Isolation of Plasma – Undiluted, Pre-Ficoll

Equipment:
- Benchtop centrifuge (Allegra X-15R, Beckman Coulter)
- Pipette Gun (Drummond)

Materials:
- Heparin Green Top Tube (Fisher, #367874)
- 1.8mL Cryotube vials (Fisher, #375418)
- 15mL conical vial (Fisher, #1495949B)
- 2mL, sterile, serological pipettes (Fisher, #356507)

Reagents:
- Ca+ and Mg+ Free PBS (Invitrogen, 10010-049)

Procedure:
1. Collect whole blood from subject into Heparin Green Top Tube
2. Centrifuge collection tubes at 200 x g for 10 minutes at RT.
3. Carefully remove collection tubes from centrifuge; plasma is the top layer above the rest of the whole blood layer.
4. Using caution not bring up the remaining whole blood layer, remove 2.5mL of plasma into a 15mL conical vial using a pipette gun and a 2mL sterile, serological pipette.
   a. If continuing on to isolate PBMCs, replace the plasma removed with an equal amount of PBS in the tubes.
5. Centrifuge the isolated plasma at 1,000 x g for 10 minutes.
6. Aliquot the plasma supernatant (refer to Appendix A for aliquot quantities and volumes) into tubes labeled with appropriate de-identified cryogenic labels.
7. Freeze at -80°C.
Appendix A – HIMC Aliquot Guidelines

<table>
<thead>
<tr>
<th>Total Plasma Volume</th>
<th>Standard Volume/Aliquot</th>
<th>Required # of vials</th>
<th>Minimum Volume per aliquot</th>
<th>Maximum Volume per aliquot</th>
</tr>
</thead>
<tbody>
<tr>
<td>up to 3ccs</td>
<td>120uL</td>
<td>4</td>
<td>120uL</td>
<td>200uL</td>
</tr>
<tr>
<td>3-5ccs</td>
<td>200uL</td>
<td>4</td>
<td>120uL</td>
<td>200uL</td>
</tr>
<tr>
<td>&gt; 5ccs</td>
<td>350uL</td>
<td>4</td>
<td>200uL</td>
<td>200uL</td>
</tr>
</tbody>
</table>

***Plasma should be processed according to HIMC SOP. Harvest plasma prior to Ficoll and before diluting whole blood. Provide a second spin on isolated plasma prior to aliquotting.

***Flu Studies: Additional aliquots of plasma may be required for other collaborators. Refer to Flu Distribution chart for those details and volume requirements.