

## **Sampling Procedure for the UK Meningitis Study**

All patients consent to giving the remainder of any routinely taken sample (**number 6** on the consent form). These are termed 'leftover samples', in reality this is normally CSF. A sample is deemed to be leftover either when the patient is ready for discharge or when the sample would be being thrown away by the laboratory anyway – whichever occurs first.

Consent to extra samples is optional (**number 8** on the consent form).

**Number 9** on the consent form refers to the deferred consent process and if this is ticked the sample **MUST** also be documented on the deferred samples log.

**All patients must have consented to take part in these substudies before samples are sent to the University of Liverpool.**

### **1) All patients - Leftover CSF (and any other samples) locating and aliquoting**

Please locate any leftover CSF. If it is NOT already frozen, label with subject ID and aliquot into 140µL samples in Eppendorf tubes (can be supplied by University of Liverpool if required). If the sample IS already frozen, DO NOT thaw in order to aliquot. If possible label with subject ID and store with samples for study.

Store CSF at -70/80°C as soon as possible (higher temperatures are acceptable if access to a -70/80°C freezer is not possible – please ensure this is recorded on the samples storage log)

### **2) Optional/Extra samples - Biomarkers of infection, proteomics and DNA analysis**

If number 8 on the consent form is initialled (or if you are taking samples using deferred consent – please ensure that number 9 is ticked when the patient does consent, if it is not please destroy the sample and ensure this is documented on the deferred sample log).

#### **A. Biomarkers of infection/genomics/PAXgene samples**

- i. Take 2.5ml of blood +/- CSF in provided PAXgene tube
- ii. Ensure complete draw into tube (incomplete filling degrades sample)
- iii. Label with study label
- iv. Invert gently 10 times at the bedside
- v. Leave tube upright at room temp for 2 hours
- vi. Store in -70/80°C freezer in an open rack (prevents differential freezing) labelled UK Meningitis study.

#### **B. Proteomics**

- i. Take EDTA sample of blood (4.5ml), label with study stickers and store in -80°C freezer.

C. DNA Analysis


- i. Take 3-5ml CSF in universal container (if patient consented prior to LP, ensuring not to take more than 17ml in total).
- ii. Label CSF with study sticker
- iii. Store in -70/80°C freezer, (preferably, if not available -20°C would suffice.)
- iv. Take 18ml of blood in EDTA tubes
- v. Label with study stickers
- vi. Store in -70/80°C freezer, (preferably, if not available -20°C would suffice.)

D. Serum separation

- i. Serum tubes should sit upright after the blood is drawn at room temperature for a minimum of 30 to a maximum of 60 minutes to allow the clot to form.
- ii. Centrifuge in a horizontal rotor (swing-out head) for 20 minutes at 1100-1300 g at room temperature. If the blood is not centrifuged immediately after the clotting time (30 to 60 minutes at room temperature), the tubes should be refrigerated (4°C) for no longer than 4 hours. After centrifuging, the clot is at the bottom of the tube, and the serum is on top of the clot). Warning: Excessive centrifuge speed (over 2000 g) may cause tube breakage and exposure to blood and possible injury. If needed, RCF for a centrifuge can be calculated. For an on-line calculator tool, please refer to:  
<http://www.mseuk.co.uk/Support/RCFCalculator/Default.aspx>
- iii. Use pipette to transfer the serum (Recommendation: do not pour!) into the labelled cryovials. (Aliquot volume is recommended to be 250 µl).
- iv. Close the caps on the vials tightly. This process should be completed within 1 hour of centrifugation. Note: Be very careful not to pick up red blood cells when aliquoting. This can be done by keeping the pipette above the red blood cell layer and leaving a small amount of serum in the tube.
- v. Check that all aliquot vial caps are secure and that all vials are labelled.
- vi. Place all aliquots upright in a specimen box or rack in an -70°C freezer. The samples should not be thawed prior to shipping. (Serum will be shipped on dry ice).

**Labelling**

Labels are provided for both the forms and the tubes. The labels for forms only need to be filled in if you are sending samples to a laboratory where they then need to identify and store the samples. They do not need to be used if you are taking the sample directly to the freezer yourself. In particular they should NOT be used to stick onto tubes as they do not stay on and prevent the sample from fitting in the storage boxes. The label for the form looks like this:

<b>UK MENINGITIS STUDY</b>	
Subject ID _____	OR
Screening Number _____	
Blood/CSF <i>(please delete)</i>	
Biomarkers	<input type="checkbox"/>
Proteomics	<input type="checkbox"/>
DNA Sampling	<input type="checkbox"/>
	

If the patient has received a subject ID please record the subject ID – if they haven’t you can record the screening number only.

Please tick which study the bloods taken are for i.e. biomarkers, proteomics, DNA sampling or all 3.

Please indicate whether it is CSF or blood. Please send separate forms for CSF and Blood.

The label for the CSF/blood tubes look like this:

<b>UK MENINGITIS</b>
<b>Sub ID OR (Screen No.)</b>
_____ ( )
<b>Date</b> _ / _ / _ _

If the patient has received a subject ID please record the subject ID – if they haven’t you can record the screening number only.

**On aliquots please ensure subject ID is on all samples.**