Procedures for receipt/intake of samples:

Samples will be received by Dr. Venkata S Abbasetti or central laboratory staff. We request investigators to email the manifest prior to shipping the samples to make sure that sample manifest is consistent with our recommendations.

Sample manifest with sample data:

<table>
<thead>
<tr>
<th>Collection StudyName</th>
<th>BioCon ID</th>
<th>Sample Type</th>
<th>Barcode crosscheck</th>
<th>Barcode Matched</th>
<th>Our numerical code</th>
</tr>
</thead>
<tbody>
<tr>
<td>EX. REGARDS, CRIC</td>
<td>Unique sample ID</td>
<td>Ex. Plasma, urine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Expansion of the above manifest: Cells highlighted in grey will be filled in by Central Lab Personnel.

<table>
<thead>
<tr>
<th>Received Date</th>
<th>Date Used</th>
<th>Number of Thaw</th>
<th>Initial Amount</th>
<th>Amont Used</th>
<th>Current Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>volume in microliter</td>
<td>volume in microliter</td>
<td>volume in microliter</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Freezer Section Name</th>
<th>Shelf</th>
<th>Rack</th>
<th>Box</th>
<th>Row</th>
<th>Column</th>
<th>Subject Comment</th>
<th>Participant ID</th>
<th>Visit</th>
<th>Aim</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Box #</td>
<td>Row position</td>
<td>Column position</td>
<td>Comment</td>
<td>Participant ID</td>
<td>study visit</td>
<td>BioCon aim #</td>
</tr>
</tbody>
</table>

The Central Lab will interface this manifest with FreezerWorks software for sample storage.

We request

- Investigators or Repositories to ship samples either on Mondays or Tuesdays of a week through Fed Ex overnight shipping.
- There must be a sender contact phone number and email on the manifest included with the samples.
- There must prior communication with Dr. Abbasetti and confirmation by both the sender and Dr. Abbasetti that the samples are to be sent.
- Samples should be sent to the following address:

Venkata Abbasetti, PhD  
Room 550,  
Harvard Insitutes of Medicine  
4 Blackfan Circle  
Boston, MA 02115  
Phone No: 617 525 5988  
Back-up phone number 318-348-6858 (Abbasetti Cell Phone)  
Secondary back-up number: 617-525-5960 (Dr. Bonventre’s lab office number)
Upon Receipt of Samples:
- The sample manifest is reviewed and checked that all the identifying information is included
- Confirmation and documentation of frozen status of samples received will be recorded in Excel Spreadsheet Column E
- A project number is assigned to the samples and the shipment will be added to our Inventory Excel spreadsheet. The Inventory Excel spreadsheet contains columns including:

  Column A: Project Number
  Column B: Study Name
  Column C: Date that Samples are Received
  Column D: Specimen type (e.g. blood, urine)
  Column E: Samples Condition when arrived
  Column F: Number of Boxes of Samples
  Column G: Total Number of Samples
  Column H: Source of Samples (Investigators or Repository Name(s))
  Column I: Freezer and location within Freezer where the Samples will be stored
  Column J: Biomarkers to be measured
  Column K: Comments regarding Assays
  Column L: Study completion date
  Column M: Date that the Data are sent

Freezer Storage:
- Before transferring to the freezer, we randomly select 10 samples from each box of the shipment including first and last sample of each box and compare it against the manifest.
- We upload the information in the Inventory Excel spreadsheet and the information in the manifest directly into Freezersworks software (Version 8.1) which also contains information of sample ID, freezer number, rack number, boxes, location of each sample in the box.
- Samples will be transferred immediately and stored in -80°C freezers.

Freezer Monitoring:
- Temperature of freezers are monitored 24/7 with TemperatureAlert monitor system. TemperatureAlert is a cellular-based, remote temperature monitoring system that connects wirelessly to a cellular network. The system works by placing sensors in the -80°C freezers, and connecting sensors to the cellular hub which transmits temperature reading hourly to SensorCloud™.
- We use SensorCloud to monitor temperatures, configure alerts when conditions fall outside of our set ranges, and set up automated alert notifications via email and text messages to Venkata Sabbisetti (co-Director of Central Laboratory), Nandita Srayoshi (Research Assistant) Eileen O’Leary (Lab manager) and Joseph Bonventre (PI). The system provides temperature reading and alerts every hour, seven days a week.
- Fail safes incorporated into the system include Backup battery included (48 hour lifespan) in case of power failure (freezers have emergency power access). In addition, if there is a missed temperature reading, Venkata Sabbisetti and lab staff will receive email and text message notifications.
- If failure occurs during holidays/weekend, central lab staff will come on-site to address issue as specified above.
In addition, the freezers are also equipped with their own temperature monitoring system and local alarms. Temperature data can be downloaded for up to 2 years.

Our threshold for investigating and manually monitor freezers is -65 °C. Once lab staff receive this notification, they will physically examine the freezer and start an independent log of temp readings, with in-person checks hourly. If the temperature goes back to -80 °C within the business day we then resume reliance on remote monitoring notifications. If the temperature continues to rise as indicated by our manual log or the SensorCloud notification to -65 °C,
- Dr Sabbisetti and the PI are notified
- samples are moved to a back-up freezer. We currently have 9 freezers with 2 back-up freezers
- repair service is called for onsite service

Sample Management:

- We use Freezerworks version 8.1 software to manage samples. Freezerworks is on Partners Healthcare network system and both Venkata Sabbisetti and Nandita Srayoshi have access to Freezerworks.
- The information in Freezerworks is stored in two data centers and backed-up off-site every night.
- Once the data are transferred and compiled, we will again cross check the numerical number on the tube with the barcode scan.

Sample Analysis:

- In addition to scanning of the barcode on each sample, each sample tube will be numerically labeled for cross verification on the day of sample analysis. These values will be populated into column D (Barcode Cross Check) and Column E (Our Numerical Code) in the manifest. The marking is done on the tube cap using a permanent marker. The manifest will be updated with this unique number along with its barcode. This allows us to cross check the same sample tube in two different ways.
- On the day of analysis samples from the original tube will be thawed and aliquoted in to 96 well V bottom Corning plastic plates on ice and immediately sealed with a 96 well rubber sealing mat. The amount of volume dispensed into 96 well plates will be dependent on the number of markers being measured. For 7-plex measurement of markers in plasma sample, 20 µl of plasma sample will be transferred and diluted to 1:5 dilution using “sample dilution” (SD) buffer.
- We transfer a total of 35 samples onto each plate. This allows us to do multiple dilutions at a faster pace (to measure markers which require different dilutions) using automated pipetting systems and also eliminates multiple freeze thaw cycles of samples. The order we aliquot the samples on the plate will be recorded in the notebook.

Assay Order:

- The order that the samples are run will depend upon availability of the samples in the lab and the priority set by the BioConII steering committee.
- Before we start the analysis of the samples, we communicate with the primary investigator associated with the samples to be sure that there is no new information that should modify
priorities

**Data Dispatch:**

The analysis of markers will be done according to SOPs for each panel, and data will be populated to the manifest for each marker. We include biomarker marker levels and %CV in the manifest.