

Appendix M The blood sample: collecting and processing the blood

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M.1 Introduction

This appendix gives further information about the blood collection procedures, including details of the selection and training of the phlebotomists, procedures for obtaining blood, procedures at the field laboratories, storage and assay auditing and the protocol for reporting clinically significant blood results to participants and their GP.

The feasibility of the blood collection protocol was tested in the Run In and all procedures were found to be suitable for use in the main stage.

M.2 Ethical approval

As described in chapter 2 of the main report,ⁱ ethical approval was granted by a Multi-centre Research Ethics Committee (MREC) for all aspects of the survey protocol, including blood sample collection by venepuncture, measurement of blood analytes and for storing blood sample residues for potential use in future analyses related to nutrition and health.

The ethical approval allowed for a maximum of 35.1mL of blood to be taken from participants aged 16 years and over, a maximum of 21.1mL from children aged seven to 15 years and a maximum of 10.9mL from children aged 1.5 to six years.

A number of the analytes measured in the blood are known to be unstable. To ensure prompt stabilisation of the blood sample, suitably located and resourced field laboratories were approached within each geographical area to process part of the blood sample from each participant within two hours of venepuncture. The recruitment of all field laboratories was subject to the signing of a service level agreement including pre-agreed remuneration for the services provided. Where field laboratories were located within the National Health Service (NHS), their Research and Development (R&D) department was contacted to seek approval for the laboratory to take part in processing; in most cases formal approval was not required.

M.3 Consent

Written consent was required for the following aspects of blood sampling:

- taking a venous blood sample
- storing blood residues for potential future analysis of additional analytes related to nutrition and health
- informing GPs of potentially clinically relevant blood results
- informing participants of potentially clinically relevant blood results

For children aged under 16 years, written consent was sought from a parent or legal guardian, with written assent from the child where possible (see chapter 2 of main report for more detail of consents).ⁱ

M.4 Exclusion from participation in venepuncture

Participants were asked a series of screening questions prior to venepuncture to assess their eligibility for giving a blood sample. Participants with a bleeding or clotting disorder or those taking anti-coagulant medications were excluded from the blood sample component.

Participants aged 16 years and over who had had a fit in the previous five years and those aged 15 years and under who had ever had a fit were also ineligible to give a blood sample. Blood samples were not taken from participants who volunteered that they had hepatitis B or HIV; however, participants were not asked about their hepatitis B or HIV status.

M.5 Equipment used

Blood samples were collected by a nurse (or in the case of young children aged 1.5 to 10 years by a paediatric phlebotomist) using a Sarstedt fixed or butterfly needle, depending on the nurse's or phlebotomist's preference. The monovette system was chosen because it is a closed system, allowing the safe collection of blood in the home. Children who had consented to provide a blood sample were offered the option of Ametop anaesthetic gel being applied prior to venepuncture.

The contents of the monovette packs varied depending upon the age of the participant. Details of the equipment nurses were provided with to take blood are provided in Appendix G of the main report.ⁱ

M.6 Phlebotomy: training, procedures and instructions

Information about the recruitment and training of nurses is provided in chapter 2 of the main report.ⁱ

Blood samples were taken by the nurse from participants aged 11 years and over. For children aged 1.5 to 10 years, samples were taken by a paediatric phlebotomist recruited for their skill and recent experience in taking paediatric blood samples.

During the first nurse visit to the participant's home the nurses were instructed to:

- Assess the participant's eligibility for blood sampling and explain the procedure in detail
- Obtain the participant's verbal agreement for a revisit for blood sampling
- Instruct the participant about overnight fasting (participants aged four years and over only)
- Record the details of the visit in the CAPI program

Prior to the phlebotomy visit the nurses were instructed to make the following preparations:

- Arrange the appointment with a paediatric phlebotomist (for participants aged 1.5 to 10 years)
- Contact the local laboratory to inform them of the intended sample delivery date and time
- Select the correct set of barcoded labels and cross through any labels that would not be required
- Select the age-appropriate monovette and microtube packs
- Freeze the cold packs for transporting the blood samples to the field laboratory

At the phlebotomy visit the nurses were instructed to:

- Re-check the participant's eligibility for blood sampling. If the participant did not meet the eligibility criteria the nurse did not attempt to take a sample
- Ensure that the participant understood the blood sampling procedures

- Confirm and obtain the appropriate written consents
- Obtain the blood sample, filling the monovette tubes in the specified priority order
- Label the monovette tubes with the appropriate barcoded labels
- Record the details of the visit in the CAPI program and complete the blood tracking forms for the field laboratory and Addenbrooke's Hospital
- Leave the £15 blood sampling promissory note with the participant

Immediately after the visit the nurses were instructed to:

- Send the monovette tubes and blood tracking forms to Addenbrooke's Hospital in the pre-addressed postal pack provided
- Take the blood specimens, microtubes, relevant labels, contaminated waste and blood tracking form to the field laboratory, to arrive no later than two hours after venepuncture
- Use Milton wipes to clean the cold packs before placing them into a new plastic bag in the freezer in preparation for the next appointment
- Use Milton wipes to clean the inside of the carrying box

The approved protocol allowed for two attempts at phlebotomy with adults, provided that the participant consented. Only one attempt was permitted with participants aged 15 years and under.

M.7 Recruitment of, and procedures at, the field laboratories and Addenbrooke's hospital

M.7.1 Recruitment of the field laboratories

Several of the analytes measured in the blood, particularly certain vitamins and vitamin status index analytes, are known to be very labile and therefore need to be stabilised by low temperature storage, chemical treatment, or both, and by separation of the cells from the plasma or serum at the earliest opportunity following venepuncture. This process was carried by suitably located field laboratories recruited to process and store blood samples for later analysis by HNR.

In order to process samples for NDNS field laboratories were required to be within two hours travelling time of the fieldwork area and have access to a refrigerated centrifuge, piston pipettes and storage facilities at or below -40°C . Where such a laboratory could not be recruited, a laboratory with facilities to store samples at a minimum of -20°C storage and to chill the centrifuge buckets and inserts to 4°C prior to processing the samples was recruited.

M.7.2 Procedures at Addenbrooke's Hospital

Following venepuncture, two blood tubes (EDTA and serum gel) from each participant's sample set were sent in the post by next day delivery to the Department of Clinical Biochemistry and Immunology at Addenbrooke's Hospital in Cambridge for immediate analysis. Samples were sent in a postal pack which met Royal Mail guidelines for sending biological samples by post.

The analysis conducted by Addenbrooke's included:

- Full blood count
- Lipid profile
- HbA1c

In addition to the above, two aliquots of serum for serum folate analysis were collected. These tubes were labelled with barcoded labels provided by the nurse and stored at -80°C . The tubes were then transported to HNR at monthly intervals and stored at -80°C for future analysis.

M.7.3 Liaison with the field laboratories

Nurses delivered the blood samples within two hours of venepuncture to the field laboratories in a cool box, together with the appropriate blood tracking form, sub-aliquot tubes and barcoded labels. Two members of laboratory staff were nominated as contact points, whom the nurse could contact to arrange sample deliveries. The nurses were required to give at least 24 hours' notice of a sample delivery and to hand the samples to one of their named contacts upon arrival at the laboratory.

M.7.4 Procedures at the field laboratories

Field laboratories were provided with a detailed processing protocol, providing instruction on the separation, aliquoting and storage of samples for NDNS, with specific instructions for washing red blood cells, labelling aliquots and sending samples to HNR on dry ice. Set-up visits at newly recruited laboratories were conducted upon request in Year 1 and were compulsory for all new laboratories in Year 2. The set-up visits were conducted by a member of HNR research staff and provided the analyst or technician at the field laboratory with instruction on the nurse liaison procedures, aliquot labelling, sample processing protocol, completion of the blood tracking form and shipment of samples on dry ice to HNR.

In addition to the initial set-up visit, a random sample of 10% of laboratories per year received a review visit to ensure that the laboratory remained properly equipped to process samples for the survey and to address any quality issues that may have arisen in the intervening period. Where a laboratory was not complying with the protocol a feedback letter was sent and changes were implemented.

Prior to the start of fieldwork in their respective areas, field laboratories were provided with aliquots of 10% w/v meta-phosphoric acid for vitamin C stabilisation in 2mL screw-capped containers. The stabilising solution was delivered frozen in dry ice from HNR and was kept frozen, below -40°C where facilities were available (and below -20°C where they were not), until use.

Immediately upon receiving a participant's blood sample, the analyst/technician was required to:

- Take a 1.3mL aliquot of heparinised whole blood (from participants aged 16 years and over)
- Centrifuge remaining blood at 2000g, 4°C , for 20 minutes
- Transfer an aliquot of 300 μL of heparinised plasma in to the meta-phosphoric acid treated microtube for subsequent vitamin C analysis
- Transfer aliquots of various volumes of plasma/serum in to the microtubes provided
- Wash the heparinised red cell pellets three times in 0.9% saline to yield a red cell concentrate depleted of buffy coat
- Label all microtubes with the barcoded labels provided, store microtubes in a polythene bag and freeze at -40°C or below (or -20°C where -40°C facilities were not available)

- Complete a blood tracking form giving processing date and time and aliquot volumes and fax back to HNR on the day of processing
- At the end of each fieldwork period, courier the samples on dry ice, as well as the original copies of the blood tracking forms, to HNR

M.8 Sample tracking, reception and storage

Blood sample tubes and documents were identified and tracked via the use of pre-printed barcode labels.

Prior to the start of each fieldwork assignment, nurses were sent a work pack containing strips of unique barcode labels for every participant within their designated fieldwork area that had agreed to a nurse visit. These labels were used to identify all blood tubes and documents associated with each participant. A unique barcode label was affixed to each blood tube collected from the participant at the time of venepuncture and each sub-sample tube taken by the field laboratory was barcode labelled by the technician/analyst at the time of processing. Blood aliquots were assigned to the appropriate analysis in priority order (see appendix L) based on the information provided in the field laboratory blood tracking form.

Upon receipt of the samples from the field laboratories at HNR, each sample tube was scanned in to a computerised sample tracking system (ItemTracker (International) Ltd, Birkenhead, UK), which cross-checked the received samples against the list of expected samples to ensure that all samples had been received and were correctly labelled.

M.9 Assay auditing

M.9.1 Assay auditing at Addenbrooke's hospital

At Addenbrooke's, analytes that were reported to participants and their GPs were analysed on the day of arrival, with the exception of vitamin B₁₂ which was analysed in batches on a monthly basis. Quality controls were run according to laboratory schedules for the individual assays. HbA1c samples were batched into work lists so quality controls were run prior to and after 50 samples. Lipids quality controls were run every 11 hours. Vitamin B₁₂ quality controls were run at the beginning and at the end of the working day. In the case of quality control failures samples were re-analysed to ensure integrity of results. Automated assays were

performed as singletons, as per normal practice with clinical analysers. External quality assurance schemes were included where available in order to confirm the accuracy of the assays.

M.9.2 Assay auditing at MRC HNR

Samples were analysed in order of receipt. Analytes that were reported to participants and their GPs were analysed monthly at HNR. Those that were not reported to participants/GPs were analysed quarterly. Internal quality control samples were included with all analytes, and external quality assurance schemes were included where available in order to confirm the accuracy of the assays. Manual assays (EGRAC, ETKAC, vitamin C, colorimetric PABA) and sTfR ELISA were performed in duplicate and if agreement between duplicates failed to meet the pre-set criteria for each assay, the assays were repeated.

M.10 Procedures for reporting results to participants and/or their GP

Consent was sought from the participant (or the parent in the case of children) to inform them and their GP by letter of their potentially clinically relevant blood results.

Results were reported in two letters, the first of which was sent approximately two months after the blood sample was taken. This letter included the results of analytes which had been measured immediately. A second letter was sent approximately three to four months after the blood was taken and included the results of the blood analyses conducted in monthly batches at HNR.

Letters to GPs and participants contained a result table together with information on the normal range for each analyte. Any result for an individual which was outside the normal range was flagged in the letter and advice for follow-up was provided if appropriate. The letter also included the contact details of the survey doctor at UCL should the participant or GP wish to discuss the results further.

If results exceeded the action limits defined by Addenbrooke's Hospital Department of Clinical Biochemistry and Immunology as the levels for serious conditions which require immediate action, HNR notified the survey doctor at UCL as a matter of urgency. The survey doctor then

notified the participant's GP or the participant/the participant's parent themselves, subject to their signed permission. The following analytes had action limits:

- Haemoglobin: low <6.0g/dL, high >20g/dL
- Platelet count: low <20 x 10⁹/L, high >1,500 x 10⁹/L
- White cell count: low <1.0 x 10⁹/L, high >50 x 10⁹/L
- Neutrophil count: <0.5 x 10⁹/L
- Creatinine: >500 µmol/L
- Fasting glucose: low 2.5mmol/L, high 10.0mmol/L if not a known diabetic or 30.0mmol/L if a known diabetic
- Haemoglobin A1c: >10%

Participants who wished neither to receive their own results, nor to have them sent to their GP were required to sign a disclaimer before a blood sample was taken. This disclaimer stated that, in line with their wishes, neither they nor their GP would be notified of any abnormality detected in the blood sample.

ⁱ http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsStatistics/DH_128166 (accessed 01/08/2011)