Guidelines for STOP-ACEi Trial Sample Preparation

As part of the STOP-ACEi trial, blood and urine samples will be collected for analysis at the central lab in Hull. This guide will give you instructions on how to prepare the samples for storage and transport to the central lab.

Types of sample

Routine tests for biochemical profile, eGFR and full blood count should be performed at each trial visit and CRP should be tested annually. These samples should be analysed locally at your site according to the normal local procedure. Please report the results in the study CRFs.

All trial participants will also have blood samples taken for analysis of Cystatin-C, NT-proBNP, ACE and renin levels. These samples will be taken at your site and sent for analysis at the central lab in Hull. These are only taken at the annual trial visits (Baseline and months 12, 24 and 36) so each trial participant will have these samples taken and analysed a total of 4 times during their trial participation.

Participants can also give optional consent to give additional serum and urine samples for future biomarker analysis. These samples will be taken at your site and sent to the central lab in Hull for storage until analysed in the future. These are only taken at some of the annual trial visits (Baseline and months 12 and 36 only) so each trial participant will have these samples taken and analysed a total of 3 times during their trial participation.

The table below summarises the types of sample being taken for STOP-ACEi.

	What will be tested	**Where analysed**	**When samples taken**
Routine tests For all trial participants.	Biochemical profile eGFR Full blood count Urinary PCR CRP (See protocol for details)	Locally, at your site.	Baseline Every 3-monthly trial visit CRP taken annually only, i.e. baseline and at months 12, 24 and 36
Standard Trial Samples For all trial participants.	Cystatin-C NT-proBNP ACE Renin levels	Centrally, at Hull lab	Baseline Month 12 Month 24 Month 36
Biomarker Samples For participants that give optional consent only	Unknown biomarkers in future analysis	Centrally, at Hull lab	Baseline Month 12 Month 36

The rest of this guide will only concern preparations for centrally analysed samples, please follow your own local procedure for the routine tests.

Preparing the tubes and labels

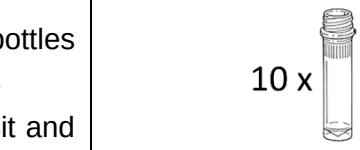
Please prepare the tubes and labels before taking any samples to avoid confusion. You will not be able to tell the difference between types of sample once they are separated so the tubes need to be labelled first. We recommend that you do this before the patient arrives.

- Please use the provided screw-cap bottles.
- You will need 4 x bottles for the standard trial samples and 6 x bottles for biomarker serum samples (= 10 bottles in total) for each visit.
- Label all bottles with the participant trial ID number, the trial visit and sample type (i.e. plasma, serum or urine) using the stickers provided.
- Put the label on the tube vertically so that the sample is visible from top to bottom on the other side of the tube.

Please do not write the hospital number or patient name on the bottles to prevent sharing patient identifiable information

- Use yellow caps for the serum samples and purple caps for the plasma samples to match the blood collection tubes. Use clear caps for urine samples

PREP TUBES



STOP-ACEi ID: __ __ __ __
Visit: __ __ Type: __

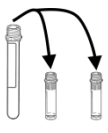
Taking the samples

Please use your own blood / urine collection tubes. Approximately 1mL sample is needed for each aliquot tube. You may need to use more blood collection tubes to get the required amount for the aliquot bottles.

	Tests	Collection Tubes required	Aliquot bottles required
Standard Trial Samples	Cystatin-C ACE	1 x gold-top serum tube 	2 x aliquot tube, yellow cap 
	NT-proBNP Renin	1 x purple-top EDTA plasma tube 	2 x aliquot tube, purple cap 
		DO NOT REFRIGERATE OR FREEZE WHOLE BLOOD SAMPLE FOR RENIN BEFORE IT IS ALIQUOTED AS THIS WILL CAUSE ERRONEOUS RESULTS	
Optional Biomarker Samples	unknown biomarkers in future analysis	1 x gold-top serum tube 	3 x aliquot tube, yellow cap 
		1 x urine collection pot 	3 x aliquot tube, clear cap 

Sample Preparation

NB. Renin samples must be prepared and frozen within 1 hour of venepuncture.

<u>Blood samples only</u>. After taking the samples, leave them to stand <u>upright</u> at room temperature for 10-20 min, but no longer than 2 hours, to allow clotting.	 CLOT
<u>Blood samples only</u>. Centrifuge samples at 3000 rpm (~1500*g*) for 5 mins.	 SPIN
<u>Blood and urine samples</u>. Aliquot the samples into the *labelled* tubes. Tubes should be ¾ full (approx. 1 mL) if possible. Cap with the appropriate caps (yellow = serum, purple = plasma, clear = urine).	 ALIQUOT
<u>Blood and urine samples</u>. Freeze the samples upright at -80°C as soon as possible after aliquoting. **Update the freezer log**.	 FREEZE

Labelling samples

Please do not write the hospital number or patient name on the sample bottles to prevent sharing patient identifiable information outside your hospital. The samples should be labelled with the patient's trial ID number, the visit number and the sample type only.

Trial sample labels are provided in section 8.2 of your Investigator Site File, alongside the Guidelines for Trial Sample Preparation. Further labels will be provided by the STOP-ACEi Trial Office at BCTU as required.

Please use the following abbreviations for visit number:
Baseline = BL
Month 12 (visit 5) = 12
Month 24 (visit 9) = 24
Month 36 (visit 13) = 36

Please use the following abbreviations for the sample type:
Serum = S
Plasma = P
Urine = U

Sample Storage

Once they are centrifuged and aliquoted, samples should be stored at -80°C in the upright position using the cardboard boxes provided.

Please keep the samples from each study visit together e.g. samples from a participant's baseline visit all on the same row.

Check all labels and freezer logs have been completed whenever new samples are added.

Complete all the details on the label on the storage box lid, remembering to write the box number and hospital name on the box itself.

Please contact the STOP-ACEi trial office if you need more sample storage boxes or bottles.

The Freezer Log

A freezer log is provided with your Investigator Site File so that you can easily keep track of what samples are held at your hospital without having to look through the freezer. A copy of this should accompany your samples when they are transported to the central lab in Hull. Please update the freezer log every time you put samples into the freezer.

Sample Transport

Please store samples at your hospital until you are contacted by the STOP-ACEi trial office to arrange transport. We expect samples to be transported approximately annually.

Dry ice and packaging will be provided by the courier that comes to collect the samples.

Questions and Answers

What if I don't have access to -80°C freezers?

The samples are not as stable at -20°C, so if you do not have access to a -80°C freezer, we will need to organise more frequent transport; approximately 3-monthly. You will still be able to do the trial at your centre and all other instructions for sample preparation still apply. Please alert the STOP-ACEi trial office if you cannot store samples at -80°C so that we can make alternative arrangements.

What if we don't use yellow capped blood collection tubes for serum or purple capped tubes for EDTA plasma at my hospital?

Different hospitals use different blood sampling tubes so there may be some differences. Please use whatever tubes for blood collection that you would use routinely for collection of serum and EDTA plasma. When aliquoting the samples, please use the provided yellow caps for serum samples, purple caps for EDTA plasma and clear caps for urine samples.

I'm running out of tubes/caps/storage boxes/labels. What should I do?

The STOP-ACEi trial office at BCTU will keep track of what consumables you need based on the number of participants entered into the trial, but if you are worried that you are running out, please contact us in case we have made an error. Please also let us know if you plan to enter lots of participants in quick succession so that we can make sure you have all the consumables you need. Remember that samples are taken at the baseline visit.

I've made a mistake with the sample preparation. What should I do?

Examples of errors in sample preparation include the following:

- Leaving sample clotting at room temperature for more than 2 hours or overnight
- Taking the wrong type of sample.
- Spilling a sample
- Refrigerating samples before they have been separated (centrifuged)
- Freezing samples before they have been separated (centrifuged)

If you make a mistake, please keep the samples and continue to process them as normal, but please make a note of any errors/problems in the study source documents and on the CRF. If you don't normally complete the CRF (e.g. you work in the hospital lab), please alert the research nurse so they can note this in the participant's records. This will help us with sample analysis at the central lab. Please also let the STOP-ACEi trial office know of any issues in sample preparation so that we can try to prevent any common errors being repeated at other hospitals.

Samples that have been frozen prior to separation are no longer viable and must be discarded. Further blood must be taken at the next visit and notes made on the study source documents and CRF.

I don't have enough serum/plasma for the required number of aliquots. What should I do?

Different hospitals use different sized blood collection tubes and some patients are more difficult to get samples from than others. You may need to use more than one blood collection tube or a larger size to get the required volume of serum or plasma for the number of aliquots required. Each aliquot needs to contain *at least* 0.7 mL, but ideally should be 1 mL.

If you haven't got enough and cannot obtain another sample, do not risk contaminating the sample with the pellet at the bottom of the tube or by pouring blood from one container to another. Please collect what aliquots you can. If you cannot collect the required number of aliquots, please make a note of this in the participant's source documents and on the STOP-ACEi CRF so that we can account for any 'missing' samples. If you don't normally complete the CRF (e.g. you work in the hospital lab), please alert the research nurse so they can note this in the participant's records.

Queries

If you have any queries about sample preparation or transport that aren't addressed here, please contact the STOP-ACEi trial office:

Birmingham Clinical Trials Unit, College of Medical & Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT.

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