Appendix H. Clinical Staff (Stage 2) documents

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National Infant Diet and Health Study

Stage 2 - Clinic Visit

Manual of Procedures

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H.1. Introduction

The purpose of this document is to detail, from start to finish, the procedures and components involved in **Stage 2 of The National Infant Diet and Health Study** that are being carried out within your clinic ((CRF)/hospital) and/or laboratory. Training is provided for key personnel ahead of the study start and we will also aim to be there for your first study day to offer support.

The study is being carried out for the Department of Health and the Food Standards Agency, by the National Infant Diet and Health Study consortium. The consortium consists of independent research institutes: Medical Research Council (MRC) Human Nutrition Research (HNR) based in Cambridge, National Centre for Social Research (NatCen) based in London, MRC Epidemiology Unit based in Cambridge, and the Human Nutrition Research Centre at Newcastle University. The study has been reviewed and approved by the Scientific Co-ordination Committee of MRC HNR and by *Cambridgeshire 4* Research Ethics Committee [REC Ref: 09/H0305/101].

The clinic visit (Stage 2) component of the National Infant Diet and Health Study is overseen and coordinated by MRC Human Nutrition Research.

This pack includes all the information you need to know about the overall study. Prior to the clinic visit the following steps will have been completed for participants:

1. Postcodes selected by National Centre for Social Research (NatCen), London
2. Children selected by HM Revenue and Customs from Child Benefit Records
3. Opt out/Advance letter sent to homes of selected children (NatCen)
4. NatCen interviewer goes to the home and with agreement interviews the parent/carer of the selected child
5. Parent/carer completes a 4 day diet diary for the selected child
6. NatCen interviewer returns to collect the diet diary and introduces Stage 2, the clinic visit. If interest is shown, further information is left with the participant and contact details are collected for the parent and child
7. Contact details for interested participants are passed to HNR from NatCen
8. HNR contact the participant by telephone to set up the clinic visit

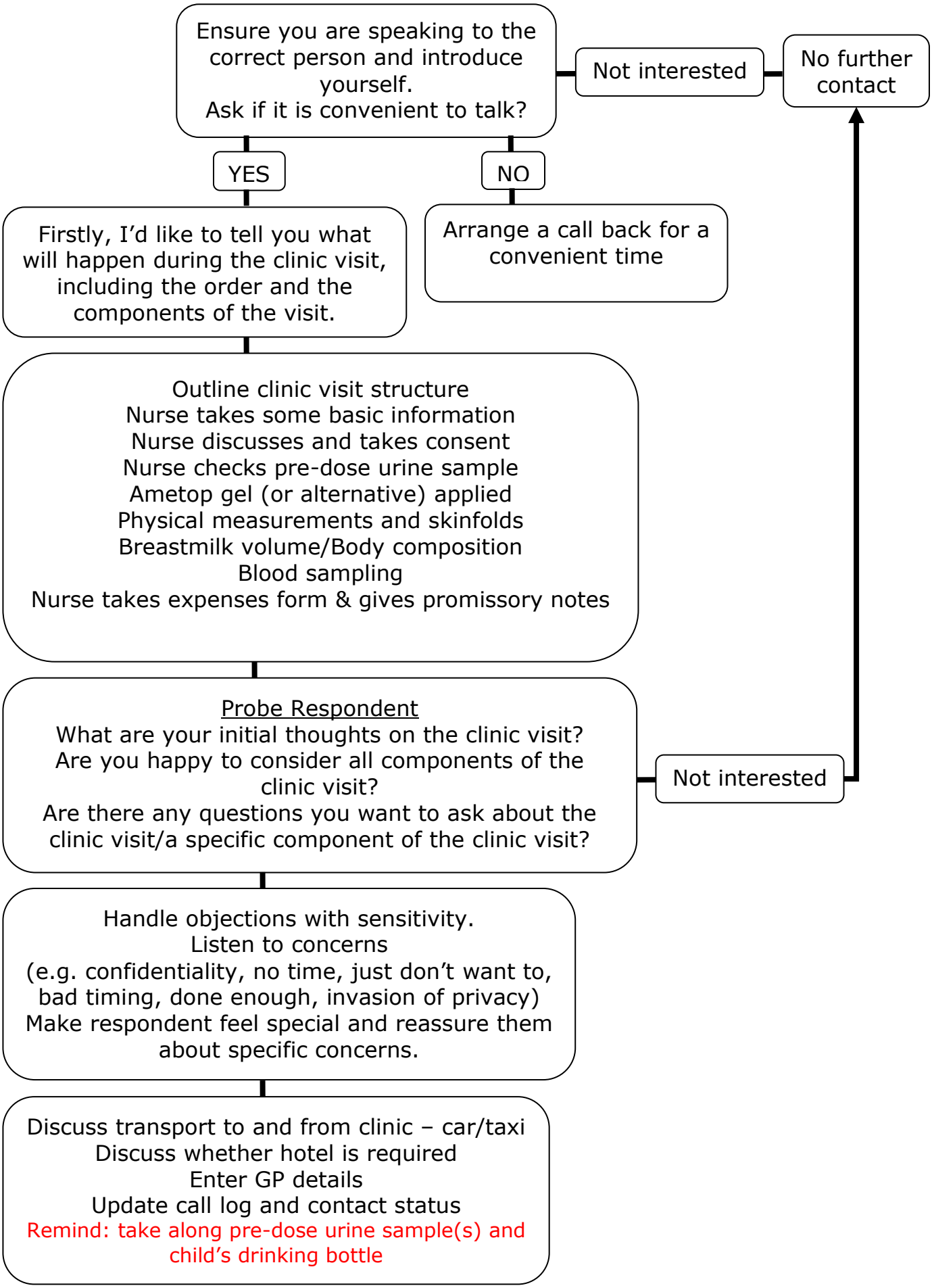
H.2. Clinic Visit - Set Up

Telephone call to participant

The research team at HNR are responsible for contacting the parent/carer of each participant by telephone prior to the clinic visit. This phone call is used to book the appointment and to determine which components of the clinic visit the participant is likely to be involved in. The participant will already have received Stage 2 leaflets [Annexes 8, 10 and 11] and instructions on how to take a pre-dose urine sample if an interest in the breast milk, fluid intake and body composition element was recorded at Stage 1. The telephone call is the first time that the parent of the participant will have the chance to ask detailed questions about the different components of the clinic visit. Each component will be discussed in full and plenty of time will be allowed to ensure the parent/carer is comfortable with each component.

Please refer to flowchart below for the format of the telephone call.

Telephone call to participant (HNR Research Team)



Data File
Following the telephone call the data below will be entered into the clinic database:

- date of phone call(s)
- participant status i.e. appointment booked, appointment refused etc
- date and time of clinic visit
- clinic visit components likely to be undertaken
- method of transport to clinic (e.g. taxi, car) and whether a hotel is required

A letter confirming the clinic visit will be sent to the participant by HNR. Text message reminders may also be sent where permission is obtained.

Taxi
HNR will already have arranged a TAXI account with your local site.

When the TAXI box is selected in the clinic database you will need to ensure a taxi is booked (to and from the clinic visit) for your participant with a local taxi company and that the participant is informed.

Expenses
HNR will issue expenses forms to participants (if required), and book hotel (if required).
Collect the expenses claim form at the participant's clinic visit and post back to HNR weekly.
In the event that it is **not** possible to collect the expenses claim form, i.e. because the mum is unsure of the return costs, HNR will provide pre-addressed/paid envelopes for the clinic to provide the parent for the return of the expenses form. **This should be a last resort.**

H.3. Informed consent

The tests involved cannot be done unless the parent of the participant has consented to the procedure and has consented for either their child's results to be sent to their GP, or alternatively, to be contacted by a study clinical advisor if their results have potentially important implications for their health or health care. Each component requires separate consent. Parents/carers may choose which components they wish their child to participate in. Consent forms are provided by HNR.

Please ensure all procedures are fully explained and time for discussion is allowed to ensure informed consent. Annex 24, the study conduct information sheet must also be given out and explained.

Informed consent must be taken at the clinic visit prior to commencing any measurements or procedures.

Procedure

1. Check the DOB and gender on the labels with the participant and place the **CON** label on page 1 of the consent form
2. Write the participant's study ID number in the appropriate place on all 3 pages of the consent form
3. Clearly write the name of the parent/legal guardian and the participant in the appropriate place on page 1 of the consent form
4. Complete each section of the consent form as appropriate and initial each statement in the relevant box
5. Strike through any sections that are not relevant to the participant e.g. if the participant is not taking part in the blood sampling protocol, strike through section **C** and **D**
6. Ensure the parent/legal guardian has clearly written their name, the date and signed each page of the consent form
7. It is equally important for the research team member, who has taken the written consent, to also print their name, date and sign each page of the consent form in the presence of the parent/legal guardian

Notes on the consent form:

1. The participants' GPs will only be informed of blood results. They will not be informed of general participation in the study in the event that a blood sample is not undertaken.

2. Section D – only needs completed if the parent/legal guardian of the participant fulfils **all** of the following criteria:

   - If the parent/legal guardian has initialled 'Yes' to consent questions 11 and 12
   - If the parent/legal guardian has initialled 'No' to consent questions 3 and 13

   *To continue with the blood sample the parent **must** complete section D. If refused, please do **not** continue with the blood sample.*

3. Top copy (original): to be faxed at end of clinic visit to HNR (see Section 8) and then posted back to HNR by pre-paid special delivery
   Middle copy: to be given to the parent/carer of the participant to keep
   Bottom copy: to be kept by clinic

H.4. List of Documents

There are a number of documents required for the National Infant Diet and Health Study. Documents are split into 1) Informational documents such as Stage 1 leaflets (for your reference) and Stage 2 leaflets (for your usage), and 2) Forms to be completed at the clinic visit. Please see list below with brief explanations:

Stage 1 – participant information sheets for reference

- Annex 6 – NatCen general information leaflet
- Annex 6A – HNR general information leaflet
- Annex 7 – Opt out/Advance letter
- Annex 8 – Stage 1 leaflet
- Annex 18A – Instruction sheet – pre-dose urine sample - Protocol 1
- Annex 18B – Instruction sheet – pre-dose urine sample - Protocol 2

Stage 2 – participant information sheets

- Annex 9 – Stage 2 leaflet – 'The Clinic Visit'
- Annex 10 – Why provide a blood sample
- Annex 11 – General info sheet about combined breastmilk, fluid intake and body composition
- Annex 19A – Instruction sheet for urine sample – Breastmilk, fluid intake & body composition
- Annex 19B – Instruction sheet for urine sample – Fluid intake & body composition
- Annex 20A – Collection form - Breastmilk, fluid intake & body composition
- Annex 20B – Collection form - Fluid intake & body composition
- Annex 21 – Ametop gel leaflet
- Annex 22 – Clinic consent 2
- Annex 23 – Clinic appointment letter
- Annex 24 – Study conduct sheet
- Annex 31a&b – Bravery certificates
- Annex 37 – Missed clinic appointment card
- Promissory notes – for promise of voucher payment, following completion of certain clinic components

Clinic – Forms/information sheets

- Clinic measurement record card
- Addendum B – Blood tracking form
- Addenbrooke's blood form
- Checklist

Laboratory Information

- Addendum A – Flow chart for sample processing
- Addendum B – Blood sample tracking form
- Addendum C – Instructions for the shipment of the National Infant Diet and Health Study serum samples

Participant Feedback

- Example of Dietary Feedback
- Example of Blood feedback
  - Full blood count
  - Iron and Vitamin D
- Example of breastmilk feedback

H.5. Clinic Visit – Order of Events

Once consent has been taken it is at your discretion which order you undertake the components of the clinic visit. The participant may choose which components to take part in and does not have to complete all of them. If the participant is undertaking the blood sampling and the mother requests the use of Ametop gel, this takes at least 20 minutes to take effect. We advise that this is applied while other procedures are carried out to allow the gel to take effect. Additionally, if the participant is taking part in the stable isotope component, you may start giving the dose at the beginning of the clinic visit and allow up to an hour to complete the dosing.

Suggested order of events (as applicable per participant)

1. **Give out study conduct information sheet – Annex 24**
2. **Give study toy**
3. **Describe components, talk through consent and allow time to read and discuss consent**
4. **Take written consent –** *see section III*
5. **Check pre-dose urine sample(s) **if not provided do this now –** *see protocol 1/2*
6. **Apply Ametop gel if desired–** *see Annex 21*
7. **Give stable isotope dose to mother first and then child. Note: dosing can continue for up to an hour –** *see either protocol 1 or 2*
8. **Take child's length –** *see protocol 3*
9. **Take mother's height –** *see protocol 4*
10. **Take mother's weight –** *see protocol 5*
11. **Take child's weight –** *see protocol 5*
12. **Take child's head circumference –** *see protocol 6*
13. **Recap – how to collect a spot urine sample –** *see either Annex 19A or B (which are provided in the Stable Isotope dosing kit)*
14. **Demonstrate how to complete urine collection forms –** *see either Annex 20A or B (which are provided in the Stable Isotope dosing kit)*
15. **Provide and discuss instructions for completion of the Breastmilk diary and place Breastmilk diary**
16. **Carry out skinfold thickness measurements –** *see protocol 7*
17. **Photocopy clinic measurement record card and SI form – give originals to mum –** *refer to chapter on 'after clinic visit'*
18. **Take blood –** *see protocol 8*
19. **Give promissory note(s) –** *see promissory notes voucher payment form*
20. **Take and check expenses claim form (if applicable)**
21. **Deliver blood to Laboratory** *– see protocol 8*
22. **Post EDTA monovette to Addenbrooke's hospital –** *see protocol 8*
23. **Fax documents to HNR** *– refer to chapter on 'after clinic visit'*
24. **Update electronic database –** *refer to chapter on 'after clinic visit'*

H.6. Individual Protocols

Protocol 1.0 Tracer water BREAST MILK Protocol

Breast Milk Volume Assessment – Protocol for Dose Administration

Introduction

All mothers who report some degree of breastfeeding will be asked whether they are willing to participate in the breast milk volume assessment component of the study. A sample of urine is required from both mother and child, and then both are given oral doses of tracer water. The mother and child will then supply further urine samples, one a day for fourteen consecutive days after drinking the tracer water, which together with the pre-dose, makes a total of fifteen urine samples each. The urine samples will be sent to HNR for analysis.

If this protocol is completed, protocol 2.0 will not apply.

Before the Clinic Visit

The mother will have been asked for verbal consent to participate, and if they have agreed she will have been supplied with a pre-dose collection kit. On arrival at the clinic the mother should have brought with her:

- 1 x sample of the mother's urine
- 1 x sample of the child's urine
- Details of when the samples were collected should be written on both the bag labels and the urine bottle storage containers
- One of her child's usual feeding bottles (ready for use). (They may not have one if they are exclusive breastfeeders)

The clinic should have received Annex 19A, 20A, the breast milk diary, prompt sheet, trial page and the tracer water dosing kits from HNR comprising:

- 1 x mother's dosing bottle containing a pre-weighed amount of tracer water.
- 1 x straw for drinking of dose
- 1 x printed urine collection form
- 1 x printed urine collection instructions.
- 14 x glass urine collection and storage bottles for mother's post dose daily urine collections (labelled days M1 to M14)
- 15 x plastic cups to aid mother urine collection (if required)
- 1 x child's dosing bottle containing a pre-weighed amount of tracer water
- 14 x glass urine collection and storage bottles for child's post dose daily urine collections (labelled days B1 to B14)
- Cotton wool
- 15 x syringes (14+1 spare) to squeeze urine out of cotton wool
- 1 x spare pair of plastic forceps
- 1 x pen
- Elastic bands to secure full boxes

The dosing bottles should be inspected for leakage against a 'fill line' on the bottle, and the whole kits should preferably be stored refrigerated (but NOT frozen) or in a cool place if not possible to do so.

*** Please note: The dosing kits should be taken out of the fridge at least an hour before the clinic visit to allow the doses to reach room temperature.**

During the Clinic Visit

Discuss and gain consent for the dosing procedure with the mother and witness the signature on the consent form. The form must be correctly filled in before any further part of the protocol can be performed.

The breast milk volume assessment components of the clinic visit are to:

- Ensure that formal *written* consent from the mother is obtained for herself and her child before administering the dose
- Check that the pre-dose samples have been properly obtained
- Weigh both the mother and the child
- Dose the mother
- Dose the child
- Hand over the equipment required for the mother to complete the method protocol for post-dose urine collection
- Hand over the breastfeeding diary and explain how to fill this in

Procedure

A urine sample from both mother and child should have been collected and brought to the clinic for checking. These bottles should be no more than ¾ full. If these pre-dose samples are not available you will have to obtain a urine sample from both mother and child **before** opening the tracer water doses. The 'pre-dose' urine sample is the most important sample of the study and should be treated as such. The respondent should store this sample and subsequent ones in the containers provided, preferably in their fridge or alternatively in a cool, dry environment such as an unheated garage. It is vitally important that the pre-dose is uncontaminated by the tracer water itself. For this reason the pre-dose samples must be obtained and stored in capped vials before the dosing bottles are opened.

With a colleague check that the respondent identity number agrees with that on the dosing kit. **The kits are tailor made for the participants and must only be used for the intended mother and child**.

Copy the pre-dose collection time information onto the appropriate sections of the urine sample collection form.

Administering the dose to the mother: -

1) Weigh all the mother's dosing materials, which will include the dosing bottle, tracer water dose plus top and straw, to 1 decimal place and record the weight on their collection form.
2) Ask the mother to drink the tracer water dose, without spilling it, using the straw provided. **Record the date and time of dosing on the urine collection form.**
3) Once the water is drunk squash the straw inside the bottle and replace the top.

4) Reweigh the empty dosing bottle, top and straw (and wrapper) and record this weight also on the collection form to 1 decimal place.

The bottle and straw do not need to be kept and can be disposed of in normal household rubbish.

Administering the dose to the child: -

Where applicable, the mother will have been asked to bring one of her child's bottles with her for dosing. The bottle should be dry, empty and ready for use. If this bottle is not available or not in a suitable condition for use, e.g. dirty or wet, then please use one of the disposable bottles already provided to the clinic by HNR. Depending on the child, a feeding tube and syringe or just the syringe provided by HNR can also be used to administer the tracer water dose. The dose can be fed to the child for up to 1 hour to maximise the intake.

***Please specify the drinking vessel used on the urine collection form.**

If using a child's drinking bottle:

1) Weigh the empty dosing bottle and teat and record on the collection form to 1 decimal place.
2) Carefully transfer the tracer water contents of the dosing bottle provided into the child's drinking bottle.
3) Weigh the child's drinking bottle with the dose in it to 1 decimal place and record this weight on their collection form.
4) Ask the mother to feed the child the water trying to avoid spillage as much as possible. **Record the date and start time at which the child drinks the water on the urine collection form. Feeding the child the dose may be continued for an hour to maximise the dose drunk.**
5) Once the water is consumed reweigh the child's empty bottle and record this weight also on the collection form.

*Please note - although it is preferable that all the dose is drunk, even if none of the dose is drunk or only a little of the dose, please continue with the protocol and re-weigh the bottle containing the leftovers to allow the determination of the actual amount consumed. *Please do not force-feed the child just to ensure total consumption. **We can still determine the breast milk intake so long as the mother has consumed her dose.**

If using a syringe and/or feeding tube:

1) Weigh the empty syringe and/or feeding tube and record on the collection form to 1 decimal place.
2) Transfer the tracer water contents of the dosing bottle provided into an empty plastic drinking cup. Using the syringe provided, carefully draw up the tracer water into the syringe. Please ensure that all of the tracer water is drawn up.
3) Weigh the syringe containing the tracer water (plus the feeding tube if used) to 1 decimal place and record this weight on their collection form.
4) Ask the mother to slowly push the plunger and feed the child with the tracer water through the syringe. If using the feeding tube then attach this on the mother's finger using the tape provided and carry on using the syringe as above.

Try to avoid spillage as much as possible. **Record the date and start time at which the child drinks the water on the urine collection form.**

5) Once finished re-weigh the child's empty syringe and/or feeding tube. Record this weight also on the collection form.

*Please note – although it is preferable that all the dose is drunk, even if none of the dose is drunk or only a little of the dose, please continue with the protocol and re-weigh the syringe containing the leftovers and/or feeding tube used to allow the determination of the actual amount consumed. *Please do not force-feed the child just to ensure total consumption. **We can still determine the breast milk intake so long as the mother has consumed her dose.**

Any problems during dosing <u>must</u> be recorded on the urine collection form e.g. child was sick, spillage etc. It is important to be honest and provide as much detail as possible when recording this to enable valid analysis. If possible an estimation of any dose losses should be made.

If the child's own bottle was used this can be rinsed out following reweighing, and given back to the mother for her to clean as normal. The disposable bottle or syringe and/or feeding tube (if used), and the bottle that contained the dose should be disposed of with the clinic's normal rubbish.

Before the mother leaves the clinic

Hand out the breastfeeding diary to the mother, go through the diary prompt sheet and explain how to fill in the diary. You can use the trial page to practice.

Please advise that this should be completed for the next 14 days along with the collection of the urine samples.

Please print your name and sign the urine sample collection form. Photocopy and retain for future use.

Pack the sample collection kits including the already collected pre-dose urine samples, with the items supplied by HNR (urine sample collection form, urine sample collection instructions, 14 x mother's glass urine collection and storage bottles for post dose daily urine collections labelled days M1 to M14 and storage box, 14 x child's glass urine collection and storage bottles for post dose daily urine collections labelled days B1 to B14 and storage box, cotton wool, 15 x syringes (14+1 spare), 1 x spare pair of plastic forceps, pen and elastic bands along with 15 x plastic disposable cups) into the cooler bags provided.

Contact Details

All dose requests, sample returns, problems and queries should be directed to one of the following contacts at HNR:

- Marilena Leventi (Tracer water study manager)
- Priya Singh (Tracer water study assistant)
- Dr Jill Sommerville (Survey co-ordinator)
- Dr Les Bluck (Senior Scientist, stable isotopes research)

They can all be contacted **during normal office hours** via HNR reception, on **01223 426356** and by asking to speak to one of the above contacts. The people above will be contacted in order of appearance if no contact is specified.

Email addresses for the contacts are also provided below:

- Marilena Leventi Marilena.Leventi @mrc-hnr.cam.ac.uk
- Priya Singh Priya.Singh @mrc-hnr.cam.ac.uk
- Dr Jill Sommerville Jill.Sommerville @mrc-hnr.cam.ac.uk
- Dr Les Bluck Les.Bluck@mrc-hnr.cam.ac.uk

HNR address: Human Nutrition Research
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge
CB1 9NL

Protocol 2.0 Tracer water BODY COMPOSITION Protocol

Body composition and Fluid Intake – Protocol for Dose Administration

Introduction

All mothers who report no degree of breastfeeding, or who decline to participate in the breast milk volume assessment will be asked whether they are willing to participate in the body composition and fluid intake part of the study. For this a sample of urine is required from the child, and then the child is given an oral dose of tracer water. The parent will then obtain further urine samples from the child, one a day for five consecutive days after drinking the tracer water, which together with the pre-dose makes a total of six urine samples in all. The urine samples will be sent to HNR for analysis.

If this protocol is completed, protocol 1.0 will not apply.

Before the Clinic Visit

The parent will have been asked for verbal consent to participate, and if they have agreed they will have been supplied with a pre-dose collection kit for their child. On arrival at the clinic the parent should have brought with them:

- 1 x sample of the child's urine
- Details of when the sample was collected should be written on both the bag label and the urine bottle storage container
- One of her child's usual feeding bottles (dry, empty and ready for use)

The clinic should have received Annex 19B, 20B and a tracer water dosing kit from HNR comprising:

- 5 x glass urine collection and storage bottles for child's post dose daily urine collections (labelled days C1 to C5)
- 1 x child's dosing bottle containing a pre-weighed amount of tracer water
- 1 x printed urine collection form
- 1 x printed urine collection instructions
- Cotton wool
- 6 x syringes (5+1 spare) to squeeze urine out of cotton wool
- 1 x pen
- Elastic bands to secure full box

The dosing bottle should be inspected for leakage against a 'fill line' and the whole kit should preferably be stored refrigerated (but NOT frozen) or in a cool place if not possible to do so.

*** Please note: The child's dosing kit should be taken out of the fridge at least an hour before the clinic visit to allow the dose to reach room temperature.**

During the Clinic Visit

Discuss and gain consent for the dosing procedure with the mother and witness the signature on the consent form. The form must be correctly filled in before any further part of the protocol can be performed.

The body composition and fluid intake components of the clinic visit are to:

- Ensure that formal *written* consent from the mother is obtained for her child before administering the dose
- Check that the pre-dose sample has been properly obtained
- Weigh the child
- Dose the child
- Hand over the equipment required for the mother to complete the method protocol for post-dose urine collection

Procedure

A urine sample from the child should have been collected and brought to the clinic for checking. The bottle should be no more than ¾ full. If this pre-dose sample is not available you will have to obtain a urine sample from the child **before** opening the tracer water dose.

The 'pre-dose' sample is the most important sample of the study and should be treated as such. The respondent should store this sample and subsequent ones in the containers provided, preferably in their fridge or alternatively in a cool, dry environment such as an unheated garage. It is vitally important that the pre-dose is uncontaminated by the tracer water. For this reason the pre-dose sample must be obtained and stored in a capped vial before the dosing bottle is opened.

Check with a colleague that the participant identity number agrees with that on the dosing kit. **The kit is tailor made for the respondent and must only be used for the intended child.**

Copy the pre-dose collection time information onto the appropriate sections of the urine sample collection form.

Administering the dose to the child: -

The mother will have been asked to bring one of the child's bottles with her for dosing. The bottle should be dry, empty and ready for use. If this bottle is not available or not in a suitable condition for use, e.g. dirty or wet, then please use one of the disposable drinking bottles already provided to the clinic by HNR. Depending on the child, a feeding tube and/or syringe provided by HNR can also be used to administer the tracer water dose. The dose can be fed to the child for up to 1 hour to maximise the intake.

***Please specify the drinking vessel used on the urine collection form. If using a child's drinking bottle:**

1) Weigh the empty dosing bottle and teat and record on the collection form to 1 decimal place.
2) Carefully transfer the tracer water contents of the dosing bottle provided into the child's drinking bottle.
3) Weigh the child's drinking bottle with the water in it to 1 decimal place and record this weight on their collection form.
4) Ask the mother to feed the child the water trying to avoid spillage as much as possible. **Record the date and start time at which the child drinks the water on the urine sample collection form.**

5) Once the water is consumed reweigh the empty bottle and record this weight also on the collection form to 1 decimal place.

*Please note – although it is preferable that all the dose is drunk, if this is not possible and half (50%) of it is consumed, please reweigh the syringe containing any leftovers and feeding tube used to allow the determination of the actual amount consumed. Please do not force-feed the child just to ensure total consumption. **In this protocol only: In the event that less than half the dose has been drunk please do not continue with the protocol and please do not ask the parent to collect the urine samples.**

If using a syringe and/or feeding tube:

1) Weigh the empty syringe and/or feeding tube and record on the collection form to 1 decimal place.
2) Transfer the tracer water contents of the dosing bottle provided into an empty plastic drinking cup. Using the syringe provided, carefully draw up the tracer water into the syringe. Please ensure that all of the tracer water is drawn up.
3) Weigh the syringe containing the tracer water (plus the feeding tube if used) to 1 decimal place and record this weight on their collection form.
4) Ask the mother to slowly push the plunger and feed the child with the water through the syringe. If using the feeding tube, attach this on the mother's finger using the tape provided and carry on using the syringe as above. Try to avoid spillage as much as possible. **Record the date and start time at which the child drinks the water on the urine sample collection form.**
5) Once finished reweigh the child's empty syringe and/or feeding tube. Record this weight also on the collection form to 1 decimal place.

*Please note – although it is preferable that all the dose is drunk, if this is not possible and half (50%) of it is consumed, please reweigh the syringe containing any leftovers and/or feeding tube used to allow the determination of the actual amount consumed. Please do not force-feed the child just to ensure total consumption. **In this protocol only: In the event that less than half the dose has been drunk please do not continue with the protocol and please do not ask the parent to collect the urine samples.**

Any problems during the dosing <u>must</u> be recorded on the urine sample collection form e.g. child was sick, spillage etc. It is important to be honest and provide as much detail as possible when recording this to enable valid analysis and if possible an estimation of any dose losses should be made.

If the child's own bottle was used this can be rinsed out and given back to the mother for her to clean as normal. The disposable bottle or syringe and/or feeding tube (if used), and the bottle that contained the dose should be disposed of with the clinic's normal rubbish.

Before the mother leaves the clinic

Please print your name and sign the urine sample collection form. Photocopy and retain for future use.

Pack the sample collection kit including the already collected pre-dose urine sample with the items supplied by HNR (urine collection form, urine collection instructions, 5 x glass urine collection and storage bottles for post dose daily urine collections labelled

days C1 to C5 and storage box, cotton wool, 6 x syringes (5+1 spare), 1 x spare pair of plastic forceps, pen and elastic bands) into the cooler bag provided.

Contact Details

All dose requests, sample returns, problems and queries should be directed to one of the following contacts at HNR:

- Marilena Leventi (Tracer water study manager)
- Priya Singh (Tracer water study assistant)
- Dr Jill Sommerville (Survey co-ordinator)
- Dr Les Bluck (Senior Scientist, stable isotopes research)

They can all be contacted **during normal office hours** via HNR reception, on **01223 426356** and by asking to speak to one of the above contacts. The people above will be contacted in order of appearance if no contact is specified.

Email addresses for the contacts are also provided below:

- Marilena Leventi Marilena.Leventi @mrc-hnr.cam.ac.uk
- Priya Singh Priya Singh @mrc-hnr.cam.ac.uk
- Dr Jill Sommerville Jill.Sommerville @mrc-hnr.cam.ac.uk
- Dr Les Bluck Les.Bluck @mrc-hnr.cam.ac.uk

HNR address: Human Nutrition Research
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge
CB1 9NL

Protocol 3.0 Infant Length Measurement

Introduction
The infant length measurement, when taken in conjunction with other growth parameters, can be used as an indicator of an infant's nutritional status. Taking this measurement across many years allows trends in infant length to be monitored and provides a means for the evaluation of current policies, interventions and treatments relating to infant health and nutrition. The measurement is taken for children aged six weeks or more and under two years.

Equipment
You will need:

- A Rollameter baby measure mat
- Kitchen roll

Preparing the participant
Explain to the parent or legal guardian of the infant the reason for taking the length measurement. Further explain that you will need their assistance in taking this measure and how they can help. Only trained staff are authorised to perform the measurement.

Procedure
1. Ask the parent to remove any bulky clothing or shoes that the infant is wearing as it may result in an inaccurate measurement. It is not necessary for them to remove the infant's nappy.

2. Unroll the Rollameter and lay it flat on any suitable flat, firm surface, preferably the floor. It is essential that the Rollameter is fully unrolled and as flat as possible. For hygiene purposes, lay one layer of kitchen roll on the mat.

3. The measurement can be taken with the infant on a Rollameter on a raised surface, e.g. a table, **ONLY** if the baby is held by an adult at all times, even if the baby has never previously rolled over.

4. Place the child on the foam bed of the Rollameter with his/her head touching the headpiece on which the name Rollameter is printed.

5. The Frankfort Plane is an imaginary line passing through the external ear canal and across the top of the lower bone of the eye socket, immediately under the eye (see Figure 1). Move the child's head so that Frankfort Plane is in a position at right angles to the floor/table. This position is important if an accurate reading is to be obtained. Ask the parent to hold the child in this position and make sure their head is in contact with the headpiece.

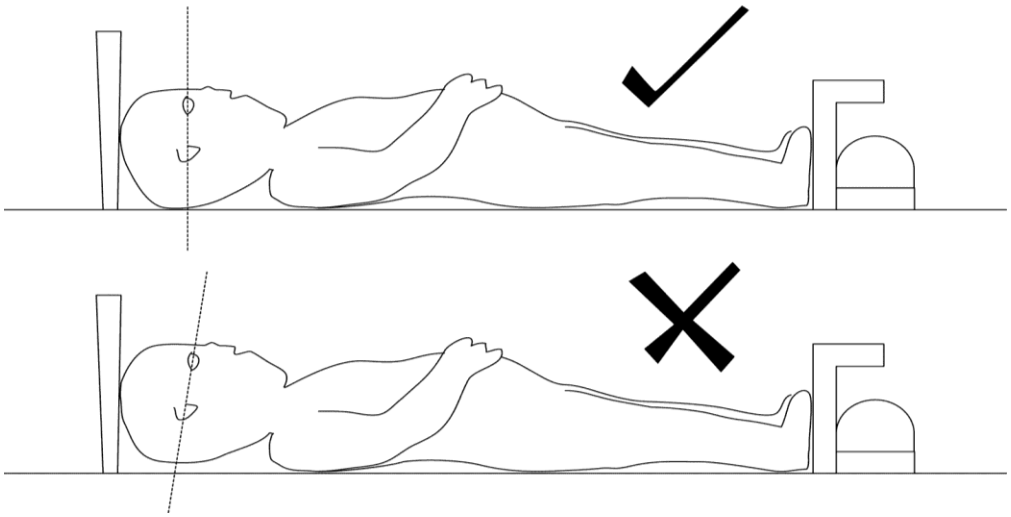


Figure 1 The infant Frankfort Plane

6. Straighten the child's leg(s) by holding the legs by the ankle(s) with one hand and applying a gentle downward pressure.

7. With your free hand, move the footrest on which the measuring tape is mounted to touch the child's heels by depressing the red button on the tape measure.

The measurement is read from the red cursor in the tape window. The measurement is recorded in centimetres and millimetres to the nearest millimetre on the clinic measurement record card. If the measurement lies between two millimetres then you should round to the **nearest even millimetre.**

Protocol 4.0 Mother's Height Measurement

Equipment
Height should be measured using a stadiometer (fixed wall height measure) to the nearest 0.1cm. The stadiometer must be checked, and any problems rectified or reported.

Preparing the Mother
Height should be measured bare-footed. Headwear must be removed. Only trained staff are authorised to perform the measurement.

Procedure
1. Ask the parent of the participant to remove their shoes in order to obtain a measurement that is as accurate as possible.

2. Assemble the stadiometer and raise the headplate to allow sufficient room for the parent to stand underneath it. Double check that you have assembled the stadiometer correctly.

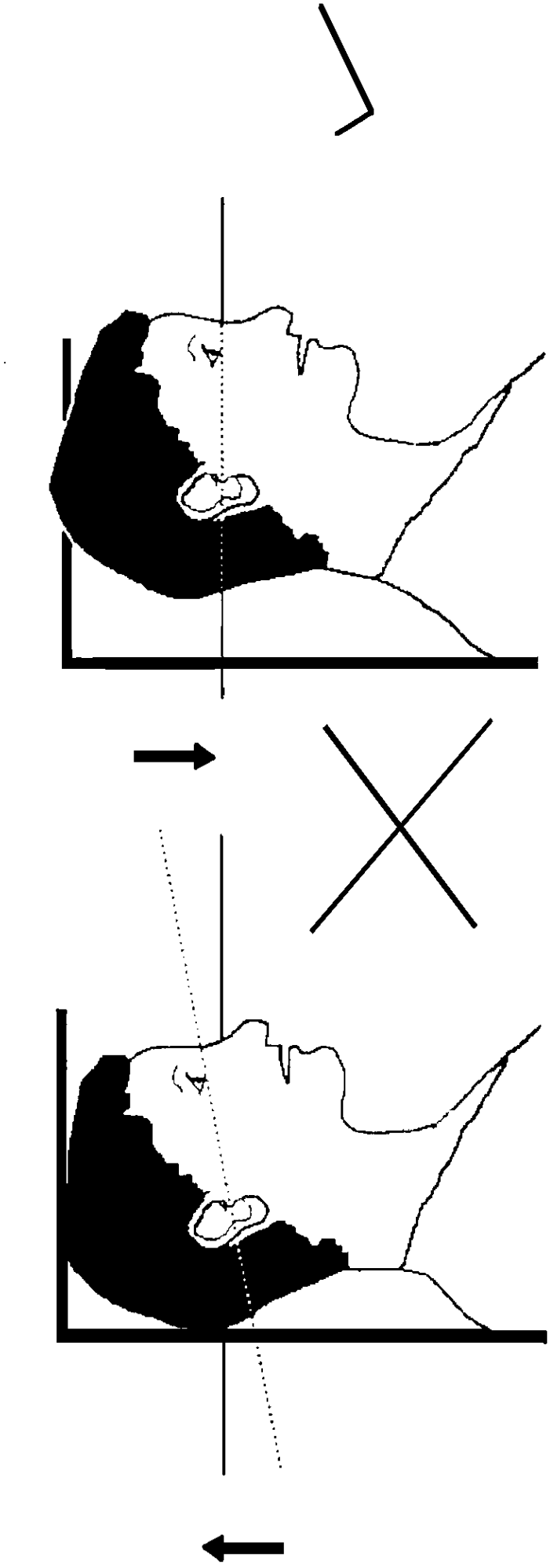
3. The parent should stand with their feet flat on the centre of the base plate, feet together and heels against the rod. The parent's back should be as straight as possible, preferably against the rod but **NOT** leaning on it. They should have their arms hanging loosely by their sides. They should be facing forwards.

4. Move the parent's head so that the Frankfort Plane is in a horizontal position (i.e. parallel to the floor). The Frankfort Plane is an imaginary line passing through the external ear canal and across the top of the lower bone of the eye socket, immediately under the eye (see diagram). This position is important if an accurate reading is to be obtained. An additional check is to ensure that the measuring arm rests on the crown of the head, i.e. the top back half. To make sure that the Frankfort Plane is horizontal, you can use the Frankfort Plane card to line up the bottom of the eye socket with the flap of skin on the ear. The Frankfort Plane is horizontal when the card is parallel to the stadiometer arm.

5. Instruct the parent to keep their eyes focused on a point straight ahead and to breathe in deeply. It can be difficult to determine whether the stadiometer headplate is resting on the parent's head. If so, ask the parent to tell you when s/he feels it touching their head by raising a hand.

6. Ask the parent to step forwards. If the measurement has been done correctly the parent will be able to step off the stadiometer without ducking their head. Make sure that the head plate does not move when the parent does this.

7. Look at the bottom edge of the head plate cuff. There is a green arrowhead pointing to the measuring scale. Take the reading from this point and record the parent's height in centimetres and millimetres. You may at this time record the parent's height onto the clinic measurement record card. Please note any problems or difficulties on the data collection form.



8. Height must be recorded in centimetres and millimetres, e.g. 176.5 cms. If a measurement falls between two **millimetres,** it should be recorded to the **nearest even millimetre.**

9. Push the head plate high enough to avoid any member of the household hitting their head against it when getting ready to be measured.

Protocol 5.0 Weight Measurement in Adults and Children

Introduction
Similar to the height measurement, the weight measurement is an indicator of and can predict the nutritional status and health of a population. When used in conjunction with the height measurement it can be used to derive the Body Mass Index, a statistical measure used to determine if an individual's weight falls within a healthy range. The method for weighing the participant in this study is to weigh the parent/guardian alone and then with the infant and a subtraction will be undertaken.

Exclusion criteria
Participants are excluded from this measurement if they are:

- Too frail or unable to stand upright
  If you are concerned that being on the scales may cause them to be too unsteady on their feet then do not weigh them. Alternatively you can place the scales next to something that they can steady themselves on.

Preparation
Weight should be measured without heavy outer garments such as jackets and cardigans, heavy jewellery, and with empty pockets. Only trained staff are authorised to perform the measurement.

Equipment
Weight should be measured using a Seca scale, Birmingham, UK. to the nearest 0.1 kg. The scales must be checked, and any problems rectified or reported.

Please check which scales you have been provided with and make sure that you are familiar with how they operate.

Calibrating the scales
The scales should be recalibrated at regular intervals (as per NHS SOPs) to ensure that they provide accurate measurements.

Procedure for adults
1. Weigh the parent/guardian on a hard and even surface if possible. Carpets may affect measurements.

2. Ask the parent/guardian to remove shoes, heavy outer garments such as jackets and cardigans, heavy jewellery, and to empty their pockets of all items.

3. Switch on the scales and wait for display to be momentarily displayed in the window. Do not attempt to weigh anyone at this point.

4. When the display reads 0.0, ask the parent/guardian to stand with their feet together in the centre and their heels against the back edge of the scales. Their arms should be hanging loosely at their sides and their head should be facing forward. Having the parent/guardian stand in this position means that the most accurate weight measurement can be obtained. Ensure that they keep looking ahead – it may be tempting for the parent/guardian to look down at their weight reading. Ask them not to do this and assure them that you will tell them their weight afterwards if they want to know.

5. The scales will need to stabilise. The weight reading will flash on and off when it has stabilised. If the parent/guardian moves excessively while the scales are stabilising you may get a false reading. If you think this is the case reweigh the parent/guardian.

6. The scales are calibrated in kilograms and 100 gram units (0.1 kg). Record the reading before the parent/guardian steps off the scales.

7. If the parent/guardian wishes, you can tell them their measurement.

8. Note any problems or difficulties on the data collection form.

Procedure for children

You will need to ask for the assistance of an adult as the following procedure requires you to measure the adult and then the adult holding the child:

1. Explain to the adult what you are going to do and the reasons why.

2. Weigh the adult as normal following the protocol as set out above. Record this weight.

3. Weigh the adult and child together and record this. Calculate the difference between the two weights to get the child's weight.

4. If the parent/guardian wishes, you can tell them the weight of their child.

Or

If the clinical research facility/hospital has a calibrated SECA baby scale, this can be used to weigh the child. Ensure this is calibrated regularly, as per local standard operating procedures .

Protocol 6.0 Head Circumference Measurement

Introduction

- Measurement of head circumference (occipital-frontal circumference) at birth and where required is a routine part of the infant's growth assessment.
- For your information: normal term newborn head circumference is 33 to 38 cm.

Responsibility

- It is the responsibility of all staff to follow the standard operating procedures as outlined in this document. It is also expected that all staff review the document at regular intervals to ensure they are up to date with current practises. Only trained staff are authorised to perform the measurements.

Equipment

- A Child Growth Foundation disposable measuring tape.

Procedure

- Ensure tape measure is correctly assembled and intact before and after each use. Tapes will be changed if calibration fails and/or tape is torn.

- Explain the procedure to the parent/carer.

- Position the child on mothers lap facing the nurse or lay the child on the changing mat. Do not leave unattended in any position they may roll off from.

- Place the tape measure around the child's head at its largest diameter above the eyebrow ridges, just above the point where the ears attach and around the occipital prominence (back of the head).

- Take **3 measurements** on each child. Note the average circumference to the nearest 0.1cm. Note any problems or difficulties on the data collection form.

- Record the head circumference on the clinic measurement record card.

Protocol 7.0 Measurement of Skinfold Thickness

Introduction

- Around half the fat in the body is located directly beneath the skin (subcutaneous fat), and its thickness provides some indication of total body fat.
- Subscapular, Triceps, Quadriceps and Flank skinfold thickness can be measured.

Responsibility

- It is the responsibility of all staff to follow the standard operating procedures as outlined in this document. It is also expected that all staff review the document at regular intervals to ensure they are up to date with current practises. Only trained staff are authorised to perform the measurements. To remain a competent, trained member of staff, it is required to measure 4 children a month. If this is not possible, practice on colleagues/family to maintain proficiency.

Equipment

- The Holtain Tanner Skinfold Caliper

Procedure

- Ensure the caliper is calibrated and zero measuring prior to each clinic. Report if inaccurate.
- Explain the procedure to the parent/carer. Demonstrate the procedure on the back of parents hand.
- Ask parent/carer to remove child's upper clothing. Ensure first that the room is warm and draught-free, do not keep the infant exposed longer than necessary, and do not leave them unattended in any position they may roll off from.
  Subscapular Skinfold:
    - Lay the infant prone on the parents/carers lap or on the changing mat on the bed. If they are old enough the measurement should be taken in the sitting position.
    - The skinfold is taken at the oblique angle below the left scapula.
    - Use your right hand to hold the calipers horizontally and apply the jaws of the calipers so they sit either side of the exact point. Slowly and gently release the caliper handle.

  Triceps Skinfold:
    - Extend the infants arm at the elbow and hold it firmly with your left hand covering the elbow. The skinfold is measured halfway between the acrominal process and the olecranon.
    - Use your right hand to hold the calipers horizontally behind their arm, and apply the jaws of the caliper so they sit either side of the exact point. Slowly and gently release the caliper handle.
- During each measurement, the caliper needle should initially fall quickly and then gradually come to rest. Take the reading as soon as the needle has stopped moving.
- Take three measurements at each site and record the results in millimeters on the clinic measurement record card.
- Record any problems or difficulties on the data collection form.

Clean the equipment with normal detergent unless the baby has an infection, in which case refer to the hospital's infection control policy and clean as per policy.

Protocol 8.0 Blood Sampling Protocol

Introduction
The blood samples taken will be used to measure various analytes, including iron, the levels of fat-soluble vitamins and haematology measures including white blood count, haemoglobin and platelets etc.

Exclusion criteria
Participants are excluded from this measurement if they are:

- Infants living in institutions, e.g. children's homes, hospitals
- Infants of people with no fixed address
- Infants with low birth weight (<2kg) and those with congenital abnormalities that affect feeding
- Infants who cannot be accompanied by a parent or legal guardian during the procedure

These infants should already be excluded by the time they reach the clinic visit.

Equipment and consumables
The blood samples will be collected using a syringe and the Sarstedt monovette blood collection system. You will be provided with the following equipment:

- 1.2ml EDTA monovette
- 2.7ml Serum monovette
- 5ml syringe
- 23g Butterfly needle
- Tourniquet
- Alcohol swabs
- Cotton wool
- Ametop gel
- Dressing for use with gel
- Pre-paid and pre-addressed envelope with the appropriate packaging for posting the EDTA Monovette to Addenbrooke's Hospital
- Blood tracking forms for Serum sample
- Addenbrooke's blood form to track the EDTA sample
- Labels (pre-printed) for blood monovettes, blood tracking forms and consent forms

IMPORTANT INFORMATION

Before starting the blood sampling procedure:

Ensure the parent has consented to at least **one (or both)** of the following:
1. Blood results to be sent to the parent
2. Blood result to be sent to their GP

If consent has not been given to at least **one** of the above statements please ensure Section D of the consent form is complete. If section D of the consent is not complete you must **NOT** continue with the blood sample.

Blood sampling procedure

The child will have a 4ml, non fasting blood sample taken. The parent or guardian must be present throughout the blood sampling procedure and can assist to distract the child during the procedure. Samples may be taken from the arm, hand, leg or foot with the parent/guardian's consent.

It is expected that most blood samples will be taken at the first attempt. However, a second attempt is permitted if the parent is willing.

1. Explain the procedure to the parent/guardian and allow time for questions. Ensure that they have been given and understood the following information leaflets:
    - Ametop gel leaflet
    - Why provide a blood sample leaflet

2. Before taking blood, you must ensure that the child's parent/guardian has fully understood the ourpose of the blood sampling and the protocols for taking it and that written consent has been given.

3. Ensure the name of the legal parent/guardian and the name and Study ID of the child is clearly written in the space provided at the top of the consent form. Check the DOB on the label corresponds with the participant and that label **CON** has been placed in the appropriate place on the consent form.

4. Ensure each statement has been initialled by the legal parent/guardian and that they have signed and dated in the appropriate place at the bottom of the consent form. Consent to notifying GP of blood results is optional.

5. It is equally as important for you to sign and date the consent form in the appropriate place at the bottom of the consent form.

6. Wash your hands and apply gloves. Ensure equipment is fully prepared and within your reach.

7. Position the child carefully on the parent/guardian's lap so that they are facing you.

8. Ask the parent to remove or displace any clothing that the child is wearing from the area selected for venepuncture.

9. Check with the parent/guardian that the infant is not allergic to plasters or latex.

10. Apply Ametop gel to the selected area for venepuncture and apply a dressing to keep the gel firmly in its place.

11. Whilst you are waiting for the Ametop gel to take effect, you can carry out other study procedures and/or label the blood monovettes and blood tracking forms with the following labels:

Item	Label
Serum monovette	SEN
EDTA monovette	EN
Addenbrooke's blood form	Adx1
Addenbrooke's blood form	Adx2
Addenbrooke's blood form	Adx3
Blood tracking form	BTF
Consent form	CON
Serum1 label for microtube	SE1 Pass to lab
Serum2 label for microtube	SE2 Pass to lab

12. Ensure the correct label goes on the correct monovettes and microtubes.

13. **Line up the top of the barcoded label with the top of the label on the monovette**. The clear area on the labels **must** be aligned with the clear area on the monovettes. This is to enable the lab to be able to see where the serum lies in the monovette after centrifugation. If this area is covered, the lab may not be able to successfully aliquot the required number of fractions.

14. Ensure the laboratory labels (SE1 and SE2) and the mauve and white lidded microtubes get passed to the lab with the blood samples.

15. When the Ametop gel has taken effect (after 20 minutes), a tourniquet can be applied (if required) and the area cleaned with an alcohol swab.

16. Using your dominant hand, puncture the skin with the needle angled at 30-40 degrees (the bevel of the needle should be uppermost) and tape to the arm. Observe for blood flow and collect the required amount of blood as directed. If the first attempt is unsuccessful, a second attempt is permitted – **ensure the parent is willing.**

17. The syringe is attached directly to the butterfly needle and filled to 4ml. Then transfer the blood to the monovette tube. The monovette systems should be used according to manufacturers' instructions (training will be given to the nurses/phlebotomists who are not familiar with the Sarstedt monovette system).

18. Monovettes are to be filled in priority order as follows:

Priority Order	Monovette	Monovette Top Colour
1	EDTA	Red
2	Serum	White

***NOTE* A minimum of 1ml is required in the EDTA monovette for any analysis to be undertaken.**

19. The tourniquet will be released (if applicable) and the butterfly needle removed and placed directly in the sharps bin. Apply pressure with a cotton wool ball before applying a plaster/dressing.

20. Once filled and capped, the monovettes should be mixed immediately by inverting several times.

21. Any contaminated waste generated should be disposed of appropriately.

22. Advise the parent/guardian how and when to remove the plaster/dressing and about bruising. To reduce any infection risk, the parent or guardian should wash their hands before removing the plaster/dressing and the plaster/dressing should be kept on for at least 15 minutes after the blood sample has been taken. Infants may initially display some redness at the site of venepuncture, however, this should soon disappear. If the redness persists for more than 2 days or appears to get worse then the parent/guardian should seek medical advice. Similarly with bruising, no action is required unless it gets worse, in which case medical advice should be sought.

23. Ensure that the Addenbrooke's blood form for the EDTA monovette has the label **Adx1** affixed in the appropriate place on the top sheet of the carbonised booklet and label **Adx2** on the second sheet and affix **Adx3** on the third sheet of the carbonised booklet in the appropriate places and fill in the **date and time the sample was taken**.

24. The labelled red top EDTA monovette should be put in the rigid plastic tube with absorbent lining and then inside the box provided. Together with the labelled and completed Addenbrooke's blood form (with all 3 copies of the carbonised booklet), both items should be put in the pre-paid and labelled jiffy bag and then mailed to Addenbrooke's hospital. (Due to samples arriving 24 hours later, please do not post on a Friday, or any day where the arrival day will be a day that the lab is closed i.e. bank holidays). Furthermore, the EDTA blood sample must be posted to Addenbrooke's the same day so the blood must be taken in time to make the same day's post.

25. The blood tracking form for the white top serum monovette should be completed whether or not a sample is obtained. The form should **always** have the following:
    - The label **BTF** affixed in the appropriate place
    - The study ID number of the child

If a blood sample is **OBTAINED**, please fill out the following:
- The date the sample was taken
- The time the blood sample was taken
- The location of venepuncture – for attempt 1 and 2 (if applicable)
- The blood sample outcome for attempt 1 and 2
- Which blood monovettes were obtained
- Whether the EDTA monovette blood sample was sent to Addenbrooke's hospital

If a blood sample was **ATTEMPTED** but not obtained, please fill out the following:

- The date the sample was taken
- The time the blood sample was taken
- The location(s) of venepuncture
- Reason for declining Attempt 2 (if applicable)
- The blood sample outcome
- Select 'NO' where it asks which blood samples were obtained

If a blood sample was **REFUSED**, please fill out the following:

- Select 'NO' where it asks which blood samples were obtained
- Ask parent reason for not wanting to do the blood component and update clinic database with reason for refusal.

26. The labelled white top Serum monovette should be taken to the processing laboratory together with the labelled and completed blood tracking form, labels SE1 and SE2 and the microtubes.

Please remember to fill in the time of delivery of samples to the laboratory.

Any queries about processing laboratory issues and consumables or general problems should be directed to one of the following contacts at HNR:

- Priti Mistry – Research Assistant 01223 437616 *(Priti.Mistry@mrc-hnr.cam.ac.uk)*
- Sue Fisher – Volunteer Suite Manager 01223 437615 *(Susan.Fisher@mrc-hnr.cam.ac.uk)*

HNR Reception: 01223 426356

H.7. Ending the Clinic Visit

- Remind the parent that a NatCen interviewer will call mid way through the urine collection to arrange pick up of the urine samples, if applicable.

- Give bravery certificate to the participant as appropriate:
  - 'has been very brave and given blood' – If a blood sample was **Obtained**
  - 'has been very brave and attempted to give blood' – If a blood sample was **Attempted** but not obtained

- Give promissory note(s) to the parent of the participant as appropriate:
  - £10 if the participant has completed the skinfold thickness testing and physical measurements
  - £30 for attempting/obtaining the blood sample.

  Please note that the promissory notes for the breast milk, fluid intake and body composition via isotopically stable water component will be given by the NatCen interviewer when they go to collect the completed urine samples.

- Check and collect the expenses claim form (if applicable)

- At the end of the clinic session, the following documents need to be **faxed** to HNR on **01223 437559**:
  - Completed consent forms – All 3 pages
  - Completed clinic measurement record card
  - For participating participants, **EITHER** completed breast milk volume measurements **OR** completed body composition and fluid intake measurements
  - The blood sample tracking form whether the blood sample has been collected or not

- Update the clinic database on **all** clinic days* with the following:
  - Date and time of clinic attended
  - The date that the expense form was received by the clinic
  - Completed components of the clinic visit
  - Participant status e.g. attended appointment, cancelled, no show

  *Occasionally, you may need to update the database on a non clinic day e.g. if a participant wants to re-arrange an appointment and contacts the clinic directly.

- If you are experiencing technical problems and are unable to update the database – you **must** inform Jonathan Last/Priti Mistry at HNR as soon as possible on 01223 426356.

- Send the **original** signed consent forms, clinic measurement record cards, blood tracking forms for those who **Attempted** or **Refused** a blood sample and the expense claim forms weekly to HNR by Special Delivery.

- Do not send the completed blood sample tracking forms for participants where a blood sample was **Obtained**. These need to be kept with the Serum microtubes and sent together at a later date on dry ice – please refer to Laboratory Manual.

H.8. Emergency Contact/Adverse Events

All adverse events **must** be logged and reported as per local procedures by:

1. In the event of an emergency/adverse event, contact the local Principal Investigator in the first instance. Name and emergency contact numbers have been given to the clinic in advance.

2. Secondly, in an emergency/adverse event, ensure that Jill Sommerville (Study Co-ordinator) or Polly Page (Head of Operations) is informed as soon as is reasonably possible on 01223 426356.

3. Additionally, an adverse events report form* **must** be completed and faxed within 24hrs of the adverse event.

Where appropriate, serious adverse events will be reported to MREC as per standard ethics procedures.

*Adverse events report forms will be given to the clinic in advance. For reference, an adverse events report form is provided overleaf.

PROTECT PRIVATE

The National Infant Diet and Health Study

ADVERSE EVENTS REPORT FORM

Clinic visit date _____ Clinic name _____

Participant ID _____

EVENT DETAILS

Where did the event take place? (please tick)

In the clinic ☐

Elsewhere ☐ Please state location _____

When did the event take place? Date _____ Time _____

When was the clinic made aware of the event? Date _____ Time _____

Who reported the event? _____

To whom? Name _____ Role _____

Who was involved in the event? Please name all, including staff and witnesses

Were any procedures being carried out prior to the event? (e.g. venepuncture)

Yes ☐ No ☐

If yes, please state _____

Did any of the following occur to the participant? (please tick all that apply)

Bruising	☐	Wrong dose	☐	Hospital stay exceeding 24hrs	☐
Bleeding	☐	Nausea/Vomiting	☐	Other, please state	
Infection	☐	Loss of conciousness	☐		
Pain	☐	Needed resuscitation	☐		

DESCRIPTION OF EVENT

Please give as much detail as possible including any significant factors leading to the event

ACTION DETAILS

Include any advice/treatment/referral given to the participant and also any follow up plans by the clinic

Has the event been reported within your organisation in accordance with local procedures?

Yes ☐ No ☐

If not, please give reasons why

Please give details of any further action to be taken and to whom the event was reported

Hospital details (please tick)

Referred ☐ Attended ☐ Treated ☐ N/A ☐

Hospital treatment:

Was the event related to the study? (please tick)

Definitely ☐
Probably ☐
Possibly ☐
Unlikely ☐
Unrelated ☐
Unknown ☐

Severity of injuries (if applicable)

Minor ☐
Major ☐
Fatal ☐
Reportable ☐

NOTE: We may be required to report this event to the project steering committee and/or external ethics committee

Reported by Name _____ Sign _____

Date _____

Once completed, please fax IMMEDIATELY to HNR on 01223 437 559

H.9. Study Contact Details

Any general queries about the study should be directed to HNR:

- Priti Mistry – Research Assistant 01223 437616
  (*Priti.Mistry@mrc-hnr.cam.ac.uk*)
- Jill Sommerville – Survey Co-ordinator 01223 426356
  (*Jill.Sommerville@mrc-hnr.cam.ac.uk*)
- Dr Ken Ong – Survey Doctor 01223 769207
  (*ken.ong@mrc-epid.cam.ac.uk*)

HNR Address
MRC Human Nutrition Research
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge
CB1 9NL

HNR Reception Tel: 01223 426356

X. Laboratory Manual

Blood Processing and Storage Protocol

A brief summary of services required

- ***Samples delivered*** to laboratory
- ***Spin samples*** to yield serum
- ***Pipette*** various aliquots into microtubes provided
- Complete ***blood sample tracking form***
- ***Freeze and store*** microtubes until a member of the HNR team arranges collection by courier

A detailed protocol for processing the blood samples is provided overleaf. From start to end the processing should take no longer than 1 hour including 20 min for centrifugation. Ideally, processing should be completed within 2 hours of the blood clotting successfully.

National Infant Diet and Health Study

Laboratory Procedures

Blood Processing and Storage Protocol

Contents

Appendices

1. Receipt of samples

The National Infant Diet and Health Study nurse(s) will take the blood sample to the laboratory as soon as is convenient after the clinic session has finished. Please make sure that a designated person is available to receive and deal with samples (unless the nurse is also processing the samples).

On receipt in the laboratory enter the time received on the blood sample tracking form (see Addendum B for reference). The serum monovette should be left for approximately 1 hour after venepuncture at room temperature to clot. It is very important that the blood clots properly before the monovette is centrifuged. The blood sample tracking form should be checked to see what time the sample was taken.

For each participant, the lab will receive a package from the nurse containing a Monovette (1.1), microtubes for sample storage (1.2), labels (1.3) and a blood sample tracking form (1.4).

1.1 Receipt of samples

The number of monovettes per participant is shown in the table below:

Age Group	Monovette				Colour Top
	Number	Volume (ml)	Monovette Type	Label	
4-18 Months	1	2.7	Plain Serum	SEN	White

1.2 Microtubes (1.5ml) for specimen storage

The nurse will hand over a pack of microtubes for aliquoting. The number of microtubes in the pack you receive is outlined in the table below:

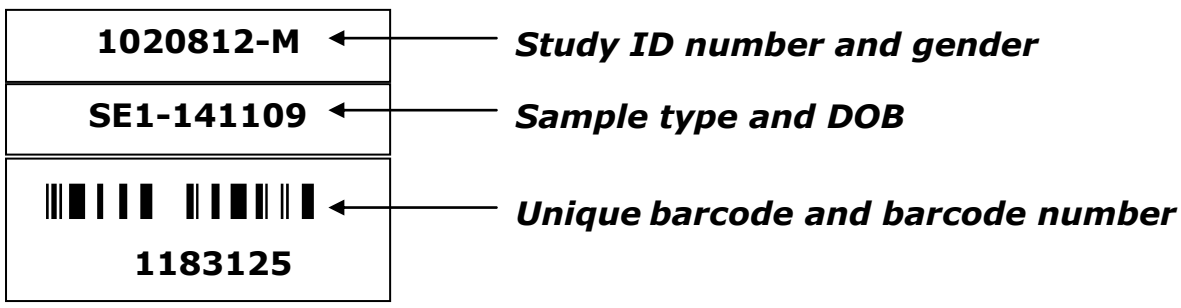
Age Group	Microtubes		Use	Label
	Number of empty microtubes	Colour of lid		
4-18 Months	1	Mauve	Serum fraction	SE1
	1	White	Serum fraction	SE2

Please ensure that when labelling the microtubes, the labels correspond to the microtube cap colour.

1.3 Labels

A set of printed barcoded labels with the participant's study ID number, gender, sample type, DOB and barcode number will be provided for each participant. If you receive more than one set of blood samples on the same day ensure that you use the **correct** set of labels for each participant and for each microtube.

Example of barcoded label



The set of labels provided is correct for the number of aliquots obtained from a participant. The second line of the label, which shows the sample type, corresponds with the aliquoting scheme on the flow chart (see Addendum A for quick reference) and the blood sample tracking form (see Addendum B) provided with each set of samples.

1.4 Blood sample tracking form

The nurse will pass on a blood sample tracking form (BTF) for the lab to fill in (see Addendum B). First, ensure the time that the laboratory received the sample is completed on the BTF. The processing table in Section 2 of the form should be completed after the serum monovette has been centrifuged and serum aliquoted.

Please use this document to note any problems with the samples on receipt, any problems with processing the samples, to record sample processing end times as well as the number and volume of sub-samples obtained and the time at which the samples were placed in the freezer for storage.

Where a partial volume has been collected, please indicate clearly in **µl** how much was obtained i.e. 100µl

2. Equipment Needed

Pipettes

Please use calibrated piston pipettes wherever possible. Volumes indicated are guidelines but note that it is necessary to record the volume of each aliquot if only a partial volume is obtained (please refer to Addendum B).

Centrifuge

Whenever possible all centrifugation steps should be conducted at 4°C. If this is not possible please **ensure the sample and associated fractions are kept cool after the blood has clotted** e.g. by keeping the sample on ice and pre cooling the centrifuge buckets and blocks in a fridge.

Freezer*

Please freeze the samples in an upright position in the Sarstedt 9x9 aliquot boxes provided for long-term storage and subsequent shipment to HNR.

* Freezer must be of the non-self-defrosting type

3. Processing Procedures

The serum monovette should be processed as soon as practicable after the blood has clotted properly (approximately 1 hr).

3.1 Spin monovette for 20 minutes at 2000g at 4°C to yield serum.

3.2 While the monovette is spinning, place the labels (SE1 and SE2) on the microtubes for serum storage, so that the bar-code runs the length of the tube. All microtubes should be labelled with the appropriate barcoded label.

3.3 Aliquoting (see Addendum A)

Monovette (Plain Serum)
Using the table below, pipette all the serum from the serum monovette in the **priority order** as follows; recording the volumes on the blood sample tracking form.

Priority Order	Label	Colour of Lid	Volume
1	SE1	Mauve	500µl
2	SE2	White	500µl

Take great care not to transfer any cells into the microtubes; if necessary re-centrifuge the sample to obtain additional serum.

Please note that the volumes specified should be followed and carefully recorded. The most important information is the **total volume** in **µl** in each microtube. If you can't measure this with the pipette, record the volume as closely as possible from the graduations on the microtube. The graduation 0.5 on the microtube is the equivalent to 500µl. If there is not sufficient serum for SE2, please do not attempt to transfer serum from SE1. SE2 is only to be filled if any serum is left after the full volume of **500µl** has been transferred to SE1.

Place all serum microtubes on ice if they cannot be transferred to the freezer immediately.

Please don't forget to fill in the blood sample tracking form. The form must be faxed to HNR on the same day, that is after completion of sample processing

(Fax no: 01223 437559)

4. Blood Sample Tracking Form

Please record the required information on the blood sample tracking form. Take a copy for your own files and fax the completed blood sample tracking form to HNR. Please retain the original blood sample tracking form with the respective samples until they are ready to be transferred by courier to HNR at the pre-arranged date. Do not send these forms separately. They will need to be packaged with the serum microtubes on dry ice.

5. Dispatch

5.1 Once blood processing for the National Infant Diet and Health Study has started in your lab, a member of HNR will be in touch to arrange a mutually convenient time for the samples to be collected. This will be on a monthly basis, unless the number of samples is very low, in which case they will be collected as appropriate.

5.2 Couriers will be arranged for door-to-door delivery wherever possible. We will arrange for the courier to deliver one or more polystyrene boxes containing dry ice to you and to collect these boxes once the samples have been packed (see Addendum C for instructions on packing samples).

5.3 Please ensure that all the samples in this consignment are for the **National Infant Diet and Health Study**, and are placed in the secondary packaging provided (Bio-hazard bags), to avoid any possibility of leakage during transport.

5.4 Please place all the **original** blood sample tracking forms of the samples and any unused ID labels in a plastic bag, between the inner thermal box and outer cardboard box. Please keep a copy of the blood sample tracking forms in your laboratory until the end of the survey, in case any information goes astray.

5.5 Do not seal around the lid of the cardboard box completely, as CO_2 gas must be able to escape.

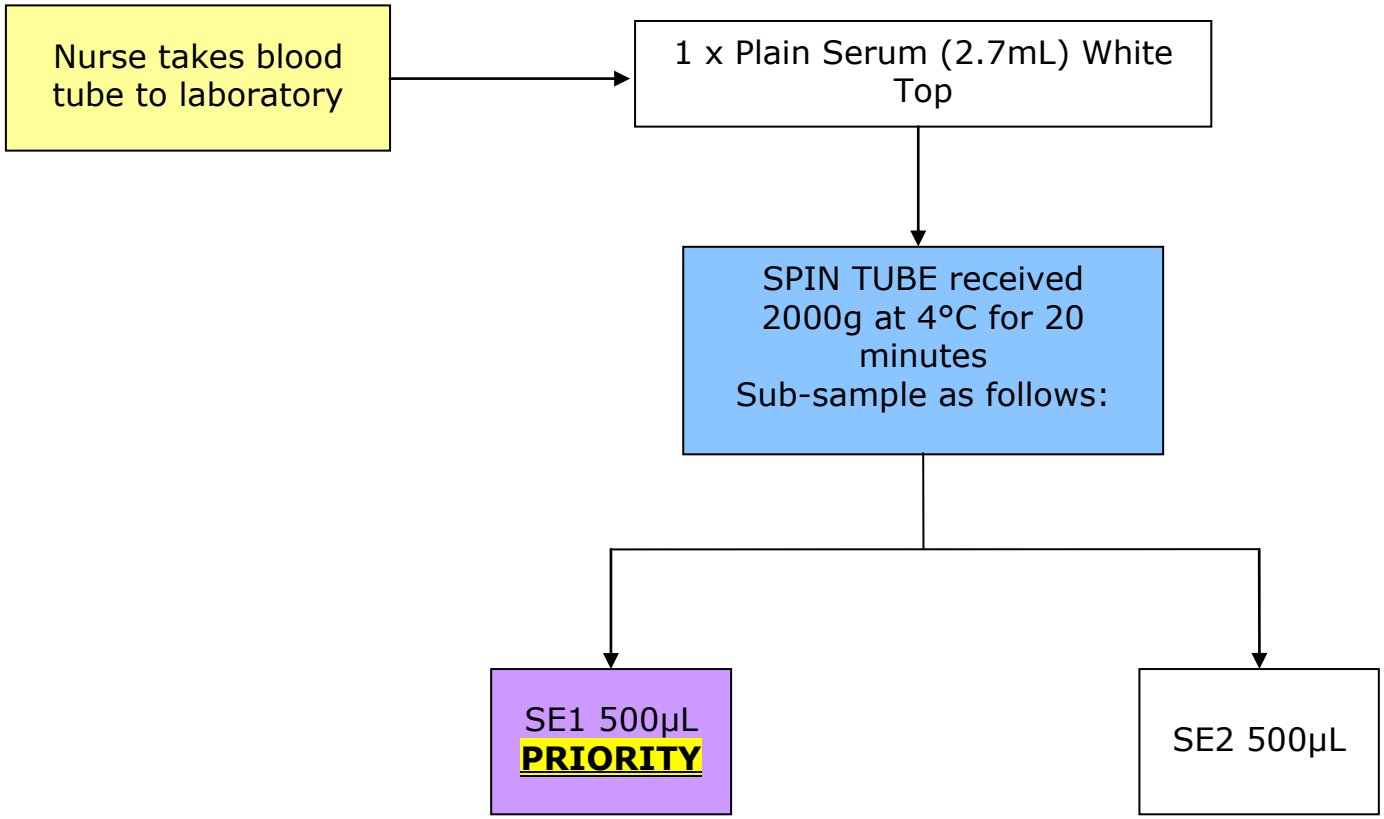
5.6 Ensure package is addressed to:

MRC Human Nutrition Research
National Infant Diet and Health Study samples
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge CB1 9NL

If you have any queries, please contact the Research Assistant
Priti Mistry at HNR 01223 437616/426356
or by email Priti.Mistry@mrc-hnr.cam.ac.uk

Thank you for your assistance

Addendum A. Flow Chart for Processing Blood Samples for Participants V2.0



Addendum B. Blood Sample Tracking Form

National Infant Diet and Health Study
BLOOD SAMPLE TRACKING FORM

Place label BTF here

Section 1: Blood collection

Study ID number: | | | | | | | |

Date sample taken: D D M M Y Y Y Y

Time sample taken: H H M M 24 hr clock

Attempt 1 – Location Arm/Hand/Leg/Foot Circle as appropriate

Attempt 2 – Not applicable/Agreed*/Declined** Circle as appropriate

Attempt 2* – Location Arm/Hand/Leg/Foot Circle as appropriate

Attempt 2** – Decline reason: Please tick as appropriate

Child was distressed		No suitable veins	
Parent changed mind		Clinician advised against	
Other (specify):			

Blood sample outcome: Please tick as appropriate	Attempt 1	Attempt 2
No problem		
Incomplete sample		
Collapsing/poor vein		
Couldn't find vein		
Baby moving		
Other (specify)		

Blood sample obtained: EDTA Y N SERUM Y N Please tick

EDTA tube sent to Addenbrooke's: YES ☐ NO ☐

Section 2: Processing

Time of arrival at lab: H H M M 24 hr clock

Following lab instructions, please indicate completion of processing for the following:

Tube	Label	Time (24 hr clock)	Volume (tick one)	Partial Volume (µl)
Serum (500µl)	SE1	H H M M	Full Partial	
Serum (500µl)	SE2	H H M M	Full Partial	

Comments on processing:

Time samples placed in the freezer: H H M M 24 hr clock

Please refer to clinic manual for further guidance

Addendum C. Instructions for the dry ice shipment of National Infant Diet and Health Study blood specimens to HNR Cambridge

Overview

Samples processed and stored for the National Infant Diet and Health Study will be shipped at agreed regular intervals from the laboratory, to MRC Human Nutrition Research in Cambridge (HNR).

HNR will inform you when the samples will need to be sent back. A pre-paid and pre-labelled cardboard box containing a thermal box with dry ice and packaging for the transport of the frozen samples will be sent over night to the laboratory. The courier used is Davies International.

On receiving the box it is the responsibility of designated laboratory staff to pack up samples as per protocol. The courier will deliver before lunch on the pre-arranged pick-up date and a 2-hour window will be agreed for the collection of packaged samples ready for over-night delivery to HNR Cambridge.

Protocol

1. A member of the National Infant Diet and Health Study team (HNR) will confirm the number of samples ready for shipment with you so that the courier can be instructed as to the appropriate quantity of secondary packaging required.

2. HNR will make all the arrangements for the collection of the samples on a pre-agreed date. The samples will be collected from you on a Tuesday, Wednesday or Thursday of the agreed week.

3. A thermal box marked and labelled for the return journey to HNR Cambridge will be delivered to the named contact at the laboratory by courier.

4. The outer cardboard box provided contains the following:

   - **Inner thermal box with dry ice**
   - **Sealable biohazard bag**

   Each sealable biohazard bag will take up to 2 Sarstedt aliquot boxes (9 x 9 aliquots)

   The thermal box you receive will vary in size depending on the number of samples to be shipped. For example, if you have told us that you are currently storing Sarstedt aliquots boxes of samples we will instruct Davies International to provide the appropriate size thermal box and dry ice.

   If for any reason, there doesn't seem enough packaging provided please only pack the appropriate number of samples as per packaging provided.

Once you have informed HNR about the mismatch we will arrange for another shipment.

5. Open the top of the cardboard box carefully and remove the biohazard bag.

6. The frozen samples are to be kept in the Sarstedt aliquot box and placed directly in the biohazard bag (max 2 Sarstedt aliquot boxes per bio bag) along with the absorbent strip and sealed as per the instructions on the bag.
NB: If no absorbent strip is provided please use a couple of sheets of blue roll or equivalent.

7. Remove half the dry ice from the box and retain. Pour the contents in to the thermal box. Place the sealed biohazard bag on top of the dry ice and pour the remaining dry ice over the bag to top up the box. **DO NOT** over fill the box and **DO NOT** seal the thermal box with any tape.

8. The blood sample tracking form (originals; one for each set of samples) should be placed in a plastic sleeve on top of the thermal box, i.e. between the inner thermal box and outer cardboard box. The top flaps of the outer cardboard box are then sealed with parcel tape. **DO NOT** tape over all edges of the cardboard box so that CO_2 can escape during transport.

9. The box will be picked up on the same day it is delivered at a pre-arranged time and couriered to HNR over night.

10. When the samples arrive at HNR, the laboratory will be informed of their arrival and any problems will be reported.

11. For any queries about the above please contact:

Priti Mistry – Research Assistant
Priti.Mistry@mrc-hnr.cam.ac.uk
Phone: 01223 437616
HNR Reception: 01223 426356

Many thanks for your cooperation and assistance with this important national study

National Infant Diet And Health Study

Bravery Award

_____

has been very brave and given blood

Sign:..... Date:.....

National Infant Diet And Health Study

Bravery Award

_____

has been very brave and attempted to give blood

Sign:..... Date:.....

NATIONAL INFANT DIET AND HEALTH STUDY

Clinic Measurement Record Card

ID: | | | | | | | |

DOB: _____

SEX: _____

CHILD'S PHYSICAL MEASUREMENTS

WEIGHT		.	kg (to the nearest 0.1)
LENGTH		.	cm (to nearest 0.1)
HEAD CIRCUMFERENCE	1	.	cm (to nearest 0.1)
	2	.	cm
	3	.	cm
		.	**average**

CHILD'S SKINFOLD MEASUREMENTS

TRICEPS	1	.	mm (to nearest 0.1)
	2	.	mm
	3	.	mm
		.	**average**
SUBSCAPULAR	1	.	mm (to nearest 0.1)
	2	.	mm
	3	.	mm
		.	**average**

MOTHER'S MEASUREMENTS

WEIGHT	.	kg (to the nearest 0.1)
HEIGHT	.	cm (to the nearest 0.1)

THE NATIONAL INFANT DIET AND HEALTH STUDY

Our ref: Study ID xxxxxxx

Name
Address line 1
Address line 2
Address line 3
Address line 4

Dear ……..

I am the paediatric phlebotomist/nurse working on the National Infant Diet and Health Study. You recently agreed to a clinic visit and an appointment was made. Unfortunately, you missed your appointment.

I would be grateful if you could telephone our survey team on freephone 0800 6785 625*, so that we can arrange a more convenient time for you to participate in the clinic visit. I look forward to meeting with you then.

*Calls from mobiles may be charged.

MRC Human Nutrition Research

National Infant Diet and Health Study

Breast Milk Volume Assessment (Tracer Water to Mother and Child)

Respondent ID Number | | | | | | | | | |

Dose bottle numbers/details .....

Date written consent obtained | | | | | | | | | | | |

Mother Information

Mother's weight on day of dosing | | | . | | | kg

Date dose taken: DAY | | MONTH | | YEAR | | | |

Time dose taken: HR | | MINS | | am/pm?

Weight of dosing materials before drinking | | | | . | | g
(bottle+screw cap+water+straw)

Weight of dosing materials after drinking | | | | . | | g
(bottle+screw cap+any remaining water+straw)

Child Information

Child's weight on day of dosing | | | . | | | kg

Date dose taken: DAY | | MONTH | | YEAR | | | |

Time dose taken: HR | | MINS | | am/pm?

Please specify drinking vessel used for dosing (tick as appropriate):

Child's own bottle ☐ Bottle provided at clinic ☐ Syringe+feeding tube ☐

Other (please specify).....

Weight of empty chosen drinking vessel | | | | . | | g

Weight of chosen drinking vessel + added dose water before drinking | | | | . | | g

Weight of chosen drinking vessel + any remaining dose after drinking | | | | . | | g

Were there any losses whilst drinking the dose? Yes / No (please circle)

If losses occurred, what happened? (E.g. spillage, dribbling, positing).....

Please estimate how much of the dose you think was lost - .....

Breast milk diary discussed, explained and placed: Y ☐ N ☐ (tick as appropriate)

Nurse name (Please print):_____Nurse signature:_____

MRC Human Nutrition Research – National Infant Diet and Health Study

Breast Milk Volume Assessment (Tracer Water to Mother and Child)

Day	**Mother's Urine Sample Label**	**Date (dd/mm/yy)**	**Time of sample collection**	***Morning* (Please ✓)***	***Afternoon* (Please ✓)***	***Comments***
Pre-Dose	Pre-M0					
1	M1					
2	M2					
3	M3					
4	M4					
5	M5					
6	M6					
7	M7					
8	M8					
9	M9					
10	M10					
11	M11					
12	M12					
13	M13					
14	M14					

Day	**Child's Urine Sample Label**	**Date (dd/mm/yy)**	**Time of last nappy change**	**Time of sample collection**	***Morning* (Please ✓)***	***Afternoon* (Please ✓)***	***Comments***
Pre-dose	Pre-B0						
1	B1						
2	B2						
3	B3						
4	B4						
5	B5						
6	B6						
7	B7						
8	B8						
9	B9						
10	B10						
11	B11						
12	B12						
13	B13						
14	B14						

Note: For collections at 12 o'clock mid-day, tick afternoon; at 12 o'clock mid-night, tick morning.

MRC Human Nutrition Research

National Infant Diet and Health Study

Body Composition and Fluid Intake (Tracer Water to Child)

Respondent ID Number ☐☐☐☐☐☐☐☐☐

Dose bottles number/details .....

Date written consent obtained ☐☐ ☐☐ ☐☐☐☐

Child Information

Child's weight on day of dosing ☐☐.☐☐ kg

Date dose taken: DAY ☐☐ MONTH ☐☐ YEAR ☐☐☐☐ Time dose taken: ☐☐ ☐☐ am/pm?

Please specify drinking vessel used for dosing (tick as appropriate):

Child's own bottle ☐

Bottle provided at clinic ☐

Syringe + feeding tube ☐

Other (please specify).....

Weight of empty chosen drinking vessel ☐☐☐.☐ g

Weight of chosen drinking vessel + added dose water before drinking ☐☐☐.☐ g

Weight of chosen drinking vessel + any remaining dose after drinking ☐☐☐.☐ g

Were there any losses whilst drinking the dose? Yes / No (please circle)

If losses occurred what happened? (E.g. spillage, dribbling, positing) .....

.....

Please estimate how much of the dose you think was lost - .....

Nurse name (Please print):.....

Nurse signature:.....

MRC Human Nutrition Research – National Infant Diet and Health Study

Body Composition and Fluid Intake (Tracer Water to Child)

Day	Child's Urine Sample Label	Date (dd/mm/yy)	Time of last nappy change	Time of sample collection	***Morning*** **(Please ✓)***	***Afternoon*** **(Please ✓)***	***Comments***
Pre-dose	Pre-C0						
1	C1						
2	C2						
3	C3						
4	C4						
5	C5						

***Note: For collections at 12 o'clock mid-day, tick afternoon; at 12 o'clock mid-night, tick morning.**