STANDARD OPERATING PROCEDURE FOR DOSE ADMINISTRATION AND 
SALIVA SAMPLING

1.0 Purpose:

1.1 This SOP describes the procedure for dose administration and sampling of saliva in studies of body composition and human milk intake assessed using deuterium dilution techniques with analysis of deuterium enrichment by Fourier Transform infrared spectrometry (FTIR).

2.0 Scope:

2.1 This procedure should be followed by all participants in projects funded by the International Atomic Energy Agency (IAEA).

2.2 Any queries comments or suggestions relating to this SOP should be addressed to a technical officer at the Nutritional and Health Related Environmental Studies section of the Division of Human Health, IAEA, Vienna, Austria C.Slater@iaea.org).

2.3 Last updated by Christine Slater 8 July 2013.

3.0 Safety requirements:

3.1 Appropriate protective clothing e.g. aprons and gloves must be worn at all times when collecting or handling samples.

3.2 No eating, drinking or applying of cosmetics is allowed during sample collection and handling.

3.3 Dispose of syringes and swabs with clinical waste (incinerate).

4.0 Associated documents:

4.1 Study report forms and SOP’s.

4.2 Local safety rules.

4.3 Standard Operating Procedure for the preparation of deuterium oxide doses.

4.4 Standard Operating Procedure for analysis of deuterium enrichment by FTIR.

5.0 Notes:

5.1 Saliva sampling should take place in a calm environment.

5.2 Good preparation before taking the samples and a clear understanding of the procedure are very important. Make sure you have all the equipment listed in sections 7 and 8 before starting.

5.3 Clearly explain the procedure to the mother before sampling.

5.4 The mother can be trained to sample saliva from her baby. She should wear gloves when doing this.
5.5 Take care with babies older than 6 months that they do not swallow the cotton wool.

5.6 Use different coloured caps for baseline and post-dose sample vials to aid identification.

5.7 Keep good records. Record all dates and times of saliva collection on the saliva report form as well as on bottles. Copy this information to a spreadsheet as soon as possible. An example of a saliva report form is shown in Appendix 1.

6.0 Quality Control:

6.1 Label vials before starting saliva sampling.

6.2 Precautions must be taken to avoid cross-contamination between baseline and post-dose samples.

6.3 To avoid contamination of samples NEVER store samples and doses together.

6.4 Keep track of samples.

7.0 Equipment:

7.1 Watch (to note time of saliva sampling).

7.2 Cool box with ice pack (for storing samples in the field until they can be frozen).

7.3 Racks for sample vials.

8.0 Consumables:

8.1 Saliva report form

8.2 Doses (prepared in the laboratory)

8.3 Drinking water

8.4 Drinking straws

8.5 Cotton wool and swabs

8.6 Hand towels

8.7 Disposable 20 mL syringes

8.8 Sample storage vials (cryovials) with screw cap

8.9 Replacement caps for baseline sample vials

8.10 Gloves

8.11 Zip-lock bags- small and large

8.12 Labels

8.13 Permanent ink pens for labelling tubes
9.0 Procedures: Saliva sampling for adults and children

9.1 Label a sample storage vial with Participant ID, Date and Time of collection. Use permanent ink marker.

9.2 Use clean gloves for each participant.

9.3 When collecting samples ensure that the participant has not eaten or drunk anything for at least 30 minutes before collection.

9.4 Give the participant a cotton wool ball to soak up saliva. Ask them to move it round their mouth until it is sodden, keeping their mouth closed while doing this.

9.5 Remove the plunger from a new 20 mL disposable syringe.

9.6 Ask the participant to transfer the cotton wool to the front of their mouth and transfer it directly from the mouth into the body of the syringe.

9.7 Replace the plunger in the body of syringe.

9.8 Remove the lid from the vial, and use the syringe plunger to extract saliva from the cotton wool into the sample storage vial. Replace the lid making sure it is properly closed. Use a coloured cap for the baseline sample.

9.9 If there is not at least 2 mL of saliva repeat above steps with a new cotton wool ball or swab. If possible collect 4 mL to allow for repeat analysis.

9.10 Discard syringe, cotton wool and gloves between participants. Do not reuse sample vials or syringes.

9.11 Dispose of syringes and swabs with clinical waste (incinerate).

9.12 Record all dates and times of saliva collection on data entry form as well as on vials.

10.0 Procedures: Saliva sampling for babies

10.1 Label a sample storage vial with Participant ID, Date and Time of collection using a permanent ink marker.

10.2 Use clean gloves for each baby.

10.3 When collecting samples ensure that it is at least 15 minutes since the baby was last fed, so that there is no residual milk or other foods in the baby’s mouth.

10.4 In babies, saliva is sampled using a cotton wool wrapped around a wooden spatula. Collect saliva by moving the swab around the baby’s mouth until the cotton wool is sodden. The time required for this will vary between babies. Be patient. It may take several attempts to collect the required volume (minimum 2 mL).

10.5 Remove the plunger from a new 20 mL disposable syringe. Remove the cotton wool from the swab and place in the barrel of the 20 mL syringe.

10.6 Replace the plunger in the body of syringe.

10.7 Remove the lid from the vial and use the syringe plunger to extract saliva from the cotton wool into the sample storage vial. Replace the lid making sure it is properly closed. Use coloured cap for baseline sample.
10.8 If there is not at least 2 mL of saliva repeat above steps with a new cotton wool ball or swab.
10.9 Discard swab, syringe, cotton wool and gloves between participants. Do not reuse sample vials or syringes.
10.10 Dispose of syringes and swabs with clinical waste (incinerate).
10.11 Record all dates and times of saliva collection on data entry form as well as on bottles.

11.0 Storage of saliva samples
Careful management and labelling of saliva samples is essential. Saliva sample vials from each participant should be stored together.

11.1 It is important to use good quality, screw-capped containers for storage of saliva samples.
11.2 Containers must be firmly closed to prevent loss of water by evaporation, and cross-contamination between samples.
11.3 Sample vials should be stored in zip-lock bags to prevent cross-contamination between participants, and between pre-dose and post-dose specimens.
   11.3.1 Place the baseline sample in a ziplock bag.
   11.3.2 Use a new bag for the post-dose samples.
   11.3.3 Place all of these together in a larger ziplock bag.
   11.3.4 Write the participant identification number on both the sample vials and the zip-lock bags.
   11.3.5 To minimise bacterial growth, saliva samples should be stored in a cool box or fridge until they can be transferred to a freezer at -20°C for storage until analysis.

12.0 Sample Tracking
12.1 Have a system in place to keep track of where samples are stored, and which samples have been analysed. This is independent of the laboratory records. An example of a page from a saliva sample tracking form is shown in Appendix 2.

13.0 Supporting information:
13.1 IAEA Human Health Series No.7: Stable Isotope Technique to Assess Human Milk Intake in Breastfed Infants.
13.2 IAEA Human Health Series No. 12: Introduction to Body Composition Assessment using the Deuterium Dilution Technique with Analysis of Saliva Samples by FTIR.
13.3 IAEA distance learning modules on Assessing Intake of Human Milk in Breastfed Infants and Assessing Body Composition by Deuterium Dilution.
The above publications can be access through the Nutrition pages of IAEA Human Health Campus: [http://nucleus.iaea.org/HHW/Nutrition/index.html](http://nucleus.iaea.org/HHW/Nutrition/index.html)
14.0 History review:

This draft was prepared at the IAEA Regional (AFRA) Training Course on Standard Operating Procedures (SOP) for Isotope Techniques in Infant and Young Child Nutritional Status, Dare es Salaam, United Republic of Tanzania, 17-21 August 2009.

It was adapted for general use by Christine Slater, Nutritional and Health Related Environmental Studies Section, Division of Human Health, IAEA, 11 January 2010.
Appendix 1  Example of a study report form for saliva sampling

RAF/6/039 Applying Stable Isotope Techniques to Improve Infant and Young Child Nutrition Interventions in AFRA Countries

Country Code: _______________________________
Couple ID: _______________________________
Responsible person: _______________________________
Dose Date: _______  Dose number: ______  Weight D₂O (g) ______

<table>
<thead>
<tr>
<th></th>
<th>Mother</th>
<th>Baby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg) Day 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg) Day 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of baseline saliva sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time dose taken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 saliva sample: Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2 saliva sample: Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 saliva sample: Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4 saliva sample: Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 13 saliva sample: Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14 saliva sample: Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 2: Example of a saliva sample tracking form

RAF/6/039 Applying Stable Isotope Techniques to Improve Infant and Young Child Nutrition Interventions in AFRA Countries

Keep samples from mother/baby pairs together. Place the mother’s and the baby’s baseline samples in a small ziplock bag. Use a second bag for the mother’s postdose samples and a third bag for the baby’s post dose samples. Place all of these together in a larger ziplock bag.

<table>
<thead>
<tr>
<th>Couple ID</th>
<th>Date of freezing baseline samples</th>
<th>Storage place</th>
<th>Name of responsible person</th>
<th>Date of freezing post dose samples with initials of responsible person</th>
<th>Date removed for analysis</th>
<th>Name of analyst</th>
<th>Storage place after analysis</th>
<th>Date of disposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1234</td>
<td>01/10/09</td>
<td>Drawer 1</td>
<td>Nadine</td>
<td>02/10/09 NC 03/10/09 NC 04/10/09 NC 05/10/09 NC 14/10/09 NC 15/10/09 NC</td>
<td>10/03/10</td>
<td>Grace</td>
<td>Drawer 4</td>
<td>10/03/10</td>
</tr>
</tbody>
</table>