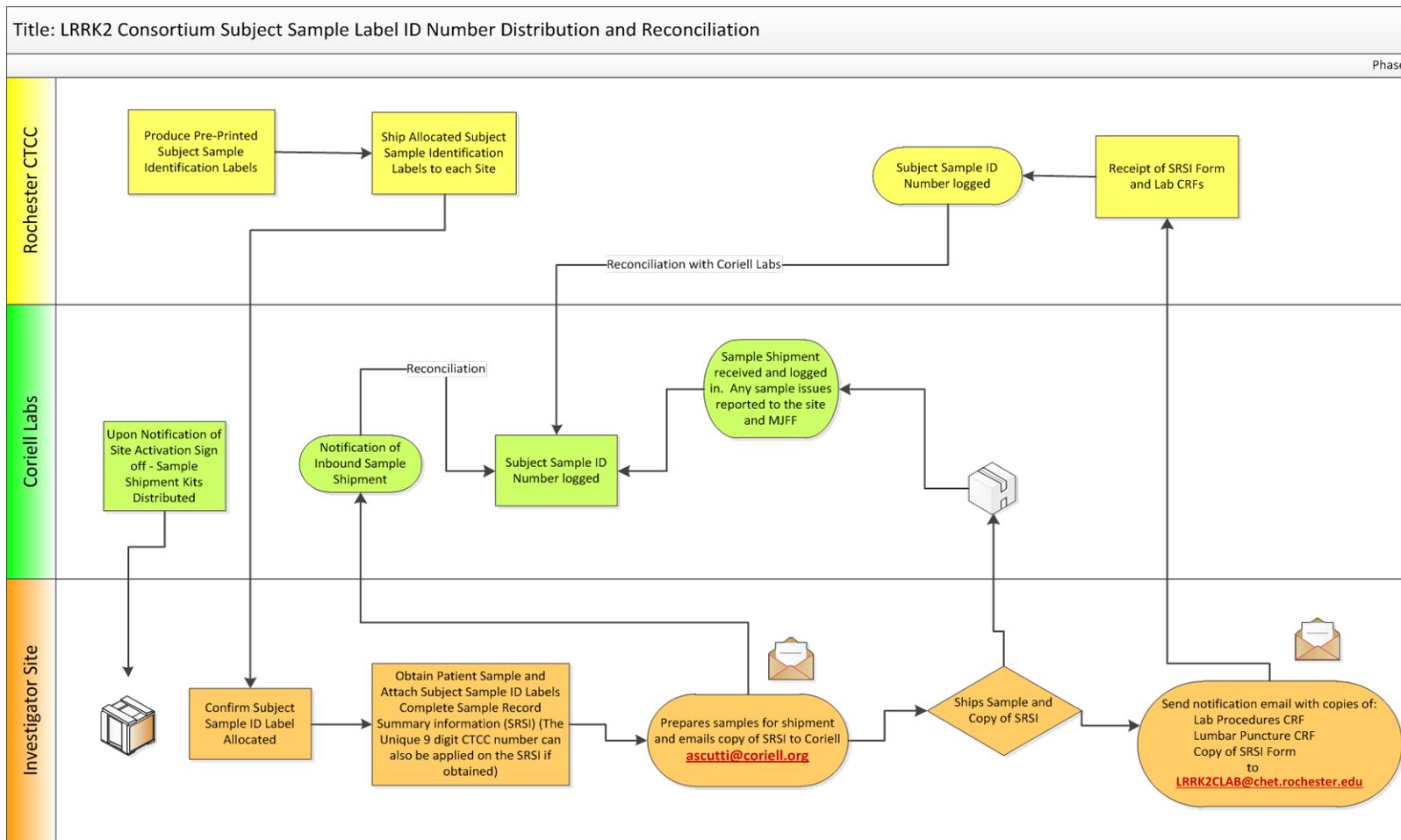


Incorrect



Correct

Appendix O: Summary of Shipment Process



Appendix P: Low-Fat Diet Menu Suggestions



PARKINSON'S
PROGRESSION
MARKERS
INITIATIVE

Play a Part in Parkinson's Research.

Low-Fat Diet Menu Suggestions

It is very important that you are fasted (no food and only drinks such as water, clear tea or black coffee) for at least 8 hours before your PPMI study visit for blood collection. However, we understand that fasting may not always be possible. If you are not able to fast, we ask that you choose from the suggested food items below. The PPMI study staff may provide additional suggestions for you.

Foods that are allowed prior to blood collection:

Sample Breakfast Items:

Dry whole-wheat toast
Fruit salad – no dressing
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Dry cereal – (without nuts/ no granola;
no milk)
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Plain oatmeal or other cooked whole
grain cereal
Topped with fresh or dried fruit (no
butter, milk or cream)
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Dry whole-wheat toast
Poached egg-whites or egg substitute
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Sample Lunch Items:

Turkey breast sandwich on whole wheat bread
Lettuce and Tomato and Mustard
Clear beverage
Flavored gelatin

Plain pasta with plain marinara sauce– no
butter or cheese
Side of steamed vegetables or green salad
Clear beverage
Flavored gelatin

Steamed chicken breast (lean, without skin)
Side of steamed vegetables or green salad
Clear beverage
Flavored gelatin

Large tossed green salad with assorted
vegetables (no dressing or cheese)
Clear beverage
Flavored gelatin

Cucumber sandwich on whole-wheat bread
Lettuce, tomatoes, shredded carrots, onions or
other vegetables
Clear beverage
Flavored gelatin

Clear broth with vegetables and pasta
Fruit salad – no dressing
Clear beverage
Flavored gelatin

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 cheeses
- All products containing cheese
- Cheeses spreads such as cream 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
- 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
- Fried snacks such as:
 - Chips
 - Crackers
 - French fries
- Gravies and sauces
- Baked goods & frosting

Appendix Q: Non-Conformance Report



This form is to be completed by biorepository personnel when a sample has been received and issues are noted. Completed form is to be emailed or faxed to submission site coordinator and cc Matt Read (MRead@prnservices.co.uk), Maurizio Facheris (mfacheris@michaeljfox.org) and Cindy Casaceli (Cindy.Casaceli@chet.rochester.edu).

Site #/Name: _____

Subject ID: _____ Visit Type: _____

Received by: _____ Date: _____

Your shipment was received with the observed problem(s) checked below. Please take note so that your future shipments are received without incident.

| | |
|--------------------------|---|
| <input type="checkbox"/> | Frozen samples shipped on Thursday, Friday or Saturday |
| <input type="checkbox"/> | Ambient temperature samples (e.g. whole blood for DNA) shipped on Fri or Sat |
| <input type="checkbox"/> | Advanced notice of shipment not provided |
| <input type="checkbox"/> | Shipment notification does not match Shipment Notification form received with samples |
| <input type="checkbox"/> | No Shipment Notification form included in package |
| <input type="checkbox"/> | Shipment Notification form incomplete |
| <input type="checkbox"/> | Package contents do not match Shipment Notification form |
| <input type="checkbox"/> | Package received has little/no dry ice |
| <input type="checkbox"/> | Signs of sample thawing present |
| <input type="checkbox"/> | Samples packaged improperly |
| <input type="checkbox"/> | Samples submitted in non-standard tubes |
| <input type="checkbox"/> | Sample tubes damaged/cracked |
| <input type="checkbox"/> | Samples not labeled/labels peeling off |
| <input type="checkbox"/> | CSF/SER/PL samples pink in color |
| <input type="checkbox"/> | Sample low volume |
| <input type="checkbox"/> | Unexpected sample(s) received (specify in Comments section below) |
| <input type="checkbox"/> | Multiple sample sets packaged in a single kit |
| <input type="checkbox"/> | Other (specify in Comments section below) |

Comments: _____

This form was () emailed () faxed to _____ on _____
 Name Date