Protocol Number 175151

SITE SAMPLE MANUAL

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Contents

Associated Forms / Templates

1. Introduction

The purpose of this document is to describe the procedures involved in collecting samples at site level for the PANTHER trial.

2. Scope

This procedure is applicable to site teams that will be collecting samples from patients during the PANTHER trial. This manual will explain the process of collection, any sampling and storage requirements and sample shipping procedures.

3. Abbreviations

BAL	Bronchoalveolar lavage
ICTU	Imperial Clinical Trials Unit
SSPM	Study Specific Procedure Manual
RCF	Relative Centrifugal Force
VTM	Viral transport medium
HTA	Human Tissue Act

4. Responsibilities

Site staff (e.g.: Research Nurse, Investigators)	• Collect samples in accordance with protocol and sample collection manual • Ship collected samples to central lab following guidance in sample manual • Maintain oversight of sample kit levels at site and inform Trial Manager/Monitor when sample kits need to be replenished
Trial Manager/Monitor	• Maintain oversight of samples kits requirements at each site. • Liaise with the central lab when sample kits are required/replenished at site
Central Lab	• Maintain oversight of samples stored at central lab and master sample log • Liaise with the trial manager/monitor when sample kits are required at each site and ship these accordingly. • Create and ship sample kits to participating sites

5. References

- Good Clinical Practice (ICH- GCP) Guidelines

- Data Protection Act (2018)
- Human Tissue Act 2004

6. Procedures

6.1. Sample Collection Tiers

All samples should be collected in accordance with informed consent rules and legislation described in the PANTHER protocol. To minimise discomfort, blood should be collected from existing central lines or arterial lines, where possible. For patients who do not have any vascular access, blood collection should be done by venepuncture. It is important that the blood is NOT haemolyzed for the analysis to be valid. If the sample is haemolyzed, another tube of blood may be drawn. If repeat sampling is not possible, then the hemolyzed sample can be processed with a note written in the Sample Collection & Shipping Form to indicate that the sample is haemolyzed.

Sites will choose a sampling tier per patient according to Table 1 and in accordance with the resources available at each site. The only sample that is mandatory for the PANTHER trial is the phenotype stratification sample (Tier 0). Therefore, all sites are required to participate in this tier for all patients as a minimum. Subsequent samples will be optional, though sites will be encouraged to participate to enhance the value of the trial.

Please note for tiers 1 & 2, the nasal swab and tracheal aspirates are optional; if site staff do not feel they have capacity to manage these they are strongly encouraged to take the plasma, serum and RNA tubes and note on the tracking form/eCRF that no tracheal aspirate or nasal swab were taken

Table 1 – Sampling Tiers

Tier	Biological samples
0	a. Baseline - Stratification sample - Lithium Heparin tube 4ml *MANDATORY for all sites*
1	a. Baseline - Stratification sample - Lithium Heparin tube 4ml b. Baseline - Lithium Heparin 6ml c. Baseline - EDTA 6ml d. Baseline - SST tube 2.5ml e. Baseline - PAXgene 2.5ml f. Baseline - Nasal swab VTM tube 1ml (where possible) g. Baseline - Tracheal aspirate* 2-5ml (where possible) * in patients on invasive mechanical ventilation
2	a. Baseline - Stratification sample - Lithium Heparin tube 4ml b. Baseline, D2 & D6 Lithium Heparin 6ml c. Baseline, D2 & D6 EDTA 6ml d. Baseline, D2 & D6 SST tube 2.5ml e. Baseline, D2 & D6 PAXgene 2.5ml f. Baseline, Nasal swab VTM tube 1ml (where possible) g. Baseline, D2 & D6 Tracheal aspirate* 2-5ml (where possible) * in patients on invasive mechanical ventilation
3	a. Baseline - Stratification sample - Lithium Heparin tube 4ml b. Baseline, D2 & D6 Lithium Heparin 6ml c. Baseline, D2 & D6 EDTA 6ml d. Baseline, D2 & D6 SST tube 2.5ml e. Baseline, D2 & D6 PAXgene 2.5ml f. Baseline - Nasal swab VTM tube 1ml (where possible) g. Baseline, D2 & D6 Tracheal aspirate* 2-5ml (where possible) h. Baseline & D2 CPT Heparin tube 2 x 8ml * in patients on invasive mechanical ventilation
4	a. Baseline - Stratification sample - Lithium Heparin tube 4ml b. Baseline, D2 & D6 Lithium Heparin 6ml c. Baseline, D2 & D6 EDTA 6ml d. Baseline, D2 & D6 SST tube 2.5ml e. Baseline, D2 & D6 PAXgene 2.5ml f. Baseline - Nasal swab VTM tube 1ml (where possible) g. Baseline, D2 & D6 Tracheal aspirate* 2-5ml (where possible) h. Baseline & D2 CPT Heparin tube 2 x 8ml i. Baseline & D2 BAL conical tubes* 50-100ml * in patients on invasive mechanical ventilation

For tiers 1, 2, 3 and 4 if not all samples can be collected sites are encouraged to collect the blood samples only, if the other samples are not feasible.

Table 2 – Sampling schedule

	Timepoints			
Sample Type	**Baseline**	**Day 2**	**Day 6**	**Notes**
Lithium Heparin	✓	✓	✓	For stratification sample, plus plasma cytokines
Serum Separation Tube	✓	✓	✓	For biomarker and antibody analyses.
EDTA Tube	✓	✓	✓	For plasma
CPT heparin	✓	✓		For isolation of immune cells
PAXgene RNA	✓	✓	✓	For RNA and DNA extraction
Nasal swab	✓			Identifying viral pathogens, pathogen studies
Tracheal aspirate	✓	✓	✓	For biomarker analyses
BAL	✓	✓		For biomarker analyses

Definitions & Clarifications

Baseline - the 24-hour period prior to randomization

Day 0 - the remainder of the calendar day after randomization took place

Day 1 - the first calendar day following randomization

Day 2 - the second calendar day following randomization (essentially the 48-hour time samples)

Day 2 samples must be drawn at least 48 hours after the first dose of investigational agent. Day 2 samples can be taken up to one day later, and Day 6 samples can be taken one day earlier or later, to work around weekend staff availability.

For example, if Day 2 is Sunday, the Day 2 blood samples may be collected on Monday. If early discharge or death is anticipated Day 6 samples can be substituted with Day 5.

Centrifuge speed – If a 2000g RCF speed is not available, a 1800g can be used and the site are advised to centrifuge for an extra 2 minutes (12 instead of 10) to ensure good separation.

VTM tubes – various viral transport medium tubes (eg Virocult, Copan Universal transport medium, Remel M4-RT and others) are used in different clinical sites. Sites should use the VTM tubes routinely available (i.e. the tubes used for nasal or pharyngeal swabs for virology) in their own hospital.

6.2. Sample Collection and Storage

Personnel must follow 'Universal Precautions' when handling blood products as well as site-specific requirements for handling each type of specimen. Samples should be processed in an appropriate work area (bench or biosafety cabinets) according to local guidelines. Any volatile cleaning agents (e.g. ethanol) used to clean biosafety cabinets/working area should be allowed to evaporate completely before opening any blood tubes or aliquoting.

Processing of blood specimens should be initiated as soon as possible (~ 30 minutes) following collection with all plasma and serum samples ideally processed and frozen within one hour. If a -70 or -80°C freezer is not immediately available the sample may be frozen in a -20°C for no longer than a week, then transferred to the -70 or -80°C. If this process was followed, please enter this detail on the Sample Collection & Shipping form and in the eCRF.

Table 3 – Screw-topped tube or Cryovial Collection

In the UK plasma will be stored in 1ml samples. THIS MAY VARY ACROSS JURISDICTIONS ACCORDING TO LOCAL RESOURCE. Please check local jurisdiction agreements.

For plasma /serum samples the number of aliquots will vary depending on a range of factors including haemoglobin and blood volume driving the haematocrit. Tubes are supplied with the expectation that the fixed volume of blood will not lead to the same plasma volume for each patient. The numbers provided reflect a likely maximum/excess.

	Number of screw-topped tubes or cryovials expected at each timepoint		
Sample Type	**Baseline**	**Day 2**	**Day 6**
Lithium Heparin	**4 screw-topped**	**4 screw-topped**	**4 screw-topped**
Serum Separation Tube	**4 screw-topped**	**4 screw-topped**	**4 screw-topped**
EDTA Tube	**4 screw-topped**	**4 screw-topped**	**4 screw-topped**

CPT heparin	**2 screw-topped + 2cryovials#**	**2 screw-topped + 2cryovials#**	**Not collected**
PAXgene RNA	**N/A (not aliquoted)**	**N/A**	**N/A**
Nasal swab	**VTM tube only**	**Not collected**	**Not collected**
Tracheal aspirate	**2 screw topped tubes (UK)# + for USA also 3 ZYMO RNA/beads tubes**	**2 screw topped tubes (UK)# + for USA also 3 ZYMO RNA/beads tubes**	**2 screw topped tubes (UK) + for USA also 3 ZYMO RNA/beads tubes**
BAL	**13 screw-topped tubes, 3 cryovials# and up to 6 15ml centrifuge tubes**	**13 screw-topped tubes, 3 cryovials# and up to 6 15ml centrifuge tubes**	**Not collected**

These samples are HTA relevant (UK) and need to be stored and logged under local HTA guidelines

Unspun BAL is HTA relevant (1 screw topped tube)

*Screw topped tubes will be provided as 1ml and in colour coded tubes for the different timepoints:-

Baseline – Red topped tube

Day 2 – Blue topped tube

Day 6 – Yellow topped tube

Cryovials will be used for where cells are being frozen down and maintained in liquid nitrogen or –150°C freezers (from CPT or BAL tubes). Cryovials in this study contain HTA relevant material

Cryovials

6.2.1. Tier 0

- a) Stratification sample

Baseline phenotyping (stratification) specimen must be collected prior to randomization. All sites will be required to process the initial blood sample for centrifugation to separate plasma and be able to measure sTNFr-1 and IL-6 for stratification.

Collection of the stratification sample

4ml Lithium Heparin Tube

Procedure

a) 4ml blood will be collected in a Lithium heparin tube for use in the point of care assay.

b) Invert the tube several times and place on ice.

c) The MultiSTAT device requires the preparation of a plasma sample from the blood. Samples should be processed within 30 minutes of blood draw.

d) To prepare plasma, centrifuge blood at 2000g in a pre-chilled (if possible) centrifuge for 10 minutes.

e) Using a Pasteur pipette, take the supernatant (plasma- see diagram below) and put into one 1ml screw topped red tube (supplied unlabelled)

f) Keep on ice until sample analysed in Multistat. Keep any residual plasma in the red-topped tubes on ice, until assay is complete (see below).

g) Please refer to Device Manual for operation of the Multistat device. After sample has been successfully analysed by the MultiSTAT it should be discarded

6.2.2. Tier 1

a) Stratification sample – same as Tier 0 above.

b) Lithium heparin sample (baseline only)

c) EDTA (baseline only)

d) Serum sample (baseline only)

e) PAXgene (baseline only)

f) Nasal swab (baseline only)*

e) Tracheal Aspirate (baseline only)*

*Nasal swabs and tracheal aspirate samples to be collected where possible. If not possible to collect then please collect plasma, serum and Paxgene tubes.

Baseline samples should all be collected prior to randomisation.

Collection of the Lithium Heparin sample

6ml of blood will be collected in a Lithium heparin tube

6ml Lithium Heparin Tube

Action	Procedure
Preparation	1. Pre-cool centrifuge to 4°C where possible 2. Label four 1 ml screw-topped tubes for plasma with kit barcodes, these will detail the timepoint, baseline, day 2 or day 6.  3. Enter barcodes into eCRF. 4. Barcode labels for the lithium heparin tube are not required, sites may need to handwrite the sample ID if they choose
Collection 	1. Obtain the designated amount of blood from the participant in the prepared tube. 2. Tube must be filled to line for reagent ratios to be correct. 3. Gently invert Li-heparin tube 8-10 times immediately after collection to disperse anticoagulant into the entire blood sample. 4. Place on ice or at 2-8 ºC until centrifugation 5. Record time of each collection on CRF.
Processing 	1. Centrifuge at 2000 x g (RCF) for 10 minutes with brake ON in pre-chilled (4 ºC) swinging bucket rotor at temperature 2. Process in Biosafety Cabinet (BSL2+) if available. 3. Keeping the tubes upright, gently remove vacutainer tube cap and transfer plasma (upper layer) in 1ml aliquots to the four labelled screw-topped tubes using a P1000 micropipette (or sterile plastic transfer pipet) being careful to avoid disturbing the interface between plasma and blood pellet. a. The last aliquot may be 0.1 to 0.99 ml. b. If the last aliquot is less than 0.1 ml, combine with previous aliquot.

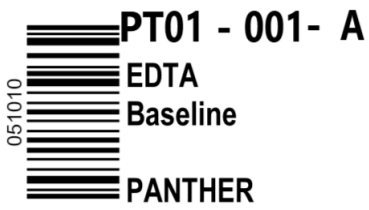
	• Cap the tubes, place in freezer box, and place at -70 or -80°C within 30 minutes of processing. 4. Store at -70 or -80°C until shipping.
eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. All samples should remain at -70 or -80ºC or colder prior to shipping. 2. In preparation for shipping the samples should be batch shipped on dry ice. 3. The samples should not be thawed prior to shipping 4. See the section on Sample Shipment in this manual for more detail.

c) EDTA sample

Collection of the EDTA sample

6ml of blood will be collected in an EDTA tube

6ml EDTA Tube

Action	Procedure
Preparation	1. Pre-chill centrifuge (to 4°C where possible). 2. Label four 1.ml screw-topped tubes for plasma with kit barcodes, these will detail the timepoint, baseline, day 2 or day 6.  3. Enter barcodes into eCRF.

	4. Barcode labels for the EDTA tube are not required, sites may need to handwrite the sample ID if they choose
Collection 	1. Obtain the designated amount of blood from the participant in the prepared tube. 2. Tube must be filled to line for reagent ratios to be correct. 3. Gently invert the EDTA tube 8-10 times immediately after collection to disperse anticoagulant into the entire blood sample. 4. Place on ice or at 2-8°C until centrifugation 5. Record time of each collection on CRF.
Processing 	1. Centrifuge at 2000 x g (RCF) for 10 minutes with brake ON in pre-chilled (4°C) swinging bucket rotor at temperature 2. Keeping the tubes upright, gently remove vacutainer tube cap and transfer plasma (upper layer) in 1ml aliquots to the four labelled screw-topped tubes using a P1000 micropipette (or sterile plastic transfer pipet) being careful to avoid disturbing the interface between plasma and blood pellet. a. The last aliquot may be 0.1 to 0.99ml. b. If the last aliquot is less than 0.1 ml, combine with previous aliquot. • Cap the tubes, place in freezer box, and place at -70 or -80°C within 30 minutes of processing. 3. Store at -70 or -80°C until shipping.
eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. All samples should remain at -70 or -80ºC or colder prior to shipping. 2. In preparation for shipping the samples should be batch shipped on dry ice. 3. The samples should not be thawed prior to shipping

	4. See the section on Sample Shipment in this manual for more detail.

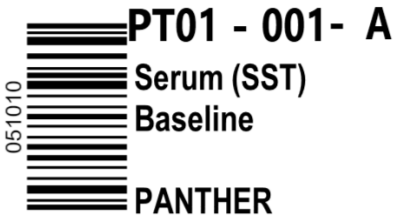
d) Serum sample (SST)

Collection of the SST sample

The SST sample can be collected at the same time or prior to first dose of investigational agents. The SST tube should be held at ***room temperature*** for up to an hour until processing.

2.5ml of blood will be collected in an SST tube

2.5ml SST Tube

Action	Procedure
Preparation	**1.** Ensure that centrifuge is **at ambient temperature.** 2. Label four 1 ml screw-topped tubes aliquot tubes for serum with kit barcodes, these will detail the timepoint, baseline, day 2 or day 6.  3. Enter barcodes into eCRF. 4. Barcode labels for the SST tube are not required, sites may need to handwrite the sample ID if they choose
Collection	1. Obtain the designated amount of blood from the participant in the prepared tube.

	2. Allow the tube to clot for at least 30 minutes in a vertical position **at room temperature**. Try not to allow the tube to stand more than one hour before centrifuging. SST tubes need to be processed within **6 hours** of collection. 3. Record time of each collection on CRF.
Processing 	1. Centrifuge at 2000 x g (RCF) for 10 minutes at ambient temperature (swinging-out rotor and brake ON) 2. After centrifugation, the gel should be intact and the cells and serum completely separated. Do not re-centrifuge the tube if the barrier is incomplete. • Aliquot *500µl* into screw-topped tubes. Prepare up to 4 vials, or as many as possible. 3. Store at -70 or -80°C until shipping.
eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. All samples should remain at -70 or -80ºC or colder prior to shipping. 2. In preparation for shipping the samples should be batch shipped on dry ice. 3. The samples should not be thawed prior to shipping 4. See the section on Sample Shipment in this manual for more detail.

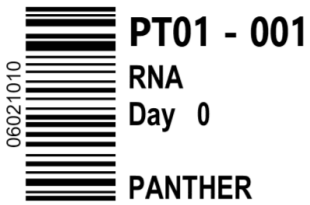
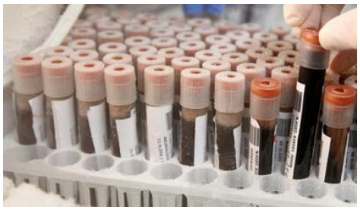
e) PAXgene RNA

Collection of the PAXgene sample

The PAXgene sample should be the last sample drawn from the patient.

2.5ml of blood will be collected in a PAXgene tube

2.5ml PAXgene Tube

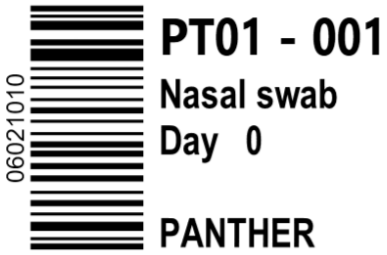
Action	Procedure
Preparation	1. Label 1 PAXgene tube with barcode provided  2. Enter barcodes into eCRF.
Collection 	1. Obtain the designated 2.5 ml of blood from the participant in the prepared tube. 2. Tube must be filled to line for reagent ratios to be correct. • Gently invert tube 8-10 times immediately after collection to disperse anticoagulant into the entire blood sample 3. Record time of collection on CRF
Processing 	1. Store tubes upright at room temperature for at least 2 hours o Tubes may be held at room temperature for up to 2 days before freezing. 2. After 2 hours at room temp, freeze tubes first at -20°C for 24 hours, then transfer them to -70°C or -70 or -80°C. 3. Freeze vials upright in a wire rack. Do not freeze tubes in a Styrofoam™ tray as this may cause the tubes to crack 4. Store at -70 to -70 or -80°C until shipping.
eCRF entry	1.Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. All samples should remain at – 70 ºC or -70 or -80ºC or colder prior to shipping. 2. In preparation for shipping the samples should be batch shipped on dry ice.

	3. The samples should not be thawed prior to shipping 4. See the section on Sample Shipment in this manual for more detail.

f) Nasal swab

Collection of the Nasal Swab sample

VTM Tube

Action	Procedure
Preparation	1. Label 1 nasal swab VTM tube with barcode provided  2. Enter barcodes into eCRF.
Collection 	1. Obtain a mid-turbinate nasal swab sample from participant. 2. Remove cap from VTM tube and insert flocked tip of nasal swab into VTM tube so that it is fully immersed in the viral transport fluid 3. Bend the handle of the swab until the tip portion breaks off into the tube and cap. 4. Record time of collection on CRF
Processing	1. Store at -70 or -80°C until shipping

eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. All samples should remain at -70 or -80ºC or colder prior to shipping. 2. In preparation for shipping the samples should be batch shipped on dry ice. 3. The samples should not be thawed prior to shipping 4. See the section on Sample Shipment in this manual for more detail.

g) Tracheal Aspirate

Collection of the Tracheal Aspirate sample *only where possible, and in UK this will be neat tracheal aspirate only. In the US sites will collect additional aspirate for storage with Zymo RNA Shield + bashing beads

The tracheal aspirate sample is only collected from patients who are mechanically ventilated.

Specimen trap

Action	Procedure
Preparation	1. Label 2 empty screw-topped tubes with barcodes provided [this step is included for UK sites] 2. Label 3 microtubes of Zymo RNA Shield (pre-filled) with bashing beads with barcodes provided [currently for US sites only as UK sites will not have these provided] 3. Enter barcodes into eCRF 4. Barcode labels for this tube are not required, sites may need to handwrite the sample ID if they choose
Collection	1. Obtain tracheal aspirate (~2-5 mL) if patient is ventilated.

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Processing **UK and US sites** US sites only 	1. Aliquot 0.5 mL tracheal aspirate to two 1mL screw topped tubes. These aliquots contain no reagent and are stored neat. Immediately transfer to -80ºC freezer# 2. (US sites only) Use a micropipette to remove 100microlitres of buffer from each of three ZYMO RNA Shield with bashing beads tubes without disturbing the beads 3. Add 0.5ml tracheal aspirate from the specimen trap into each of the three tubes 4. Vortex tubes for 10 seconds 5. Immediately transfer to -80ºC freezer
eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. All samples should remain at -70 or -80ºC or colder prior to shipping. 2. In preparation for shipping the samples should be batch shipped on dry ice. 3. The samples should not be thawed prior to shipping 4. See the section on Sample Shipment in this manual for more detail.

#HTA relevant samples

6.2.3. Tier 2

a) Stratification sample – same as Tier 0 above.

b) Lithium heparin sample – same as Tier 1 above +Day 2 & 6

c) EDTA – same as Tier 1 above +Day 2 & 6

d) Serum sample – same as Tier 1 above +Day 2 & 6

e) PAXgene – same as Tier 1 above +Day 2 & 6

f) Nasal swab – same as Tier 1 above (baseline only)*

g) Tracheal Aspirate – same as Tier 1 above +Day 2 & 6*

*Nasal swabs and tracheal aspirates only where possible

6.2.4. Tier 3

a) Stratification sample – same as Tier 0 above.

b) Lithium heparin sample – same as Tier 1 above +Day 2 & 6

c) EDTA – same as Tier 1 above +Day 2 & 6

d) Serum sample – same as Tier 1 above +Day 2 & 6

e) PAXgene – same as Tier 1 above +Day 2 & 6

f) Nasal swab – same as Tier 1 above (baseline only)*

g) Tracheal Aspirate – same as Tier 1 above +Day 2 & 6*

h) CPT heparin sample – baseline + Day 2

*Nasal swabs and tracheal aspirates only where possible

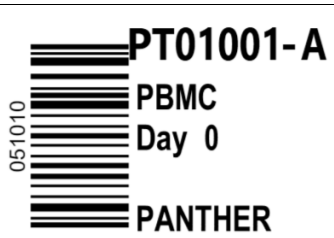
h) CPT heparin

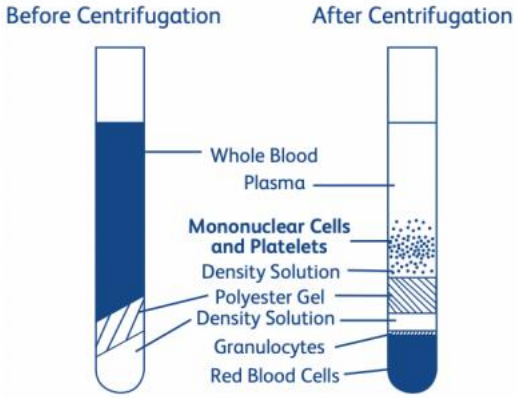
Collection of the CPT heparin sample

Heparin tubes may be stored, temporarily, at room temperature and shipped on day of collection or overnight to a central processing facility.

8ml CPT heparin tube

Action	Procedure
Preparation	1. Label 2 screw-topped tubes and 2 cryovials with kit barcodes provided.

	 2. Thaw freezing medium to room temperature 3. Fill Mr Frosty cell freezer to the correct level with isopropanol 4. Enter barcodes into eCRF. 5. Barcode labels for the CPT heparin tube are not required, sites may need to handwrite the sample ID if they choose
Collection 	1. Obtain the designated amount of blood from the participant in the prepared tube. i.e., vacuum is exhausted, and blood ceases to flow 2. Tube must be filled for heparin ratio to be correct. 3. Gently invert collection tubes 8-10 times immediately after collection to disperse preservative into the entire blood sample 4. Store upright at room temperature 5. Record time of each collection on CRF.
Processing 	NOTE: This centrifuge step of PBMC isolation process must be completed within 2 hours of blood collection. PBMC – Peripheral Blood Mononuclear cells 1. Remix the blood samples immediately prior to centrifugation by gently inverting tubes 8 to 10 times. 2. Centrifuge at 1500g for 25 minutes at room temperature. Ensure the centrifuge brake is OFF.

	3. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer (see figure below)  4. Prepare and label one new 15ml tube per sample 5. Add 10ml HBSS or RPMI medium to the Falcon tubes or conical centrifuge tubes with cap and place on ice HBSS – Hanks' Balanced Salt Solution RPMI – Roswell Park Memorial Institute Medium 6. Bring the sample tube to eye level and very gently use an 1ml pipette to slowly remove the mononuclear cells and platelets (PBMC) layer of white blood cells – the white fluffy layer of cells above the polymer gel. NOTE: you will need to go through the layer of plasma with the pipette button down in order to avoid disturbing the layers 7. Collect the cell layer with an 1ml pipette and transfer to a 15 mL conical centrifuge tube (falcon tube) with cap, containing the 10ml HBSS or RPMI. 8. AVOID: Avoid taking up any polymer gel during the pipetting 9. Wash cells: ○ Add PBS or HBSS to the 15mL conical tube to bring the volume to 15mL. Cap tube, then mix cells by inverting the tube 5 times. ○ Centrifuge for 10mins at 400g with brake ON.

Samples in DNA/RNA Shield **Cryovials for PBMC A and PBMC B** 	○ Aspirate as much supernatant as possible without disturbing the cell pellet. ▪ Carefully pipette out any remaining supernatant around the pellet, if applicable. ○ Add PBS or HBSS to bring volume to **10mL**. Cap tube, then mix cells by inverting the tube at least 5 times and tapping the tube. ▪ Resuspend the cell pellet by gently vortexing or tapping the tube. ○ **Split the 10mL** resuspended pellet volume into two 5mL aliquots in two 15mL conical tubes. One of these will be used for freezing PBMCs and the other will be lysed PBMCs in Shield. ○ Centrifuge the two 15mL conical tubes for 10 minutes at 400g with brake ON. Aspirate as much supernatant as possible without disturbing cell pellet. 10. Resuspend cell pellet in the desired medium ○ Take the PBMC in Shield aliquot and add 500uL of 1x DNA/RNA Shield (where available) ▪ Aliquot two 250uL aliquots into 1mL screw topped tubes ▪ Label as SHIELD1 and SHIELD2 ▪ Store at -80oC ○ For the other cell pellet add 500μL of freezing media (10% DMSO in FBS) ▪ Aliquot two 250μL aliquots into 1mL cryovials ▪ Label as PBMC1 and PBMC2# ▪ These PBMCs will need to be stored in Mr. Frosty in -70 or -80 freezer for 24 hours-72 hours, then moved to Liquid Nitrogen tank storage or –150oC freezer.
eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.

Shipping	1. Ship PBMCs in liquid nitrogen to central lab. 2. Must be batch shipped. 3. Ship lysates in RNA/DNA Shield along with plasma

#HTA relevant samples

6.2.5. Tier 4

a) Stratification sample – same as Tier 0 above.

b) Lithium heparin sample – same as Tier 1 above +Day 2 & 6

c) EDTA – same as Tier 1 above +Day 2 & 6

d) Serum sample – same as Tier 1 above +Day 2 & 6

e) PAXgene – same as Tier 1 above +Day 2 & 6

f) Nasal swab – same as Tier 1 above (Baseline only)*

g) Tracheal Aspirate – same as Tier 1 above +Day 2 & 6*

h) CPT heparin sample – same as Tier 3 above

i) Bronchoalveolar Lavage (BAL) sample – baseline and Day 2

*Nasal swabs and tracheal aspirates only where possible

i) Bronchoalveolar Lavage

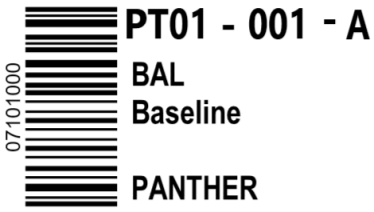
Collection of the Bronchoalveolar Lavage sample

The BAL sample is only collected from patients who are mechanically ventilated.

Specimen trap

Conical tube

Action	Procedure
Preparation	1. Barcode labels for the BAL tube are not required, sites may need to handwrite the sample ID if they choose

	2. Label 10x1ml screw topped tubes for cell-free BAL aliquots, 1x1ml screw topped tube for microbiome, 2x1ml screw-topped tubes for Tri-Reagent/RNA-later/Trizol lysates, 3 cryovials for cell pellets, and up to 6x15ml conical centrifuge tubes for cell free BAL (depending on BAL return volume) with kit barcodes provided  Store tubes in the refrigerator at 4°C. 3. 4. Prepare: ice, ice container, 100 mcl and 1000 mcl pipettes and corresponding tips, cryovial rack, thawed freezing media, Mr. FrostyTM (it provides a rate of cooling very close to -1°C/minute, the optimal rate for cell preservation), and aliquot storage box. Place screw-topped tubes, cryovials and 15ml centrifuge tubes in their racks on ice 5. Prepare: protective personal equipment (laboratory coat, gloves, eye protection and face mask) and further protection equipment if required by the known potential presence of a specific biological hazard. 6. Ensure freezing media is thawed from -20°C to 4°C in adequate amount. 7. Cool a centrifuge to 4°C. 8. Enter barcodes into eCRF.

Collection  	1. Obtain the bronchoalveolar lavage fluid sample from the participant via a specimen trap into 1 or 2 50 ml conical tubes and place it on ice. ○ Samples must be obtained from wedged position in the right middle lobe or lingula. ○ Three separate 50mL aliquots of 0.9% saline should be instilled and recovered by suction ○ Samples must be placed on ice and directly transferred to lab for processing 2. Record time of collection on CRF
Processing	1. Process samples inside a biosafety cabinet, wearing the appropriate protective personal equipment. 3. Gently and thoroughly mix the BAL sample. 4. Withdraw 100microlitres of the well-mixed sample and perform cell count using haemocytometer and trypan blue under microscope or automated cell counter (such as the Countess) where possible If site is unable to carry out this cell count please proceed to step 6, and note on eCRF 5. Record the BAL white cell counts in the eCRF. Include total cell count and percentage live and dead. Record in eCRF if cell count is not done 6. Collect 1ml aliquot of raw BAL fluid sample for microbiome analysis, and store in the designated screw-topped tube storage box in the -70 or -80°C freezer # 7. Decant 10ml of BAL and spin at 1000g for 5mins at 4°C with brake ON 8. Pipette off the supernatant and aliquot into 10x1ml aliquots in screw-topped tubes. Freeze at -70 or -80°C 9. Resuspend the cell pellet in 2ml TriReagent or RNAlater for mRNA/DNA analysis. Aliquot into 2x1ml screw-topped tubes

	10. Centrifuge the rest of the sample at 500 RCF for 5 minutes at 4°C. 11. Decant the supernatant in the 50 ml conical tube, leaving the cell pellet intact. 12. Resuspend the cell pellet in 1.5 ml of freezing media. Use 3 cryovials to store 3 500microlitre aliquots# 13. Immediately place the cell pellet aliquots in Mr. Frosty and then transfer it, within 5 minutes to prevent degradation, in the -70 or -80°C freezer for at least 24-72 hours. 14. Cells should then be transferred to liquid nitrogen or –150oC freezer. 15. Record the number of cell pellet aliquots. 16. ***If site does not have access to a Mr Frosty, or liquid nitrogen/-150oC freezer but can collect BAL and process it otherwise to collect cell free BAL, omit steps 10-15 and proceed to step 17. Indicate on the CRF that cellular samples were not stored*** 17. Centrifuge the remaining /decanted BAL at 1000 RCF for 5 minutes at 4°C . 18. Aliquot the supernatant into 10-15ml aliquots in 15ml centrifuge tubes. Store at -70 or -80°C until shipping
eCRF entry	1. Ensure the data is updated in the eCRF, date, time and sample IDs for each sample.
Shipping	1. Ship on dry ice to central lab. 2. Must be batch shipped. 3. All specimens should remain at -70 or -80ºC prior to shipping. 4. The samples should not be thawed prior to shipping. 5. Please note any variations or deviations from these instructions, problems, or issues.

#HTA relevant samples

6.3. Sample Storage

Details regarding sample storage are provided in each sample section above. In general, once processed samples should be stored at -70°C to -70 or -80°C

as soon as possible to maintain the integrity of the sample until shipping. If this is not possible, then storing in a -20°C for up to a week (7days), then transferring to a -70 or -80°C is acceptable.

6.4. Ordering Consumables

Once the site is ready to be activated an array of sample kits will be provided based on samples that can be collected from the site. For example, sites will let the PANTHER team know whether it is likely that they can collect BAL samples, if this is the case then BAL consumables will be provided within the kit.

6.5. Sample Shipment

Throughout the trial samples will be collected from each site and shipped on dry ice to Queen's Belfast. The trial team will liaise with each site in planning the shipment. As part of each shipment sites must ensure the following are contained:-

- Place all tubes/aliquots required to be shipped carefully in the provided packing boxes.
- Include a copy of the completed sample site_tracking_shipping document for each patient.

Sample pickup requests must be arranged with Judit Barabas via email (Judit.Barabas@qub.ac.uk). The request should include:

- Contact information (name, phone, email)
- Pickup address
- Instructions for the delivery person (e.g., parking, access details)
- Details of the samples to be collected (type: plasma, blood, etc.)
- Number of boxes/bags and their dimensions

Samples must be handed directly to the courier. Completed tracking logs must accompany the shipment, placed in waterproof packaging (e.g., polypackets) inside the box, or sent electronically to Judit Barabas (Judit.Barabas@qub.ac.uk).

7. Revision History

Version	Date Effective	Reason for update (page and section of change)
V1.0	08 OCT 2025	First version
V2.0	09 JAN 2026	Clarifications of no. of cryovials and box layouts

Attachment A

Sample Collection and Shipping form

Site _____ Tier 1

Patient ID _____

PANTHER ID _____

	BASELINE (DAY 0)				DAY 2				DAY 6			
	Sample taken (Y/N)	Date taken:	No. of tubes	Comments (e.g haemolyzed)	Sample taken (Y/N)	Date taken:	No. of tubes	Comments (e.g haemolyzed)	Sample taken (Y/N)	Date taken:	No. of tubes	Comments (e.g haemolyzed)
Lithium heparin Str					NA	NA	NA	NA	NA	NA	NA	NA
Lithium heparin					NA	NA	NA	NA	NA	NA	NA	NA
EDTA					NA	NA	NA	NA	NA	NA	NA	NA
Serum					NA	NA	NA	NA	NA	NA	NA	NA
RNA					NA	NA	NA	NA	NA	NA	NA	NA
Nasal swab					NA	NA	NA	NA	NA	NA	NA	NA
Tracheal aspirate					NA	NA	NA	NA	NA	NA	NA	NA
DCP Heparin	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
BAL	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Sample Storage	Circle	
Stored in -80°C	Yes	No
Stored in -20°C then -80°C	Yes	No

SAMPLES SHIPPED:

Sent by: _____ Date: _____

Received by: _____ Date: _____

Tier_0 | Tier_1 | Tier_2 | Tier_3 | Tier_4

Ref: SOP_CR012 Study Specific Procedure Manuals

SOP_TEM_CR008, v7.0 Effective 1st October 2024

PANTHER Site Sample Manual, v2.0 9th January 2026

Attachment B

Sample box layouts:

Tier 1-2:

Baseline Lihep A	Baseline Lihep B	Baseline Lihep C	Baseline Lihep D		Baseline EDTA A	Baseline EDTA B	Baseline EDTA C	Baseline EDTA D	
Baseline Serum A	Baseline Serum B	Baseline Serum C	Baseline Serum D						
Day 2 Lihep A	Day 2 Lihep B	Day 2 Lihep C	Day 2 Lihep D		Day 2 EDTA A	Day 2 EDTA B	Day 2 EDTA C	Day 2 EDTA D	
Day 2 Serum A	Day 2 Serum B	Day 2 Serum C	Day 2 Serum D						
Day 6 Lihep A	Day 6 Lihep B	Day 6 Lihep C	Day 6 Lihep D		Day 6 EDTA A	Day 6 EDTA B	Day 6 EDTA C	Day 6 EDTA D	
Day 6 Serum A	Day 6 Serum B	Day 6 Serum C	Day 6 Serum D						

Tier 3-4:

Baseline Lihep A	Baseline Lihep B	Baseline Lihep C	Baseline Lihep D		Baseline EDTA A	Baseline EDTA B	Baseline EDTA C	Baseline EDTA D	
Baseline Serum A	Baseline Serum B	Baseline Serum C	Baseline Serum D						
Day 2 Lihep A	Day 2 Lihep B	Day 2 Lihep C	Day 2 Lihep D		Day 2 EDTA A	Day 2 EDTA B	Day 2 EDTA C	Day 2 EDTA D	
Day 2 Serum A	Day 2 Serum B	Day 2 Serum C	Day 2 Serum D						
Day 6 Lihep A	Day 6 Lihep B	Day 6 Lihep C	Day 6 Lihep D		Day 6 EDTA A	Day 6 EDTA B	Day 6 EDTA C	Day 6 EDTA D	
Day 6 Serum A	Day 6 Serum B	Day 6 Serum C	Day 6 Serum D						
Baseline BAL A	Baseline BAL B	Baseline BAL C	Baseline BAL D	Baseline BAL E	Baseline BAL F	Baseline BAL G	Baseline BAL H	Baseline BAL I	Baseline BAL J
Baseline BAL K	Baseline BAL L	Baseline BAL M	**Baseline PBMC (Shield) A**	**Baseline PBMC (Shield) B**	Baseline PBMC A	Baseline PBMC B	BAL cells	BAL cells	BAL cells
Day 2 BAL A	Day 2 BAL B	Day 2 BAL C	Day 2 BAL D	Day 2 BAL E	Day 2 BAL F	Day 2 BAL G	Day 2 BAL H	Day 2 BAL I	Day 2 BAL J
Day 2 BAL K	Day 2 BAL L	Day 2 BAL M	**Day 2 PBMC (Shield) A**	**Day 2 PBMC (Shield) B**	Day 2 PBMC A	Day 2 PBMC B	BAL cells	BAL cells	BAL cells