The NIHR Southampton Biomedical Research Centre (BRC) has a tight quality assurance system for the writing, reviewing and updating of Standard Operating Procedures. As such, version-controlled and QA authorised Standard Operating Procedures are internal to the BRC.

The Standard Operating Procedure from which information in this document has been extracted, is a version controlled document, managed within a Quality Management System. However, extracts that document the technical aspects can be made more widely available. Standard Operating Procedures are more than a set of detailed instructions; they also provide a necessary record of their origination, amendment and usage within the setting in which they are used. They are an important component of any Quality Assurance Framework, but in themselves are insufficient and need to be used and interpreted with care.

Alongside the extracts from our Standard Operating Procedures, we have also made available here an example Standard Operating Procedure and a word version of a Standard Operating Procedure template. Using the example and the Standard Operating Procedure template, institutions can generate their own Standard Operating Procedures and customise them, in line with their own institutions.

Simply offering a list of instructions to follow does not assure that the user is able to generate a value that is either accurate or precise so here in the BRC we require that Standard Operating Procedures are accompanied by face-to-face training. This is provided by someone with a qualification in the area or by someone with extensive experience in making the measurements. Training is followed by a short competency assessment and performance is monitored and maintained using annual refresher sessions. If you require any extra information, clarification or are interested in attending a training session, please contact Dr Kesta Durkin (k.l.durkin@soton.ac.uk).

This document has been prepared from Version 1 of the BRC Standard Operating Procedure for preparation and administration of deuterated water and collection of saliva samples. It was last reviewed in September 2015 and the next review date is set for September 2017. The version number only changes if any amendments are made when the document is reviewed.

NIHR Southampton Biomedical Research Centre

Procedure for PREPARATION AND ADMINISTRATION OF DEUTERATED WATER AND COLLECTION OF SALIVA SAMPLES

BACKGROUND

This procedure is to be used for preparation and administration of labelled water and the processing of resulting samples. Deuterated (heavy) water for the purpose of measuring total body water (TBW) has been used for over forty years. The principle states that the volume of TBW is equal to the amount of deuterium consumed, divided by the concentration of deuterium in the body after equilibration.

PURPOSE

To ensure correct preparation and administration of deuterated (labelled) water and the processing of resulting samples.

SCOPE

This procedure applies to any study that requires preparation and administration of labelled water and the processing of resulting samples within the BRC.

RESPONSIBILITIES

It is the responsibility of the measurer to use this procedure for the preparation and administration of singly labelled water and the processing of resulting samples. It is the responsibility of the principle investigator to ensure that staff members who are working on specific studies have adequate experience and are competent to do so.

PROCEDURE

Deuterium oxide 99.9 atom % can be bought from Sigma Aldrich in different volumes ranging from 10g to 4kg. The product code is 151882

Salivettes are from Sarstedt. "Cotton swab salivettes without preparation". Product code is 51.1534 for 500/case (100/bag).

The deuterium concentration in the resulting saliva samples makes these samples suitable for analysis by FA-MS (Flowing Afterglow Mass Spectrometry) or IR-MS (Isotope Ratio Mass Spectrometry), on sample dilution.

Deuterium is not a hazardous substance and the drink should be made up in a kitchen NOT a laboratory.

Preparation of the water

1. Label an empty dosing bottle with the date, participant's details and your contact details.
2. With gloved hands, label and then weigh an empty dosing bottle with the lid and a drinking straw on the nutrition kitchen scales (to 3 decimal places).
3. Record the weight of the bottle, lid and straw.
4. Transfer the appropriate dose of deuterium based on the participant's body weight (table 1), into the weighed and labelled dosing bottle.

Subject Weight	Dose of deuterium to drink through straw
Less than 30 kg	10 ml
30 – 40 kg	12 ml
41 – 50 kg	15 ml
51 – 70 kg	20 ml
71 – 100 kg	29 ml
More than 100 kg	33 ml

Table 1. Dosing volumes in ml based on body weight in kg

5. Label another dosing bottle with the date, participant's details and your contact details and put in 20ml of tap water.
6. Wash your hands and explain the procedure to the participant.
7. Check that the participant has not had anything to drink for 2 hours prior to administration and has not brushed their teeth for at least 30 minutes before drinking the water.
8. Ask them to empty their bladder.
9. Take the cotton swab from the first of 2 salivettes (labelled with Pre-dose, date, subject identification and visit number).
10. Ask the participant to move the swab around their mouth until wet and then replace the wet swab into the upper portion of the salivettes. The swab should **not** be chewed. Repeat this using the second salivette so that you have obtained 2 saliva-soaked cotton wool swabs.

11. Centrifuge the collected saliva samples following the instructions in the section on "centrifugation of saliva samples" below (5.2.2).
12. Ask the participant to drink the full volume of deuterium through the straw that was weighed with the bottle.
13. When they have ingested all the solution, ask them to rinse their mouth with 20ml of tap water to remove any residual deuterium.
14. Record the time that the dose was given.
15. Keep the straw in the bottle and weigh the bottle and lid with the straw remaining in the bottle.
16. Calculate the weight drunk by subtracting this value from the weight of the dosing bottle containing the deuterated water drink, lid and straw. Record these details.
17. Obtain three more sets of saliva samples ideally from 2 salivettes per time-point: 2, 3 and 4 hours post-dose.
18. If the participant finds it difficult to soak 2 cotton swabs with saliva then one well soaked one should be satisfactory for the 2, 3 and 4 hour time points. It is essential to ensure there is enough saliva collected from the participant before they drink the deuterium (pre-dose saliva sample) so 2 salivettes worth of saliva at this time-point is essential. No less that 0.5 ml of saliva from any time-point should be stored in each glass vial.

Centrifugation of saliva samples

Perform this in the WTCRF sample processing laboratory "category 2" laboratory

19. Take the cotton swab after the participant has moved it around their mouth to thoroughly wet it and place it back into the salivette.
20. Making sure that you have balanced the weight in the centrifuge using the scales by the side of the centrifuge in the WTCRF sample processing lab, place the salivettes into the appropriate centrifuge buckets.
21. Centrifuge at 4°C for 8 minutes at 2060 x g (3000 rpm) following the WTCRF SOP for use of the "Beckman Counter Allegra 6R" centrifuge.
22. Remove from the centrifuge and check that all the saliva is now in the bottom portion of the salivette. Remove the top half (comprising the lid, upper plastic section and swab) and discard.
23. Split the saliva sample (in the bottom portion of the salivette) equally between 2 appropriately labelled glass vials using a pipette. Parafilm the lid to prevent any evaporation prior to analysis, whilst in storage.
24. Store the samples at -80°C