## COMET Trial

### Timing and bottles

<table>
<thead>
<tr>
<th>Time from randomisation</th>
<th>Relation with cooling</th>
<th>Bottles and blood</th>
</tr>
</thead>
</table>
| 0 hours                 | Before start of cooling | 1. 0.5 ml in PAXgene tube  
                        |                       | 2. 0.5 ml in EDTA (Neonatal tube) |
| 80 hours                | After re-warming is complete (if in the cooling arm) | 1. 0.5 ml in PAXgene tube  
                        |                       | 2. 0.5 ml in EDTA (Neonatal tube) |

*Body temperature can affect Gene expression. Hence it is important that all babies are normothermic at the time of blood sampling.*

<table>
<thead>
<tr>
<th>Purpose</th>
<th>PAXgene sample</th>
<th>EDTA sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vial to be used</td>
<td>Select the PAXgene tube (fluid filled tube with black marked line) from the blood collection bag</td>
<td>Select small EDTA tube (purple) from the blood collection bag</td>
</tr>
</tbody>
</table>
| Volume of blood | - Add 0.5mls of blood to the vial with PAXgene solution.  
- The total volume should reach exactly the black line on the vial  
- The PAXgene solution to blood ratio is important so do not under or overfill the tube | - Add 0.5 ml of blood to the tube |
| Mixing | - Close the tube & invert gently few times  
- Do Not Shake | - Close the tubes & invert few times. Do Not Shake |
| Label | - Record the date and time of collection on the label provided and attach to sample  
- No centrifuging or pipetting is required | - Record the date and time of collection on the label provided and attach to sample  
- Centrifuge it at 2000rpm for 10 minutes. Immediately after centrifuging remove the plasma and transfer to the clear bottle provided, using a pipette and label the clear bottle. Transfer ONLY plasma. Keep the residue in the EDTA bottle.  
- Store both the EDTA bottle with the residue in and the plasma in the clear bottle. |
### Storage

- Keep the samples in the NICU fridge (4 °C) soon after collection.
- The samples need to be transferred to a -20 °C freezer within 48 hours of the collection. The samples can be kept at -20 °C degrees for up to 3 months, before transferring to a -80 °C freezer. Please make sure samples are kept in dry ice when transferred between -20 °C and -80 °C freezers.
- Alternatively, they can be transferred directly to a -80 °C freezer for long term storage.

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