

GREAT-2 Laboratory Manual V3 01-12-23

Study Title	GREAT-2
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IRAS number	1005993
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Associated Documents	GREAT-2 Samples Logs GREAT-2 DHL User Guide Sputum Sample Shipment GREAT-2 Sputum Shipment Requirements GREAT-2 Sputum Sample Shipment GREAT-2 Blood Sample Shipment

Contents

1. Procedures	2
2. Equipment.....	2
2.1. Bloods	2
2.2. Sputum.....	2
2.3. Urine pregnancy test.....	2
3. Sample ID	2
4. Sample collection.....	3
5. Research Sample Processing.....	5
6. Labelling Research Samples	6
7. Storing Research Samples.....	6
8. Shipping Sputum Samples.....	6
9. Blood Sample Collection & Shipments	7
Appendix 1 Obtaining Blood Samples When Monovette Tubes Are Used.....	8
Appendix 2 PAXgene tubes, how to collect blood.....	9

1. Procedures

- Ensure all equipment used is within expiry date
- Obtain blood samples as per local venepuncture Standard Operating Procedure.
- Obtain sputum samples as per local Standard Operating Procedure.
- Dispose of all clinical equipment as per local policy.
- Deal with any needlestick injury or body fluid spillage as per local policies.

2. Equipment

2.1. Bloods

- Blood tubes as per visit (research blood tubes are provided)
- Venepuncture equipment (e.g. needles, tourniquet, cotton wool etc.)
- Collection set compatible with vacutainer tubes must be used. For PAXgene
- Sample labels (provided)
- Sample Logs (provided)
- Sample storage boxes (provided)
- -20°C freezer
- -80°C freezer

2.2. Sputum

- Sputum sample pots (provided)
- Sample labels (provided)
- Fridge
- DHL shipment documentation: GREAT-2 DHL User Guide Sputum Sample Shipment & GREAT-2 Sputum Shipment Requirements
- DHL medical express packaging (provided)
- Category B UN3373 labels (provided)
- Cool packs (provided)

2.3. Urine pregnancy test

- Urine pot
- Urine pregnancy test kit.

3. Sample ID

- Sample ID is made up of:
 - site number – participant number – visit number – sample number
 - e.g. sample ID 01-01-02-01 would be site 01 – participant 01- visit 02- sample number 01

















4. Sample collection
















The total volume required for research blood samples at each visit is stated in the table below. Research blood samples should be collected for each visit using the blood tubes provided in the lab packs.

Samples highlighted in **blue** should be carried out using local NHS Lab facilities


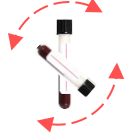











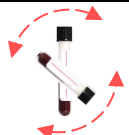





*Women of childbearing potential

† The sputum sample collected at visit 1 will be culture and/or PCR tested as per local laboratory protocol. This sample will not be shipped.

Visit	NHS Labs	Research Bloods	Research Sputum Sample
1	Bloods: <ul style="list-style-type: none"> FBC Pregnancy test* U&Es LFT Sputum: <ul style="list-style-type: none"> <i>P. aeruginosa</i> testing† 	 EDTA 4ml  SST 5ml	
2 Pre-dose	Urine: <ul style="list-style-type: none"> Pregnancy test* 	 PAXgene 2.5 ml  SST 17ml	 Min 0.5 g
2 Post-dose	Nil	SST 3.5 ml 	
3	Nil	 SST 7 ml	 Min 0.5 g
4	Nil	 SST 3.5 ml	 Min 0.5 g
5 Pre-dose	Bloods: <ul style="list-style-type: none"> FBC U&Es LFT Urine: <ul style="list-style-type: none"> Pregnancy test* 	 Paxgene 2.5 ml  SST 12 ml	 Min 0.5 g

5 Post-dose	Nil	SST 3.5 ml 	
Visit	NHS Labs	Research Bloods	Research Sputum Sample
6 Pre-dose	Bloods: <ul style="list-style-type: none"> • FBC • U&Es • LFT Urine: Pregnancy test* 	SST 13.5 ml 	 Min 0.5 g
6 Post-dose	Nil	SST 3.5ml 	
7	Bloods: <ul style="list-style-type: none"> • FBC • U&Es • LFT Urine: Pregnancy test* 	Paxgene 2.5 ml  SST 12 ml 	 Min 0.5 g
9	Bloods: <ul style="list-style-type: none"> • FBC • U&Es • LFT Urine: Pregnancy test* 	Paxgene 2.5 ml  SST 17 ml 	 Min 0.5 g
Unscheduled NHS bloods as clinically indicated		SST 5ml 	 Min 0.5 g

5. Research Sample Processing

TUBES	INVERT TUBE	ALLOW TO STAND	CENTRIFUGE	TRANSFER	LABEL	FREEZE
 PAXgene	 8-10 times	 Stand upright min 2 hours	N/A			 Store upright Transfer to -20°C freezer within 2-72 hours from collection. After minimum time overnight at -20°C and maximum time of 2 weeks at -20°C, the sample should be transferred to -80°C.
 SST	 5 times	 Stand upright 30 mins	 1100 to 1300 xg for 15 mins	 Transfer 0.5 ml serum per cryovial	 As many cryovials as required for available serum	 Store upright Transfer to -80°C freezer within 1 hour from collection
 EDTA	 8-10 times	N/A				 Store upright Transfer to -80°C freezer within 1 hour from collection
 Sputum	N/A					 Refrigerate 2-8°C Arrange shipment to Queens University Belfast on day of collection

6. Labelling Research Samples

- All study samples should be labelled with the labels provided.

<p>GREAT-2 Whole Blood (EDTA)</p> <p>Sample ID: ____-____-____-____</p> <p>Date: ____-____-____</p>
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<p>GREAT-2 Serum 0.5 ml</p> <p>Sample ID: ____-____-____-____</p> <p>Date: ____-____-____</p>
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<p>GREAT-2 PAXgene</p> <p>Sample ID: ____-____-____-____</p> <p>Date: ____-____-____</p>

<p>GREAT-2 Sputum</p> <p>Sample ID: ____-____-____-____</p> <p>Date: ____-____-____</p>
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- The appropriate sample label should be used for whole blood, serum, PAXgene, or sputum sample.
- All cryovials originating from one SST vacutainer should be listed together on one row of the sample log.
- Ensure labels are attached **prior** to freezing. Ensure that the label is **firmly rubbed** on to the tube to prevent it coming off.
- Complete each label with full sample ID and date as below. Ensure label is completed **prior** to freezing and appropriate cryo-marker pen is used.
- Date to be filled in day/month/year i.e 30-05-2022
- All samples should be logged on the appropriate GREAT-2 Sample Log.

7. Storing Research Samples

- The blood samples should be stored in the storage boxes provided.
- Research blood samples should be stored in separate boxes according to sample type. EDTA & PaxGene samples can be stored together at -80°C.
- The storage boxes should be labelled with study title, site number, sample type and box number.
- Storage location should be recorded on the GREAT-2 Sample Log.

8. Shipping Sputum Samples

From sites to Queens University Belfast

- Research sputum samples (visit 2 onwards) must be shipped on day of collection to the Belfast Laboratory.
- DHL medical expressing packaging, cool packs and Category B UN3373 labels will be provided.
- Collection must be arranged for Monday-Thursday to arrive next day.
- Please see the **GREAT-2 DHL User Guide Sputum Sample Shipment** for details on how to arrange a DHL Medical Express sample shipment.
- The person responsible for sputum shipments must register for a DHL account and be authorised on the GREAT-2 DHL account number.
- Please see the **GREAT-2 Sputum Shipment Requirements** document for the government shipment requirements and regulations for packaging category B biological

samples. The outer packaging **must** contain a Category B UN3373 Biological sample label.

- All samples must be accompanied by:
 - Completed Sample Shipment Sputum Document & Sputum Sample Log (paper)
 - The Sample Shipment Sputum Document & Sputum Sample Log (scanned copy) should be emailed to great-2-tm@dundee.ac.uk and Chloe McLaughlin Chloe.McLaughlin@qub.ac.uk

For any sputum sample queries or issues please contact the Belfast Laboratory team

Chloe McLaughlin email: Chloe.McLaughlin@qub.ac.uk
phone number: +44 02890 972091 (office)

Vanessa Brown email: V.Brown@qub.ac.uk
phone number: +44 28 9097 2064 (office)
+44 7580 677315 (mobile)

9. Blood Sample Collection & Shipments

PK blood sample collection

PK and ADA samples should be taken at the same time. Samples should be collected from the opposite arm to the infusion. The pre-dose samples should be taken within 1 hour of the start of infusion. Post infusion samples should be taken between 10 minutes and 1 hour after the end of infusion. All times of sample collection and start/end of infusion should be recorded.

Shipping blood samples from sites to University of Dundee

All research blood samples will be stored at site for the duration of the trial. At the end of the study the Trial Manager, Tayside Clinical Trials Unit, will organise a courier to transport frozen samples on dry ice to University of Dundee research labs.

- Collection will be arranged for Monday-Wednesday to arrive next day.
- All samples must be accompanied by:
 - Completed Sample Log (paper)
 - Completed Blood Sample Shipment Document (paper)
 - The Sample Log (scanned copy) & Blood Sample Shipment document (scanned copy) should be emailed to great-2-tm@dundee.ac.uk and Merete Long MLong001@dundee.ac.uk

Appendix 1 Obtaining Blood Samples When Monovette Tubes Are Used

This applies in sites where the NHS Labs use monovette blood collection tubes.

- The Research Samples **MUST** be collected in the tubes provided and therefore venepuncture should be carried out using a compatible blood collection system.

Visits 2, 5 & 6 (pre-dose) Visits 7 & 9

- A vacutainer blood collection system "Butterfly" must be used.

See Appendix 2 for special requirements when collecting the PAXgene RNA sample.



Visits 1, Visit 2 (post-dose) Visits 3 & 4 Visits 5 & 6 (post-dose) Unscheduled visits

- A vacutainer needle and holder can be used



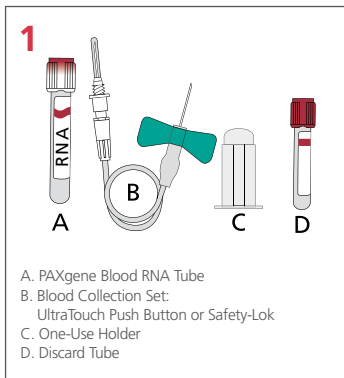
When NHS blood samples are also required and if your lab will only accept monovette tubes then these can be used with the vacutainer system.

- Pre-vacuum the monovette tube by pulling the plunger out.
- Attach to the vacutainer needle as below, fill tube and send to NHS Lab as local practice.



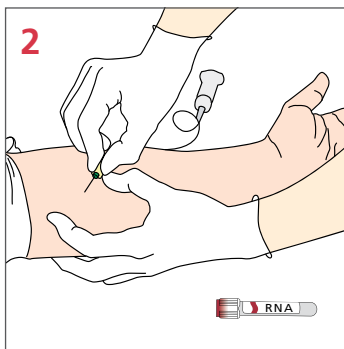
Appendix 2 PAXgene tubes, how to collect blood

How to Collect Blood Using the PAXgene® Blood RNA Tube For Molecular Diagnostic Testing



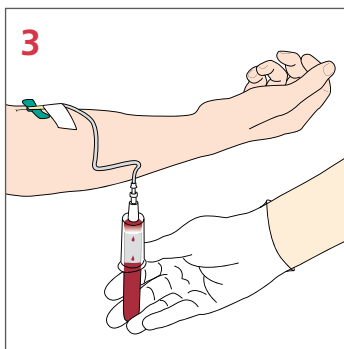
Required Items:

- 1a.** Ensure that the PAXgene Blood RNA Tube (A) is at room temperature (18°C–25°C) prior to use and is properly labeled with patient identification.
- 1b.** If the PAXgene Blood RNA Tube is the only tube to be drawn, a small amount of blood should be drawn into a “Discard Tube” (D) prior to drawing blood into the PAXgene Blood RNA Tube. Otherwise, the PAXgene Blood RNA Tube should be the last tube drawn in the phlebotomy procedure.



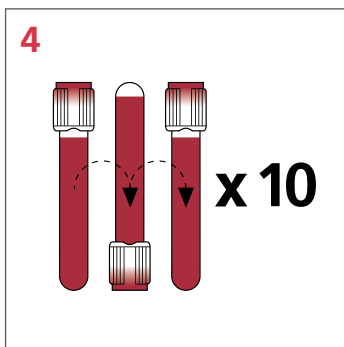
Venipuncture:

- 2.** Using a blood collection set such as the BD Vacutainer UltraTouch Push Button or BD Vacutainer Safety-Lok Blood Collection Set (B) and the One-Use Holder (C), perform venipuncture using your institution’s recommended procedure for standard venipuncture.



Blood Collection:

- 3a.** Hold the PAXgene Blood RNA Tube vertically, below the blood donor’s arm, during blood collection.
- 3b.** Allow at least 10 seconds for a complete blood draw to take place. Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.



After Blood Collection:

- 4a.** Gently invert the PAXgene Blood RNA Tube 8 to 10 times.
- 4b.** Store the PAXgene Blood RNA Tube upright at room temperature (18°C–25°C) for a minimum of 2 hours and a maximum of 72 hours before processing or transferring to refrigerator (2–8°C) or freezer (–20°C).

Before Blood Collection



RNA Stabilization Reagent

After Blood Collection



RNA Stabilized Whole Blood
