

COMET Trial

Standard Operating Procedures for blood collection – Version 2 (06.8.2018)

Timing and bottles

Time from randomisation	Relation with cooling	Bottles and blood
0 hours	Before start of cooling	<ol style="list-style-type: none"> 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)	<ol style="list-style-type: none"> 1. 0.5 ml in PAXgene tube 2. 0.5 ml in EDTA (Neonatal tube)

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

Purpose	PAXgene sample	EDTA sample
Vial to be used	Select the PAXgene tube (fluid filled tube with black marked line) from the blood collection bag	Select small EDTA tube (purple) from the blood collection bag
Volume of blood	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • Add 0.5 ml of blood to the tube
Mixing	<ul style="list-style-type: none"> • Close the tube & invert gently few times • Do Not Shake 	<ul style="list-style-type: none"> • Close the tubes & invert few times. Do Not Shake
Label	<ul style="list-style-type: none"> • Record the date and time of collection on the label provided and attach to sample • No centrifuging or pipetting is required 	<ul style="list-style-type: none"> • 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.

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Storage	<ul style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> 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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