

Appendix P The blood sample: collecting and processing the blood

P.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.

P.2 Ethical approval

As described in appendix B ethical approval was granted by a Multi-centre Research Ethics Committee (MREC)¹ 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:²

- 34.9mL of blood to be taken from participants aged 16 years
- 21.5mL from children aged 7 to 15 years
- 11.1mL from children aged 1.5 to 6 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.

P.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.

P.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.

P.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 for whom consent had been given to provide a blood sample were offered the option of Ametop anaesthetic gel being applied prior to venepuncture. All participants were offered the option of Cryogestic local anaesthetic spray.

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 L and details of the monovette packs are provided below in table P.1

Table P.1: NDNS RP Year 7 and 8 monovette packs

Tube number	Age group (years)		
	1.5-6 years	7-15 years	16 years and over
1	2.6mL EDTA	2.6mL EDTA	2.6mL EDTA
2	4.0mL Serum	7.5mL Serum	9.0mL Serum
3	4.5mL Lithium Heparin	7.5mL Lithium Heparin TM	7.5mL Lithium Heparin TM
4		2.7mL Lithium Heparin	7.5mL Lithium Heparin TM
5		1.2mL fluoride	1.2mL fluoride
6			4.5mL Lithium Heparin*
7			2.6mL EDTA*

*Samples from these blood monovettes will be stored as repository tubes.

P.6 Phlebotomy: training, procedures and instructions

Information about the recruitment and training of nurses is provided in appendix B. 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

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- obtain the participant's verbal agreement for a revisit for blood sampling
- instruct the participant about overnight fasting (non-diabetic participants and diabetics who are willing to fast, 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 unless the nurse was qualified to take paediatric blood themselves (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
- leave the £15 blood sampling promissory note with the participant

Immediately after the visit, the nurses were instructed to:

- send the specified monovette tube and blood tracking forms to Addenbrooke's in the pre-addressed postal pack provided

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- take the remaining monvettes (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 with all participants, provided that the participant and parent/legal guardian of children aged under 16 years consented.

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

P.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 out by suitably located field laboratories recruited to process and store blood samples for later analysis by MRC EWL.

In order to process samples for the NDNS RP, 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 was recruited 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.

P.7.2 Procedures at Addenbrooke's

Following venepuncture, one EDTA tube from each participant's sample set was sent in the post by next day delivery to Addenbrooke's for immediate analysis. Each sample was 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 and glycated haemoglobin (HbA1c). In addition to the analysis conducted at Addenbrooke's, two aliquots of whole blood for folate analysis were collected and preserved with ascorbic acid. These tubes were labelled with barcoded labels provided by the nurse and stored at -80°C. The tubes were then transported to MRC EWL at monthly intervals and stored at -80°C before being sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, USA for analysis.

P.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.

P.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 the NDNS RP, with specific instructions for washing red blood cells, labelling aliquots and sending samples to MRC EWL on dry ice. Set-up visits at newly recruited laboratories were compulsory for all new laboratories. The set-up visits were conducted by a member of MRC EWL 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 MRC EWL.

In addition to the initial set-up visit, each year a random sample of laboratories 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 MRC EWL 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 1.3mL aliquot of heparinised whole blood (from participants aged 16 years and over)
- centrifuge remaining blood at 2000g, 4°C, for 20 minutes
- label plasma/serum tubes with the barcoded labels provided, transfer plasma/serum to the tubes provided (one per Monovette)
- transfer aliquot of 300µL of heparinised plasma in to the meta-phosphoric acid treated microtube for subsequent vitamin C analysis
- wash the heparinised red cell pellets three times in 0.9% saline to yield a red cell concentrate depleted of buffy coat
- store plasma/serum tubes 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 plasma / serum volumes and fax/email back to MRC EWL 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 MRC EWL

P.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 O) based on the information provided in the field laboratory blood tracking form.

Upon receipt of the samples from the field laboratories at MRC EWL, 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. The plasma or serum supernatant from each tube was received as a single aliquot for subsequent thawing and sub-aliquotting at MRC EWL. This facilitated the most efficient use of serum and plasma.

P.9 Assay auditing

P.9.1 Assay auditing at Addenbrooke's

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 at the start of a batch and then run after each group of 50 samples. Vitamin B₁₂ quality controls were run at the beginning and at the end of the working day. Where a result of the quality control sample was outside the acceptable range, samples were re-analysed to ensure integrity of results. Automated assays were performed as singletons, as per normal practice with clinical analysers for these assays. External quality assurance schemes were included where available in order to confirm the accuracy of the assays.

P.9.2 Assay auditing at MRC EWL

Samples were analysed in order of receipt. Analytes that were reported to participants and their GP were analysed monthly at MRC EWL. Those that were not reported to participants/GPs were analysed in batches at intervals. Internal quality control samples were included with all analytes to monitor precision and external quality assurance schemes were included where available in order to confirm the accuracy of the assays. Manual assays (erythrocyte transketolase activation coefficient (ETKAC), erythrocyte glutathione reductase activation coefficient (EGRAC), vitamin C, and holotranscobalamin (holoTC) enzyme-linked immunosorbent assay were performed in duplicate and if agreement between duplicates failed to meet the pre-set criterion for each assay, the assay was repeated.

P.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. An example of a feedback letter is provided in appendix J.

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 MRC EWL.

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 reference 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 should the participant/parent or GP wish to discuss the results further.

If results exceeded the action limits defined by Addenbrooke's as the levels indicative of serious conditions which could require immediate action, MRC EWL notified the survey doctor as a matter of urgency. The survey doctor then notified the participant's GP or the participant/the participant's parent or guardian, subject to their signed permission. The following analytes had action limits:

- haemoglobin: low <60 g/L, high >200 g/L
- 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 20.0mmol/L if a known diabetic
- HbA1c: >10% or >82 mmol/mol
- 25-hydroxy vitamin D (2 OHD): <12.5 nmol/L

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.

¹ Ethical approval for Years 1 to 5 was obtained from the Oxfordshire A Research Ethics Committee (Ref. No. 07/H0604/113). Ethical approval for Years 6 to 9 was obtained from Cambridge South NRES Committee (Ref. No. 13/EE/0016).

² Due to a change in processing method, the number of serum tubes was reduced from 2 to 1 in Year 6 onwards and the volume of the tube was larger. See tables P.1 and P.2 for information about the number and volume of tubes used in each survey year.