Appendix P
Blood sample collection and processing in National Diet and Nutrition Survey 2019 to 2023

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 and transporting blood samples, procedures for sample processing, storage and assay auditing and the protocol for reporting clinically significant blood results to participants and their GP.

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

P.2 Consent

Separate written consent was required for each of the following aspects of blood sampling:

- taking a venous blood sample and analysing it for biomarkers related to nutrition
- storing samples 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.3 Screening for 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 biomedical fieldworker visit, including blood sampling by venepuncture (through a needle in a vein). Participants were provided with an information sheet about this stage of the survey (appendix C). At the biomedical fieldworker visit, participants were asked a series of screening questions to confirm their eligibility for giving a blood sample. Participants were excluded from the blood sampling component if they:

- had a bleeding or clotting disorder
- were taking anti-coagulant medication
- 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 had a (non-febrile) seizure within the last 2 years

P.4 Blood tube packs

Details of the blood tube packs for each age group are provided in table P.1. Appendix I details the equipment provided to biomedical fieldworkers to take blood samples. Due to supply issues at the end of 2022, the SEN1 tube was replaced with a 6 mL serum red top tube for 23 participants aged 18 years and over.

Table P.1 Blood tube packs

Blood Tube:	Label:
Participants aged 16 years and over **1.** 2 mL EDTA (lilac top) **2.** 6 mL Silica Serum Trace Element (royal blue top) **3.** 6 mL Lithium Heparin (green top) **4.** 6 mL Silica Serum Trace Element (royal blue top) **5.** 6 mL Lithium Heparin (green top) **6.** 4 mL EDTA (purple top)	EN1 PROJ2170 SEN1 LHN1 SEN2 LHN2 EN2
Participants aged 7 to 15 years **1.** 2 mL EDTA (lilac top) **2.** 6 mL Silica Serum Trace Element (royal blue top) **3.** 6 mL Lithium Heparin (green top) **4.** 6 mL Silica Serum Trace Element (royal blue top)	EN1 PROJ2170 SEN1 LHN1 SEN2
Participants aged 1.5 to 6 years **1.** 2 mL EDTA (lilac top) **2.** 6 mL Silica Serum Trace Element (royal blue top) **3.** 4 mL Lithium Heparin (green top)	EN1 PROJ2170 SEN1 LHN1

P.5 Training, procedures and instructions

Information about the recruitment and training of biomedical fieldworkers is provided in appendix B. Detailed blood sampling protocols can be found in appendix L.

Blood samples were taken by the biomedical fieldworker from participants aged 11 years and over. Biomedical fieldworkers qualified and experienced in paediatric phlebotomy took blood samples from younger children. Otherwise, blood was taken by a NatCen paediatric phlebotomist who accompanied the biomedical fieldworker on the visit. Protocols and procedures are in line with official Royal College of Nursing (RCN) guidelines.

Participants aged 6 years and over were offered the option of Cryogesic local anaesthetic spray. 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 (such as frostbite or skin reaction). 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. Blood was collected using the BD Vacutainer system.

Prior to their visit, biomedical fieldworkers made the following preparations:

- for participants aged 18 months to 10 years arrange the appointment with a paediatric phlebotomist, unless the biomedical fieldworker was qualified to take paediatric blood themselves
- contact the local laboratory (if field laboratory processing protocol, see section P.6) 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 or posting the blood samples

At the visit the biomedical fieldworker protocol included:

- answering any participant questions
- obtaining written consent and assent as appropriate
- collecting the blood sample, filling the blood tubes in the specified priority order
- labelling the blood tubes with a barcode
- recording the details of the visit in the CAPI program and completing the blood tracking forms for the respective laboratories receiving the blood samples
- leaving the blood sampling promissory note or gift card with the participant

Immediately after the visit the biomedical fieldworkers were instructed to prepare the specified blood tubes and blood tracking forms for delivery to the field laboratories or postage (see section P.6).

P.6 Procedures for blood transport and processing

In year 12 the protocol was the same as in previous survey years. Biomedical fieldworkers delivered all but one of the blood tubes within 2 hours of venepuncture to field laboratories for sample processing. One further EDTA tube was posted directly to MRC Epidemiology Biorepository using standard first-class post for full blood count and preparation of an aliquot for whole blood folate analysis.

In year 13 a postal processing protocol was introduced, replacing the field laboratories, to improve cost efficiency, standardisation of processing and to allow greater flexibility for fieldwork. All samples were mailed overnight, chilled, using a next-day delivery service to the NDNS central laboratory (MRC Epidemiology Unit, Cambridge) for processing.

In order to evaluate the impact of the change in specimen transport on biomarker concentrations, the different transport protocols were compared in a nested study performed between November 2021 and March 2022, in parallel with year 13 fieldwork. Further details can be found in the blood sample transport evaluation report.

The two sample processing protocols are described in more detail in sections P.6.1 and P.6.2.

P.6.1 Field laboratory sample processing protocol (year 12)

Recruitment of field laboratories

Initial processing and storage of blood samples for nutritional biomarker analysis or long-term storage was performed by field laboratories as had been the case since year 1 of NDNS. Suitably located and resourced field laboratories were recruited and appointed 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 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 NDNS, 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.

Set-up visits were conducted at newly recruited laboratories and laboratory staff at the field laboratory were provided with instructions on the biomedical fieldworker liaison procedures, aliquot labelling, sample processing protocol, completion of the blood tracking form and shipment of samples to MRC Epidemiology Unit on dry ice.

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

Biomedical fieldworker liaison with field laboratories

NatCen biomedical fieldworkers delivered the blood samples within 2 hours of venepuncture. Samples were delivered 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 field laboratory staff were nominated as contact points at respective field laboratories whom the biomedical fieldworker could contact to arrange sample deliveries. Biomedical fieldworkers were required to give at least 24 hours advance notice of a sample delivery to a field laboratory and to hand the samples to one of their named contacts upon arrival at the laboratory.

EDTA blood tube for full blood count and whole blood folate aliquot

One EDTA tube from each participant's sample set was sent by first class post to MRC Epidemiology Unit. Each sample was sent in a postal pack which met Royal Mail guidelines for sending biological samples.

On arrival, 2 aliquots of whole blood (each 100 µL) were removed and preserved with 1 mL 1% ascorbic acid for whole blood folate analysis. The remaining whole blood in the EDTA blood collection tubes was delivered to Addenbrooke's Hospital Pathology Department for analysis of full blood count.

Procedures at the field laboratories (LH and serum tubes, 2nd EDTA tube)

Prior to the start of fieldwork in their respective areas, field laboratories were sent, on dry ice, aliquots of 10% w/v metaphosphoric acid (MPA) for vitamin C stabilisation in 2 mL screw-capped containers prepared at MRC Epidemiology Unit. MPA tubes were kept frozen, below -70°C where facilities were available, otherwise below -20°C, until use.

Immediately upon receiving a participant's blood sample, the field laboratory staff member 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
- 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 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 -40 °C 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 email back to MRC Epidemiology Unit 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 MRC Epidemiology Unit

On receipt at MRC Epidemiology Unit, the frozen samples processed by the field laboratories were stored at below -70°C. Since plasma and serum were received as single aliquots, when sufficient samples had been received tubes were thawed and sub-aliquoted to facilitate the most efficient use of serum and plasma for analysis and long-term storage.

P.6.2 Postal processing protocol (year 13 onwards)

Transport of samples

After blood sample collection, the fieldworker placed all blood specimens in absorbent pouches (Product number AB010, Alpha Laboratories, Hampshire, UK) and then in clear zip seal bags, wrapped in a double layer of bubble wrap and placed in a foam insulated box (GFS-3, Chilled Packaging, Corby, UK) containing two cold packs frozen overnight in a domestic -20°C freezer (Figure 1). The sealed boxes were taken by the biomedical fieldworkers to a local Post Office and sent by Royal Mail guaranteed next day delivery to MRC Epidemiology Unit. A small number of samples were hand-delivered by biomedical fieldworkers to the MRC Epidemiology Unit, when visits were in the local area and/or Royal Mail issues meant postage was not possible that day.

Figure 1. Blood tubes were packaged according to UK guidelines with appropriate labelling. Tubes were surrounded by absorbent material, placed in a sealed bag, wrapped in bubble wrap and placed between two

cold packs (frozen at -20 °C) and the box sealed for transport.

Note that from May 2022, a third cold pack was introduced into all packages.

Sample processing at MRC Epidemiology Unit

On arrival and immediately following unpacking (the morning after specimen collection), the temperature of one specimen tube from each box was measured with an infrared thermometer.

For the EDTA blood tube, the same protocol was used as described in section P.6.1 for the preparation of samples for full blood count and whole blood folate analysis.

The remaining blood tubes were centrifuged at 2000 *g*, 4°C, for 20 minutes and plasma and serum removed into 2 mL microtubes or 2D-barcoded Fluidx aliquots (removing the requirement for a thaw/freeze cycle as performed in the field laboratory protocol in P.6.1) for subsequent analysis or long-term storage, respectively. For vitamin C analysis, 400 µL of LH plasma was added to 400 µL 10% w/v metaphosphoric acid (MPA). The heparinised red cell pellets were washed as described above in P.7.1.1.

All aliquots were subsequently stored at below -70°C until analysis or onward shipping to analysing laboratories.

P.7 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, biomedical fieldworkers were sent a work pack containing sheets of unique barcode labels for every participant within their designated fieldwork area that had agreed to a biomedical 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 processing laboratory (field and postal protocols) was barcode labelled by the technician or analyst at the time of processing.

Upon receipt at MRC Epidemiology Unit, the samples were imported into a laboratory information management system (LIMS) (LabVantage Solutions Limited, High Wycombe, UK). All samples were cross-checked against a list of expected samples in the study database to ensure that all had been received and were correctly labelled. A set number of sub-aliquots were created providing there was sufficient sample volume (see tables P.1 to P.3 in appendix P).

P.8 Procedures for reporting results to participants and GPs

Consent was sought from the participant (or the parent in the case of children) to inform them

and/or their GP by letter of blood results. The blood results reported were a predefined sub-set of the measured analytes and were potentially relevant to health when taken in conjunction with the participant`s health and treatment records, which the NDNS team did not have access to.

Results were reported in 2 letters:

- letter 1: results of full blood count
- letter 2: results of the blood analyses conducted in batches (vitamin B12, ferritin, 25-hydroxyvitamin D, lipids, serum 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 coordinator should the participant (and their parent or guardian in the case of children) or GP wish to contact the survey. Examples of feedback letters are provided in appendix J.

If results exceeded pre-defined action limits potentially indicative of serious conditions which could require urgent action, the survey clinician team was notified. A member of the survey clinician team then notified the participant's GP or the participant (their parent or guardian in the case of children), subject to their signed permission.

The following analytes had action limits (applicable to all ages):

- 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
- triyglycerides: >10 mmol/L

Participants aged 16 years and over 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 or 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 or guardian did not agree to this, a blood sample was not taken from the child.