



**STRESS-L TRIAL**  
**Study into the Reversal of Septic Shock with Landiolol (Beta Blockade)**

Manual for collection and storage of biological samples at STRESS-L sites

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## 1. Contact details

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Email:	<a href="mailto:STRESS-L@warwick.ac.uk">STRESS-L@warwick.ac.uk</a>	<a href="mailto:Tony.Whitehouse@uhb.nhs.uk">Tony.Whitehouse@uhb.nhs.uk</a>
Role:	Trial Manager	Trial CI

<b>Primary contacts for delivery of Research STRESS-L Serum Samples:</b>		
	<b>Dr Jane Steele Director, Advanced Therapies Facility</b>	<b>Dr Kirsty McGee Lead Research &amp; Skills Training Technician</b>
Address	Human Biomaterials Resource Centre (HBRC) 6 Mindelsohn Way Birmingham B15 2SY	Institute of Inflammation and Ageing (IIA), University of Birmingham Research Labs, Queen Elizabeth Hospital, Birmingham, B15 2WB
Tel:	07584 312396	0121 371 3264
Email:	<a href="mailto:hbrc-tissuebank@contacts.bham.ac.uk">hbrc-tissuebank@contacts.bham.ac.uk</a>	<a href="mailto:k.c.mcgee@bham.ac.uk">k.c.mcgee@bham.ac.uk</a>

## 2. Introduction

The purpose of this Manual is to describe the collection, processing, storage and transportation of samples for the STRESS-L trial.

## 3. General requirements for sampling

Please also refer to the trial assessments schedule in the main Trial Protocol (section 4, table 1).

The **mandatory research blood samples** will be collected on days 0, 1, 2, 4, 6 and the End of Noradrenaline Treatment (EONT) Visit (if this does not fall on a blood sampling day) for the STRESS-L trial and sent to the University Hospitals of Birmingham NHS Foundation Trust. These samples will answer some of the secondary outcomes and the following tests will likely be carried out but will be dependent on available technology at the time of analysis: catecholamines, Troponin-T, creatine kinase (CK-MB), beta natriuretic peptide (BNP), cytokine analysis (probably IL-1 beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, TNF-alpha, TNF-beta, and IFN-gamma).

If a participant has consented to **additional optional blood samples** for donation to the Human Biomaterials Resource Centre (HBRC) at the University of Birmingham, these will be collected on day 0, day 1 and EONT visit. The HBRC is a licensed biobank and these samples will be used in future ethically approved research (including genetic studies).

- 1 set of research blood samples (approximately 12 ml total volume) on days 0, 1, 2, 4, 6 and EONT Visit (if this does not fall on a blood sampling day); plasma taken to assess secondary mechanistic outcomes for the trial.
- 2X 2.5 ml additional optional blood samples will be taken on day 0, 1X 2.5 ml blood sample will be taken on day 1 and 1X 2.5 ml will be taken on EONT; whole blood to be frozen for donation to the Human Biomaterials Resource Centre (a licensed biobank) at the University of Birmingham for future ethically approved research (including genetic studies).

To ensure staff safety and reduce the risk of viral transmission, blood samples **should NOT be taken for COVID-19 positive patients**.

## 4. Collection, processing and storage of blood samples

### 4.1 Consumables

The majority of consumables will be supplied to STRESS-L sites by the Warwick Clinical Trials Unit (WCTU).

To request a resupply of consumables, please complete the request form located on the STRESS-L website:

<https://warwick.ac.uk/fac/sci/med/research/ctu/trials/stressl/health/samplesupplies/>

Consumable	Used for
Green Lithium Heparin Vacutainer	Collection of plasma
PaxGene RNA Blood Tube	RNA
PaxGene DNA Blood Tube	DNA
Cryovials	Storage of plasma
Plastic or cardboard storage boxes with internal racks	Storage of cryovials
Racking/boxes for Paxgene blood tubes (need to fit 13mm and 16mm blood tubes)	Storage of PaxGene Tubes ( <u>must not</u> be stored in Styrofoam racking)

## 4.2 Sampling schedule

- Blood samples will be taken from a participant on day 0, 1, 2, 4, 6 and EONT visit (if this does not fall on a blood sampling day).
- Use the following blood tubes to collect up to a maximum of 20 ml of blood:

Time points	0	1	2	4	6	EONT
PaxGene RNA Blood Tube (2.5mL)	1	1				1
PaxGene DNA Blood Tube (2.5mL)	1					
Green Lithium Heparin Blood Tube (4mL)	3	3	3	3	3	3
Total volume (mLs)	17	15	12	12	12	14.5

## 4.3 Isolation of Plasma from Green Lithium Heparin Vacutainers

### PURPOSE:

This SOP outlines the reagents and procedures required for isolation of plasma from whole blood in Green-topped Lithium Heparin vacutainers.

### REAGENTS:

- Whole blood (Green Lithium Heparin vacutainer) X3

### MATERIALS:

- 1 ml Cryovials (Green lidded)
- Cryovial storage box
- Calibrated pipettes
- Disposable pipette tips
- Centrifuge (Room Temp)
- Cryovial Labels
- -80 (or -70°C) Freezer

### GENERAL POINTS:

Blood samples must always be treated as potentially infectious. Wear appropriate Personal Protective Equipment (PPE) when handling samples and reagents.

Ensure all waste is disposed of in the appropriate manner (as per local SOPs).

**Prior to starting:**

- Place green lithium heparin tube/s on a rotator at room temperature upon arrival into the lab until ready to process.

**Standard Operating Procedure (SOP):**

1. Place tubes into a balanced centrifuge and spin at room temperature for 10 minutes at 1500 x g.
2. Carefully remove the plasma layer, taking care not to disturb the bottom layer, and transfer 500µl aliquots into 8 cryovials.
3. Store at -80 (or -70°C) in the racked storage box provided. Place 4 cryovials into storage box A and 4 cryovials into storage box B (back up samples).

**4.4 Freezing of PAXgene Blood RNA and DNA tubes**

*(Information taken directly from PreAnalytix Blood RNA/DNA handbooks, which also includes information on specimen collection).*

**GENERAL POINTS**

Blood samples must always be treated as potentially infectious. Wear appropriate PPE when handling samples and reagents.

Ensure all waste is disposed of in the appropriate manner as per local SOPs.

1. Store the PAXgene® Blood Tubes upright at room temperature (18°C to 25°C) for a minimum of 2 hours and a maximum of 72 hours before processing or storage.
2. Stand the PAXgene® Blood Tube upright in a plastic or wire rack. **Do not freeze tubes upright in a styrofoam tray as this may cause the tubes to crack.**
3. The PAXgene® Blood Tubes can be stored at -20°C and below. If tubes are to be kept at temperatures below -20°C, freeze them first at -20°C for 24 hours, then transfer them to -80°C (or -70°C) for longer term storage.

N.B the frozen PAXgene tubes are subject to breakage upon impact. To reduce the risk of breakage during shipment, frozen tubes should be treated in the same manner as glass tubes.

#### 4.5 Documentation and labelling

A sample storage form will be used to record all samples retained for trial participants on an ongoing basis. A separate sample transport form will be completed when arranging for samples to be couriered for central analysis in Birmingham.

Labels for the cryovials and paxgene RNA/DNA tubes are provided in section 8 of the Investigator Site File (ISF). These labels will document the time point, Participant ID, Participant's initials and Sample Date (see example below).

Labels should be completed (using the permanent waterproof pen provided or a similar one in your Department) and attached to the appropriate vials **before** samples are taken.

#### STRESS-L Trial Mandatory Research Samples (mechanistic outcomes)

STRESS-L RESEARCH SAMPLE  
DAY 0 - PLASMA Sample  
PARTICIPANT ID:  
PARTICIPANT INITIALS:  
DATE:

STRESS-L RESEARCH SAMPLE  
DAY 0 - PLASMA Sample  
PARTICIPANT ID:  
PARTICIPANT INITIALS:  
DATE:

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STRESS-L RESEARCH SAMPLE  
DAY 1 - PLASMA Sample  
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PARTICIPANT ID:  
PARTICIPANT INITIALS:  
DATE:

#### 5. Sample transportation

A member of the coordinating centre team will contact research sites to organise collection and transportation of samples in batch, by specialist courier.

Frozen samples may be left in their storage boxes/racks when placing in the dry ice. Please do not try to overfill the transport box containing dry ice with too many samples. Please ensure the samples are completely covered. There should be at least 2cm of dry ice around the samples, ensuring they do not thaw during transport. We recommend requesting assistance from laboratory personnel when packaging samples. Please remember that dry ice is very cold and can cause cold burns. **Use gloved hands to spread the dry ice** (or other suitable implement). Care should be taken not to have prolonged contact with the dry ice.

The sample transport form listing all the samples to be transported must be completed. This form must be sent with the samples (but kept away from the dry ice). Ensure copies of the sample storage and transport form are sent to the coordinating centre team and also retained in the ISF.

On receipt of samples, each consignment will be checked to ensure that copies of all the relevant sample transport forms are enclosed. If there is any discrepancy between samples recorded on a sample transport form and samples included in the transportation, the person responsible for despatching the shipment will be contacted in order to resolve inconsistencies.

### **5.1 Courier details for transport**

Courier details are to be provided by the STRESS-L coordinating centre team prior to arranging collection of samples from site.



# STRESS-L Sample Storage Form



## STRESS-L Sample Storage Form

<b>Study title:</b>	<b>STRESS-L: STudy into the REversal of Septic Shock with Landiolol (Beta Blockade)</b>	<b>Investigator name:</b>	
<b>Site Number:</b>			

Participant ID	Initials	Date of Consent DD/MM/YYYY	Date sample collected DD/MM/YYYY	Time point / day	Sample type 1= Plasma 2= Paxgene RNA 3= Paxgene DNA	Number of samples collected	Storage location & type of facility (please specify full address)	Date stored	Stored by (Initials)

Please file a copy of the form in the ISF and email a copy to [STRESS-L@warwick.ac.uk](mailto:STRESS-L@warwick.ac.uk)

# STRESS-L Sample Transport Form



## STRESS-L Sample Transport Form

<b>Study title:</b>	<b>STRESS-L: S</b> Tudy into the <b>RE</b> versal of Septic Shock with Landiolol (Beta Blockade)	<b>Name of person sending samples:</b>	
<b>Investigator name:</b>		<b>Address:</b>	
<b>Site Number:</b>		<b>Email and telephone:</b>	

*Please record 1 sample collection tube/cryovial per line*

Participant ID	Initials	Date of Consent DD/MM/YYYY	Date sample collected DD/MM/YYYY	Time point / day	Sample type 1= Plasma 2= Paxgene RNA 3= Paxgene DNA	Number of samples	Date transported to central site for analysis	Lab use only <i>(initial against each sample to confirm receipt)</i>

*Please attach a copy of this form with the samples and email a copy to [STRESS-L@warwick.ac.uk](mailto:STRESS-L@warwick.ac.uk). The original should be retained in the ISF.*

To be completed on receipt (Birmingham labs)			
<b>Name of recipient:</b>		<b>Date of receipt:</b>	<b>Time of receipt:</b>
<b>Condition of samples received:</b>	<input type="checkbox"/> Suitable <input type="checkbox"/> Not suitable	<b>Comments (including action taken):</b>	