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 for Year 10 (2017/18) and Year 11 (2018/19) of the NDNS RP. Detail of the methodology for Year 9 can be found in appendix O and P of the Years 1 to 9 report.[1]

Appendix Q provides an overview of the methods of blood analysis and the associated quality control and quality assessment procedures.

P.2 Ethical approval

As described in appendix B ethical approval was granted by a Multi-Centre Research Ethics Committee (MREC)[i] for all aspects of the survey protocol.

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.

[i] 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 11 was obtained from Cambridge South NRES Committee (Ref. No. 13/EE/0016).

P.4 Exclusion from participation in venepuncture

Towards the end of the final interviewer visit, participants were asked if they would be willing to take part in the next stage of the survey: a nurse visit, including blood sampling. At the nurse visit, participants were asked a series of screening questions prior to venepuncture to assess their eligibility for giving a blood sample. Participants were excluded from the blood sampling component if they:

- had a bleeding or clotting disorder
- volunteered that they had hepatitis B or HIV; however, participants were not asked about their hepatitis B or HIV status
- were aged 16 and over and had had a fit in the previous 5 years or were aged 15 years and under and had ever had a fit (Years 9 and 10)
- had had a (non-febrile) seizure within the last 2 years (Year 11)

P.5 Blood tube packs

Details of the blood tube packs for each age group are provided below in tables P.1 (for Years 9 and 10) and P.2 (for Year 11). The change from monovette to vacutainer in Year 11 was made to bring the NDNS RP into line with the protocol and equipment used on other NatCen surveys. Appendix I details the equipment provided to nurses to take blood samples.

Table P.1 NDNS RP Years 9 and 10 Monovette packs

	Age group (years)		
Tube number	1.5-6 years	7-15 years	16 years and over
1	2.6 mL EDTA	2.6 mL EDTA	2.6 mL EDTA
2	4.0 mL Serum	7.5 mL Serum	9.0 mL Serum
3	4.5 mL Lithium Heparin	7.5 mL Lithium Heparin (for trace metals)	7.5 mL Lithium Heparin (for trace metals)
4		2.7 mL Lithium Heparin	7.5 mL Lithium Heparin (for trace metals)
5		1.2 mL fluoride	1.2 mL Fluoride
6			4.5 mL Lithium Heparin*
7			2.6 mL EDTA*
Total blood volume collected	*11.1 mL*	*21.5 mL*	*32.3 mL*

*Samples from these blood monovettes are stored as repository tubes.

Table P.2 NDNS RP Year 11 Vacutainer packs

	Age group (years)		
Tube number	1.5-6 years	7-15 years	16 years and over
1	2 mL EDTA	2 mL EDTA	2 mL EDTA
2	6 mL Silica Serum Trace Element	6 mL Silica Serum Trace Element	6 mL Silica Serum Trace Element
3	4 mL Lithium Heparin	6 mL Lithium Heparin	6 mL Lithium Heparin
4		6 mL Silica Serum Trace Element*	6 mL Silica Serum Trace Element*
5			6 mL Lithium Heparin*
7			4 mL EDTA*
Total blood volume collected	*12 mL*	*20 mL*	*30 mL*

*Samples from these blood vacutainers are 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 those aged 10 years or younger, blood was taken by a NatCen paediatric phlebotomist who accompanied the nurse on the visit.[ii] Participants aged 6 years and over were offered the option of Cryogesic local anaesthetic spray.[iii] Children who did not opt for the Cryogesic spray or who were too young to be offered it) were offered the option of Ametop anaesthetic gel being applied prior to venepuncture.

In Year 10, blood was collected using a Sarstedt fixed or butterfly needle, depending on the nurse's or phlebotomist's preference. In Year 11, blood was collected using the BD Vacutainer system.[2]

During their first visit to the participant's home (please see section B.5.2 of appendix B for more detail) nurses were instructed to:

- assess the participant's eligibility for blood sampling and explain the procedure in detail

[ii] Nurses qualified and experienced in paediatric phlebotomy took blood samples from children. Protocols and procedures are in line with official Royal College of Nursing (RCN) guidelines.

[iii] The spray is not suitable for use on younger children as their perception of the 'freezing / coldness' is much more varied and thus there could be more risk of harm (frostbite / skin reaction etc).

- obtain the participant's verbal agreement for a revisit for blood sampling
- if the participant was aged 4 years and over, instruct them about overnight fasting (including participants with diabetes who were willing to fast)
- 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/nurse 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 blood tube 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 written consent and assent as appropriate
- obtain the blood sample, filling the blood tubes in the specified priority order
- barcode label the blood tubes
- record the details of the visit in the CAPI program and complete the blood tracking forms for the respective laboratories receiving the blood samples
- leave the blood sampling promissory note/gift card with the participant

Immediately after the visit the nurses were instructed to:

- send the specified blood tube and blood tracking forms to Addenbrooke's in the pre-addressed postal pack provided
- take the remaining blood specimens, microtubes, relevant labels, contaminated waste and blood tracking form to the field laboratory, to arrive within 2 hours after venepuncture

- use Milton wipes to decontaminate cold packs and carrying box in preparation for the next appointment

The approved protocol allowed for 2 attempts to obtain the blood sample 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

Initial processing and storage of blood samples for nutritional biomarker analysis or long-term storage were performed by field laboratories. Suitably located and resourced field laboratories were recruited. 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 NHS, their Research and Development (R&D) department was contacted to inform and seek approval where required for the laboratory to take part in processing; in most cases formal approval was not required.

In order to process samples for the NDNS RP, field laboratories were required to be within 2 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 the laboratories were asked to chill the centrifuge buckets and inserts to 4˚C prior to processing the samples.

P.7.2 Procedures for posted samples

Following venepuncture, one EDTA tube from each participant's sample set was sent by first class post. Each sample was sent in a postal pack which met Royal Mail guidelines for sending biological samples.

In Years 9 and 10, samples were sent directly to Addenbrooke's Hospital Pathology Department for full blood count and glycated haemoglobin (HbA1c) analysis.

Year 11 samples were sent directly to the MRC Epidemiology Biorepository for processing and onward delivery to Addenbrooke's Hospital Pathology Department for full blood count.

In addition to the analysis conducted at Addenbrooke's, 2 aliquots of whole blood for folate analysis were collected and preserved with ascorbic acid (performed by the receiving laboratory, Addenbrooke's Pathology Department in Years 9 and 10 and MRC Epidemiology Biorepository in Year 11). The tubes were stored at -70°C before one of each tube was sent to the Centers for Disease Control and Prevention (CDC) in Atlanta, USA for analysis of whole blood folate. The second, spare back-up aliquot was stored at the MRC Epidemiology Biorepository.

P.7.3 Liaison with the field laboratories

Nurses delivered the blood samples within 2 hours of venepuncture to the field laboratories in a cool box with cool packs, together with the appropriate blood tracking form, sub-aliquot tubes and barcoded labels. One or more 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' advance 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 the laboratories for analysis (MRC EWL (Years 9 and 10), MRC Epidemiology Biorepository (Year 11)) on dry ice.

Set-up visits at newly recruited laboratories were compulsory. The set-up visits were conducted by a member of MRC EWL / MRC Epidemiology Biorepository 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 the laboratories for analysis.

All samples and forms coming in from field laboratories were checked and any issues followed up by email/phone or a visit to ensure laboratory compliance to protocols and to address any quality issues.

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 2 mL screw-capped containers. The stabilising solution was delivered frozen in dry ice from MRC EWL (Years 9 and10) or MRC Epidemiology Biorepository (Year 11) and was kept frozen, below -70˚C where facilities were available (and below -20˚C where they were not), until use.

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

- remove a 1.3 mL aliquot of heparinised whole blood (from participants aged 16 years and over)
- centrifuge remaining blood at 2000 *g*, 4˚C, for 20 minutes (within 2 hours of venepuncture)
- label plasma / serum tubes with the barcoded labels provided, transfer plasma/serum to the tubes provided (1 per blood tube)
- transfer aliquot of 300 µL of heparinised plasma in to the meta-phosphoric acid containing microtube for subsequent vitamin C analysis
- wash the heparinised red cell pellets 3 times in 0.9% saline to yield a red cell concentrate depleted of buffy coat
- store plasma, serum and red cell pellet tubes in a polythene bag and freeze at -40˚C or below (or -20˚C where -40ºC facilities were not available). This was to be done within 2 hours of centrifugation
- complete a blood tracking form giving processing date and time and plasma / serum volumes and fax/email back to the processing lab on the day of processing if possible
- 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 the laboratories for analysis

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 sheets 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.

In Year 10, upon receipt of the samples from the field laboratories at MRC EWL, the samples were then cross-checked in Excel 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-aliquoting at MRC EWL. This facilitated the most efficient use of serum and plasma. The aliquots destined for sub-aliquoting were weighed to determine the volume of the aliquot and therefore the number and volume of sub-aliquots. Each sample tube was scanned into a computerised sample tracking system (ItemTracker (International) Ltd, Birkenhead, UK).

In Year 11, upon receipt of the samples from the field laboratories at MRC Epidemiology Biorepository, the samples were imported into a laboratory information management system (LIMS) (LabVantage Solutions Limited, High Wycombe, UK) which recorded all samples and cross-checked them 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-aliquoting at MRC Epidemiology Biorepository. This facilitated the most efficient use of serum and plasma. A set number of sub-aliquots were created providing there was sufficient sample volume.

An aliquoting priority order ensured that the highest priority analysis aliquots were created first. Blood aliquots were assigned to the appropriate analysis in priority order (see tables for this appendix) based on the information provided in the field laboratory blood tracking form.

P.9 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 3 letters:

- Letter 1: Results measured on arrival (full blood count and in Years 9 and 10 only, HBA1c)
- Letter 2: Results of the blood analyses conducted in batches (vitamin B12, ferritin, 25-hydroxyvitamin D, lipids)
- Letter 3: Serum folate results (and in Years 9 and 10 only, red cell folate)

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 letters also included the contact details of the survey doctor (Years 9 and 10)/survey coordinator (Year 11) should the participant/parent or GP wish to discuss the results further. If results exceeded pre-defined action limits potentially indicative of serious conditions which could require immediate action, the survey clinician team was notified. A member of the survey clinician team then notified the participant's GP or the participant/the participant's parent or guardian, subject to their signed permission.

For Year 10, the following analytes had action limits[iv]:

- haemoglobin: low <60 g/L, high >200 g/L
- platelet count: low <20 x 10^9/L, high >1,500 x 10^9/L
- white cell count: low <1.0 x 10^9/L, high >50 x 10^9/L
- neutrophil count: <0.5 x 10^9/L
- creatinine: >500 µmol/L
- fasting glucose: low 2.5 mmol/L, high 10.0 mmol/L if not a known diabetic or 20.0 mmol/L if a known diabetic
- HbA1c: >10% or >82 mmol/mol
- 25-hydroxy vitamin D (25-OHD): <12.5 nmol/L

Following clinical review action limits were updated in Year 11 as follows:

- haemoglobin: low <70 g/L, high >190 g/L
- platelet count: low <20 x 10^9/L, high >1,500 x 10^9/L
- white cell count: low <1.0 x 10^9/L, high >50 x 10^9/L
- neutrophil count: <0.5 x 10^9/L
- 25-hydroxy vitamin D (25-OHD): no action limit[v]:

Participants aged 16 years or older 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. However, children aged under 16 years were only allowed to take part in the blood protocol if their parent/guardian agreed to a survey clinician contacting them if it was necessary to discuss any findings that were directly relevant to their child's health. If the parent did not agree to this, a blood sample was not taken from the child participant.

[iv] Action limits applied to all ages.
[v] As with all analytes reported to the GP/participant, 25-hydroxy vitamin D was flagged in the standard letter if it fell outside of the reference range (25-150 nmol/L).

References

[1] National Diet and Nutrition Survey Years 1 to 9 of the Rolling Programme (2008/2009 – 2016/2017): Time trend and income analyses https://www.gov.uk/government/statistics/ndns-time-trend-and-income-analyses-for-years-1-to-9.

[2] BD blood collection tubes. https://www.bd.com/en-uk/products/blood-and-urine-specimen-collection/blood-collection-tubes