

## **Appendix 2: Blood sampling instruction for inflammation analysis and long term storage**

*Only study staff who are trained in venepuncture may obtain research blood sample and only staff who have completed training in use of local centrifuge are permitted to undertake this task.*

# Inflammatory markers: Plasma Collection

## Collection and Preparation of Blood from Patients in R4VAD study (Evaluation of IL-1 $\beta$ and future markers)

Collection times: **Study visit 1 (6+/- 2 weeks post baseline visit)**

**CHECK:** Participant **MUST** give consent to provide blood samples

### 1) Collection Tubes

a) Take 2 x 9ml EDTA Monovette from the 'R4VAD Blood Study 1 Participant Pack'.

### 2) Collection of blood.

a) Collect 2 x **9 ml blood** following locally agreed protocols into both 9ml EDTA Monovettes and invert slowly x 5.

B Complete the sample labels with participant number, site, initials, **date and actual collection time** to the label and place tube in sample transfer box.

e.g. For Peter Smith (participant 5), collected on 25<sup>th</sup> July 2012 at 7 a.m.

<b>P:</b> 5	<b>Site:</b> Salford
<b>Initials:</b> P.S.	
<b>Date:</b> 25/7/12	<b>Time:</b> 07:00

*\*Sample numbering and site code to be confirmed by Trial Centre*

**NOTE:** Ensure all sample/participant numbers and exact clock times are noted in the research record notes and sample collection logs.

### 4) Sample preparation

**NOTE:** Samples should be processed within 1 hour of collection or a maximum of 6 hours if stored upright in cool conditions e.g. cold room or fridge.

a) Transport tubes in the sample transfer box to the sample preparation area within local site.

b) Complete a R4VAD Blood Study 1 Sample Collection sheet.

e.g. attached

### c) Turn on centrifuge.

d) In the sample preparation area, **prepare the following** from the 'R4VAD Blood Study 1 Participant Pack' **for each sample collected:**

i) 2 x white-capped bijou, to decant plasma

ii) 2 x transfer pipettes per sample.

iii) 6 white-topped freeze vials (aliquots) for IL-1 $\beta$  sample and 6 blue-topped freezer vials (aliquots) for Future Markers sample

Tube rack, disposal bag (available on site)

e) EDTA Monovette to be balanced with **balance tubes\* and centrifuged** for 15 min at 2,000 x g (**NOTE:** check local conversion to RCF depending on diameter of centrifuge rotor). *\*if spinning both samples together, no need for balance tubes but must check equal volume in each tube*

### f) After centrifugation – prepare each sample separately:

#### Sample 1 – IL-1 $\beta$ sample:

- i. Transfer approx. 4.5 ml EDTA plasma from EDTA tube in the sterile bijou, using a sterile transfer pipette and taking great care not to disturb cell layer.

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- ii. Using a new transfer pipette, divide the plasma from 'decant' bijou between the 6 **white-top freezer vials** (depending on volume available).
- iii. Add the following information to the partly pre-printed labels provided, **using a non-water-soluble pen: Participant initials and ID, Site, the Date the sample was taken.** Write the **Participant ID, Site code** on the lid, using a permanent marker.

**e.g.** For Peter Smith (participant 5), collected on 25<sup>th</sup> July 2012 day the completed labels should read.

PS Salford  
Date: 25/07/12  
IL-1 $\beta$  study

5  
Sal

**Future Marker sample:**

- i. Repeat the steps above and decant into 6 **blue-topped freezer vials** (depending on volume available)

PS Salford  
Date: 25/07/12  
Future Marker Study

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i) Place filled vials/aliquots in a rack (or designated storage box) in designated **-70°C to -80°C freezer.**

5) **Switch off** centrifuge, check carriers for breakages or blood spillage.

6) Clean centrifuge and wipe all working surfaces according to local procedures.

Contact details for queries about the laboratory protocol and to arrange bulk transfer of stored samples:

Margaret Hoadley or Sylvia Scarth 0161 206 4270 or Sharon Hulme 0161 206 5755

Email: [Margaret.hoadley@manchester.ac.uk](mailto:Margaret.hoadley@manchester.ac.uk)

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Sample Collection Log

R4VAd

**Plasma IL-1 $\beta$  clear topped freezer vials (6 vials dispensed from decant bijou)**

STUDY ID:	Subject Initials	Consent Date	Collection date	Collection time	Processing time	Processed by	Comments	Samples filed	Data entered	Entry checked
5	PS	01/06/2012	25/07/2012	07:00	07:30	M.Mouse	e.g haemolysed sample, only 4 vials	Lab staff only		

**Future markers blue topped freezer vials (6 vials dispensed from decant bijou)**

STUDY ID:	Subject Initials	Consent Date	Collection date	Collection time	Processing time	Processed by	Comments	Samples filed	Data entered	Entry checked
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