Appendix Q Methods of blood and urinary analysis and quality control (QC) and quality assessment (QA)

Q.1 Introduction

This appendix provides an overview of the methods of analysis and the associated quality control and quality assessment procedures. The collection, transport and processing of blood and urine samples are described in appendices M and P. A list of analytes measured and the priority order of analysis are provided in the tables that accompany appendix P.

This appendix focusses on Year 10 (2017/18) and Year 11 (2018/19) of the NDNS RP. The methodology outlined in this appendix is consistent with Year 9 (2016/17) unless stated otherwise. Detail of the methodology for Year 9 can be found in appendices N and Q of the Years 1 to 9 report.[1]

For NDNS RP up to and including Year 10, analyses were conducted at MRC Elsie Widdowson Laboratory (MRC EWL) or partner laboratories. From Year 11 onwards, responsibility for analysis of blood analytes in the NDNS RP moved to National Institute of Health Research Cambridge Biomedical Research Centre (NIHR BRC) Nutritional Biomarker Laboratory (NBL). An essential part of the laboratory transitions was continuity of procedures and quality to ensure data generated across all years of the NDNS RP could be combined for statistical analysis. Equipment, standards, quality control (QC) and quality assessment (QA) materials from the MRC EWL were gifted to the NBL and for the majority of analytes allowed continuity of method and equipment. Where there was not continuity of staff with specialist knowledge and experience of the NDNS RP assays, there was extensive cross-training of staff at MRC EWL prior to its closure. Sub-contracted laboratories remained the same for full blood count, vitamin B12 and whole blood folate. Assays previously performed at MRC EWL were additionally sub-contracted, namely lipids, CRP and ferritin and selenium, zinc and urinary iodine. A summary of changes to the analysing laboratories and/or methods, are summarised in table Q.1 and are detailed under the respective sections for each analyte.

As part of the transition to the new laboratory, and to ensure data continuity, each method underwent re-evaluation, which consisted of an assessment of precision and accuracy and a re-analysis of a subset of Year 10 samples at the Year 11 analysing laboratory the results of which were compared to results obtained originally at MRC EWL. Data from the re-evaluation were reviewed by the NDNS RP external quality assurance advisor, Elaine Gunter (Specimen Solutions, LLC) and presented to the NDNS Project Board. Accuracy and precision were acceptable for all analytes and performance consistent with results obtained from MRC EWL.

Each section below provides an outline of the analytical method, notes any changes between Years 10 and 11 and summarises QC and QA data for Years 10 and 11 of the NDNS RP. Information regarding Year 9 and preceding NDNS RP years, including QC and QA information and any changes during the first 9 years (i.e. in analytical procedures where the analytical portfolio was amended or where methods were superseded) are described in the Years 1 to 9 report.[1]

Table Q.1 Summary of analytes and methods of analysis during NDNS RP Years 10 and 11

Analyte	Appendix section	Year 10	Year 11	Method changes from Y10 to Y11
Blood analytes				
Full blood count	Q.3.1	Addenbrooke's	Addenbrooke's	No change
HbA1c	Q.3.2	Addenbrooke's	Not measured	n/a
C-reactive protein	Q.3.3	MRC EWL	CBAL	Different instrument
Ferritin	Q.3.4	MRC EWL	CBAL	Plasma to serum
Serum cholesterols (total, HDL, LDL)	Q.3.5	MRC EWL	CBAL	Different instrument
Triglycerides	Q.3.6	MRC EWL	CBAL	Different instrument
Glucose	Q.3.7	MRC EWL	Not measured	n/a
ETKAC (vitamin B1)	Q.3.8	MRC EWL	NBL	No change
EGRAC (vitamin B2)	Q.3.9	MRC EWL	NBL	No change
Vitamin B6 (PLP and 4PA)	Q.3.10	MRC EWL	NBL	Changes to protocol
Folate (serum)	Q.3.11	MRC EWL	NBL	No change
Whole blood folate	Q.3.12	CDC	CDC	No change
Vitamin B12	Q.3.13	Addenbrooke's	Addenbrooke's	No change
Holotranscobalamin	Q.3.14	MRC EWL	NBL	No change
Vitamin C	Q.3.15	MRC EWL	NBL	No change
Retinol (vitamin A)	Q.3.16	MRC EWL	NBL	No change
α- and γ-tocopherol (vitamin E)	Q.3.16	MRC EWL	NBL	No change
Carotenoids	Q.3.16	MRC EWL	NBL	No change
25-hydroxyvitamin D	Q.3.17	MRC EWL	NBL	No change
Creatinine	Q.3.18	MRC EWL	Not measured	No change
Selenium	Q.3.19	MRC EWL	University Hospitals Southampton	Plasma to serum; different instrument
Zinc	Q.3.19	MRC EWL	University Hospitals Southampton	Plasma to serum; different instrument
Urinary analytes				
Iodine	Q.3.20	MRC EWL	University Hospitals Southampton	Different instrument

Abbreviations: Addenbrooke's Pathology Department, Cambridge; Core Biochemical Assay Laboratory, Addenbrooke's Hospital, Cambridge; EGRAC, Erythrocyte glutathione reductase

activation coefficient; ETKAC, Erythrocyte transketolase activation coefficient; MRC EWL, Medical Research Council Elsie Widdowson Laboratory, Cambridge; NBL, NIHR BRC Nutritional Biomarker Laboratory, Cambridge; CDC, Centers for Disease Control and Prevention, Atlanta, Georgia).

Q.2 Notes on interpretation of external quality assessment (EQA) schemes

Different schemes within the UK National External Quality Assessment Services (NEQAS (https://ukneqas.org.uk/)) and other EQA schemes (described in the relevant sections) have varying approaches to reporting and presenting performance data. In this report, performance data are presented as the % bias and SD of % bias from the method mean or the all-laboratory trimmed mean (ALTM) calculated in-house or using the scheme-specific scores; the notes below provide a summary of information to interpret the scheme-specific scores.

Q.2.1. Performance scores for C-reactive protein

Performance criteria for NEQAS are based on the following scores: MRBIS (mean running bias) and MRVIS (mean running variance index score). Performance of the laboratory over the given time period is characterised as follows:

Ideal: MRVIS < 50
Good: MRVIS 50 – 100
Adequate: MRVIS 101 – 200
Poor: MRVIS > 200 or SDBIS > 200

Q.2.2. Performance scores for fat-soluble vitamins, vitamin D, vitamin B12, holoTC, serum folate

NEQAS scores for these schemes are reported as A, B and C scores:

A – Accuracy. The A score has been transformed and uses a ‘degree of difficulty’ factor. A scores are broadly comparable across analytes and provide an overall performance A score which assesses bias, consistency of bias and clinical acceptability of performance over approximately 6 months. Bias is usually calculated to the all laboratories trimmed mean (ALTM)

B – Bias calculated as the mean bias over last 6 months (%). Bias is calculated as specimen % bias = [(result-target)/target]*100

C – Consistency of bias represented by the SD of bias over last 6 months (%)

Q.3 Methods

Details of the analysis method and the associated QC procedures for each analyte are given in section Q.3.1 onwards. Internal QC samples were run in every batch to assess assay imprecision for each analyte. Accuracy was assessed by comparisons with certified reference materials, target values from external QC materials and/or results obtained by other laboratories by taking part in EQA schemes for those analytes where such schemes were available.

Q.3.1 Full blood count including haemoglobin and haematocrit

Full blood count was measured at Addenbrooke's Hospital using a Siemens Advia 2120, which uses the Coulter Principle to count the red blood cells (RBC), mean cell volume (MCV), white blood cells (WBC) and platelet counts. Haemoglobin was measured by photometric measurement. Other parameters such as the mean cell haemoglobin (MCH), haematocrit (Hct) and red cell distribution width (RDW) were calculated from the above measured parameters.

Haemoglobin was measured spectrophotometrically at 525 nm by a photocell in a sample that was diluted 1:256 (final) with isotonic diluent and lysing solution. The red cells were destroyed with a lysing agent releasing the haemoglobin into solution, which enabled the WBC count to be estimated using the Coulter Principle (impedance counting of the WBCs) without interference by red cells. The same lysing reagent also converted the haemoglobin to cyanmethaemoglobin.

Q.3.1.1 Internal QC for full blood count

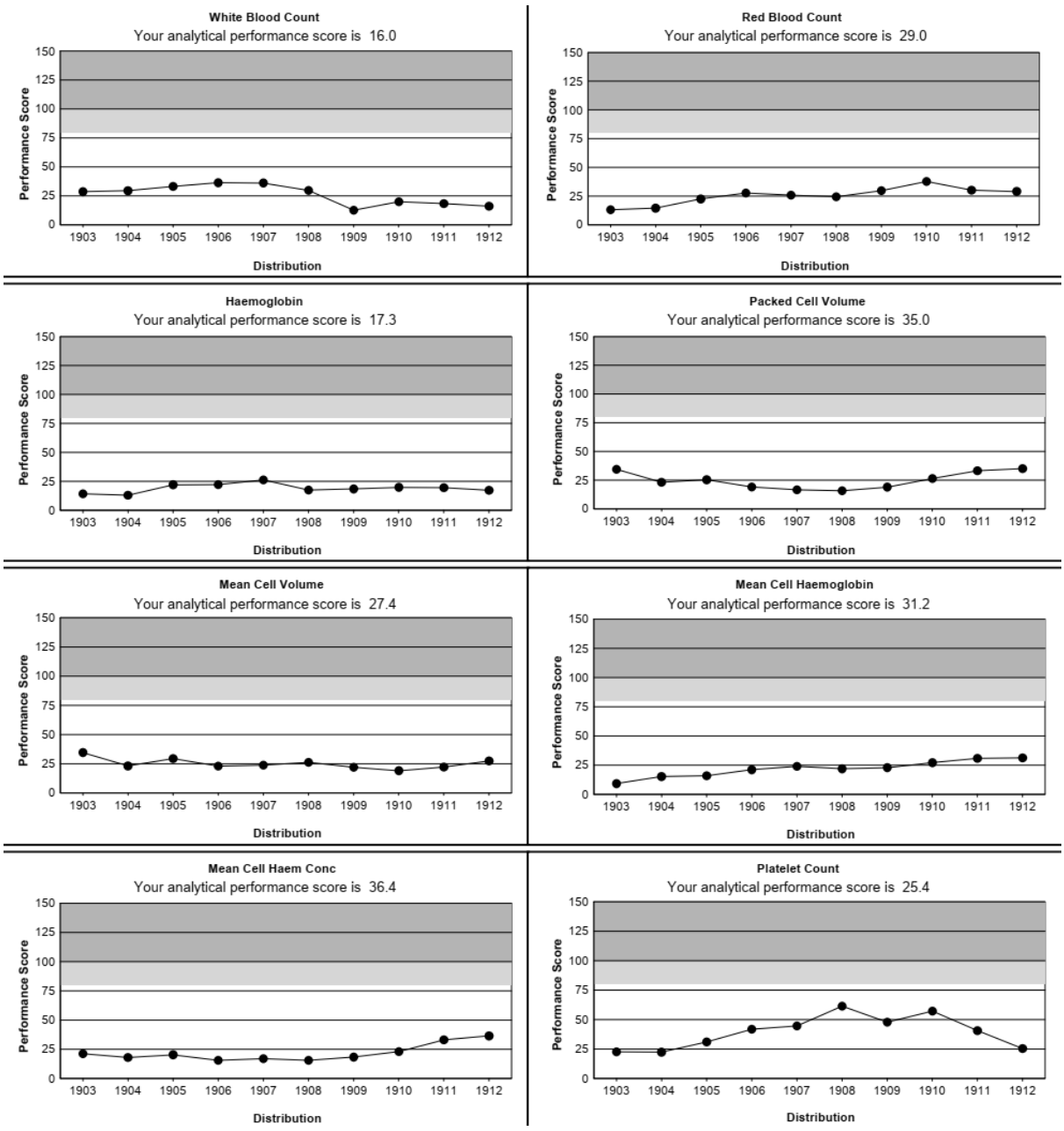
The QC results of the instruments measuring full blood count at Addenbrooke's are monitored continually, and analysis is stopped if the results are not satisfactory, ensuring that results are only reported if the analysis is within the QC parameters set by the Laboratory Manager. However, it is not possible to extract these results for reporting for each specific time period relevant to the NDNS RP analyses. Representative laboratory assay CV%s are 3.1% (RBC), 1.5% (MCV), 4.3% (WBC), 5.0% (platelet count), 3.1% (MCH), 2.4% (RDW) 1.8% (haemoglobin) and 3.3% (haematocrit).

Q.3.1.2 External QC for full blood count

Quality of results was also assessed externally through NEQAS. NEQAS results are compared against the ALTM calculated for laboratories in the NEQAS scheme using the same analyser and method as that used by Addenbrooke's. Figure Q.1 shows, as an example, the cumulative performance charts from UK NEQAS returns between January and December 2019 for 1 of the 5 Siemens Advia 2120 instruments in use in the laboratory). The "distribution" axis indicates the year and month of the UKNEQAS return. Results within the white area of the charts indicate acceptable performance as determined by UKNEQAS. "Performance index" is derived by the

NEQAS administrators as a function of the deviation of the laboratory from the consensus mean. The dark shaded area indicates unacceptable performance and the paler area indicates a borderline situation.

Figure Q.1 Illustrative overall performance charts for NEQAS for 2019, spanning NDNS RP Year 11



Q.3.2 Haemoglobin A1c (HbA1c)

In Years 9 and 10 Haemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (HPLC) at Addenbrooke's, using the Tosoh Automated Glycohemoglobin Analyser. Results are traceable to the US National Glycohemoglobin Standardization Program and to the International Federation of Clinical Chemistry. HbA1c was not measured in Year 11.

Q.3.2.1 Internal QC for HbA1c

The internal QC results in table Q.2 show good precision for Year 10.

Table Q.2 Internal QCs for HbA1c (NDNS RP Year 10)*

HbA1c	Tosoh low (G8L) LOT AB6030	Tosoh high (G8L) LOT AB6030	Bio-Rad Low (G8L) LOT 33941	Bio-Rad High (G8L) LOT 339142
Mean mol/mol	30.1	83.0	34.4	78.9
SD	0.78	0.79	0.61	0.89
%CV	2.6	0.9	1.8	1.1
n	195	200	200	201

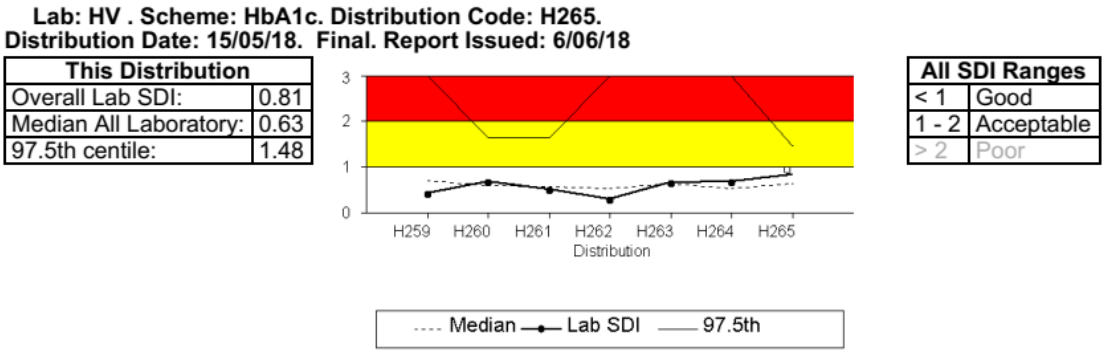
*Example – 1 of 4 instruments, QCs used between June and December 2017

Q.3.2.2 External QA for HbA1c

The HbA1c external assessment scheme is operated by Wales External Quality Assurance Scheme (WEQAS) and calculates standard deviation index (SDI) for each distribution – this is "total error" and includes both inaccuracy and imprecision. The SDI is calculated as (laboratory result – target value) / WEQAS SD. Figure Q.2 is an example of an external QA plot for the instruments at Addenbrooke's and a summary of the scores for each of 4 Addenbrooke's instruments in one of the quarterly distributions. Scores <1 indicate good performance. The solid line shows the performance of Addenbrooke's and the dotted lines are the national median and

97.5 percentile performance. These graphs demonstrate that Addenbrooke's performs close to the median UK performance for this assay.

Figure Q.2 External QA for HbA1c (NDNS RP Year 10)



Section SDI scores for this distribution

Section	EXL	G8 Left-10173304	G8 Right -12842102	G8 Three - 14144110	WDEC/WESTON G8
Overall	**1.51**	**1.00**	**0.52**	**0.52**	**0.52**
HbA1c IFCC	1.51	1.00	0.52	0.52	0.52

Q.3.3 Serum C-reactive protein (CRP) using an extended-range assay

C-reactive protein (CRP) was assayed using a high-sensitivity (extended range) assay (RCRP, DF34) on a Siemens system. In Years 9 and 10, this analysis was performed at MRC EWL on a Siemens Dimension Xpand and for Year 11 at the Core Biochemical Assay Laboratory (CBAL), Cambridge, on a Siemens Dimension EXL200 clinical chemistry analyser. The CRP method is based on a particle enhanced turbidimetric immunoassay (PETIA) technique, giving high sensitivity by extending the detection range from 0.5 mg/L to 250 mg/L. Latex particles coated with anti-CRP antibody aggregate in the presence of CRP in the sample. The increase in turbidity that accompanies aggregation is proportional to the CRP concentration.

Assay results are reported to 1 decimal place and, consistent with reporting in previous NDNS RP years, values less than 1.1 mg/mL were given an assigned value of 0.7 mg/mL[i,ii] to reflect the high imprecision at values less than 1.1 mg/mL.

Q.3.3.1 Internal QC for CRP

Internal QC material for CRP was from Bio-RadBio-Rad Laboratories Ltd, Hertfordshire, UK (Bio-Rad) and consisted of Liquichek Immunology Control, level 1

[i] Here and throughout, assigned values are calculated as the LOQ/√2
[ii] For a description of EQA scheme scores, see section Q.2

and 3 (product code 594) and Liquichek Cardiac Markers Plus Control, level 2 (product code 180). Table Q.3 shows imprecision data in Year 10 and Year 11.

Table Q.3 Internal QC for CRP (NDNS RP Years 10 and 11)

CRP	Year 10						Year 11		
	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	Q3_2	QC1	QC2	QC3
Mean (mg/L)	1.8	1.6	17.8	19.8	50.2	47.7	7.6	52.9	3.4
SD (mg/L)	0.30	0.23	0.30	0.31	1.00	0.98	0.46	1.74	0.51
% CV	16.7	14.4	1.7	1.6	2.0	2.1	6.1	3.3	14.7
n	12	4	13	4	10	4	40	40	40

Q.3.3.2 External QC for CRP

External QC was achieved through the NEQAS CRP and ultra-sensitive CRP schemes (table Q.4).

Table Q.4 External QC for CRP (NDNS RP Years 10 and 11)

CRP NEQAS	Year 10		Year 11	
Score[iii]	CRP	Ultra-sensitive CRP	CRP	Ultra-sensitive CRP
Mean Running Variance Index Score (MRVIS)	45	158	75	150
Mean Running Bias Index Score (MRBIS)	20	118	54	85
Standard Deviation Bias Index Score (SDBIS)	52	152	109	145

Q.3.4 Plasma ferritin

Plasma ferritin was measured in Years 9 and 10 at MRC EWL on a Siemens Dimension Xpand and for Year 11 on a Siemens Dimension EXL200 analyser at CBAL. The assay uses chromium dioxide particles coated with specific antibodies to human ferritin. The assay is coupled to a colour reaction and the concentration of ferritin in the sample is proportional to the intensity of colour produced in the reaction. Ferritin is quantitated by comparison to calibrants of known concentration.

Q.3.4.1 Internal QC for plasma ferritin

Control serum in Year 10 was obtained commercially (Bio-Rad Lyphocheck Immunoassay Plus), containing low, medium and high concentrations of ferritin and was included in each run. At CBAL in Year 11, QC material was Bio-Rad Lyphochek

[iii] For a description of EQA scheme scores, see section Q.2

Immunoassay Plus Control - level 1 and 3 (product code 370) and Bio-Rad Lyphochek Anemia Control (product code 500). The results in table Q.5 indicate good between-batch consistency for ferritin results during Years 10 and 11.

Table Q.5 Internal QC for ferritin (NDNS RP Years 10 and 11)

Ferritin	Year 10			Year 11			
	QC1	QC2	QC3	QC1	QC2	QC3 (Nov18 to Apr19)	QC3 (Apr19 to Jul19)
Mean (µg/L)	62.6	111.9	290.6	74.7	342.2	9.2	6.6
SD (µg/L)	2.61	11.90	12.50	2.48	12.95	0.61	0.51
% CV	4.2	10.6	4.3	3.3	3.8	6.6	7.7
n	20	20	20	40	42	23	13

Q.3.4.2 External QA for plasma ferritin

External QA was through the NEQAS Haematinics scheme. Table Q.6 shows the percentage bias relative to the target concentration in ferritin results during Years 10 and 11.

Table Q.6 Summary of NEQAS bias assessment for ferritin (NDNS RP Years 10 and 11)

Ferritin	Year 10	Year 11
Mean % bias	-0.9	5.6
SD of % bias	7.0	8.67
n	36	21

Q.3.5 Serum total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol

Total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by Siemens clinical chemistry analyser. In Years 9 and 10, analysis was performed at MRC EWL on a Siemens Dimension Xpand and for Year 11 on a Siemens Dimension EXL200 analyser at CBAL.

The total cholesterol method (Siemens CHOL DF27) uses cholesterol esterase to catalyse the hydrolysis of cholesterol esters to produce free cholesterol which, along with pre-existing free cholesterol, is oxidised in a reaction catalysed by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase, the hydrogen peroxide thus formed is used to oxidize N,N-diethylaniline-HCl/4-aminoantipyrine to produce a chromophore that absorbs at 540 nm.

The AHDL cholesterol assay (Siemens DF48B) is a method for directly measuring HDL cholesterol concentrations. The method is based on accelerating the reaction of cholesterol oxidase with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent. In the first reaction, non-HDL unesterified cholesterol is subject to a cholesterol oxidase reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a colourless product. The second reagent consists of a detergent capable of solubilising HDL specifically, cholesterol esterase and chromagenic coupler to develop colour for the quantitative determination of HDL-C.

Low-density lipoprotein (LDL) concentration was calculated by CBAL using the Friedmann calculation: LDL = total cholesterol – TAG/2.2 – HDL cholesterol. If a TAG result was more than 4.5 mmol/L then LDL cholesterol was not calculated and no LDL cholesterol result was recorded for data analysis.

Q.3.5.1 Internal QC for total cholesterol

Internal QC material was Liquid Unassayed Multiqual (product code 697) (Bio-Rad). Results are shown in tables Q.7 to Q.10 for total and HDL cholesterol and Years 10 and 11, respectively.

Table Q.7 Internal QC for total cholesterol (NDNS RP Year 10)

Total cholesterol	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	QC3_2
Mean (mmol/L)	3.32	3.52	4.51	5.00	7.18	7.52
SD (mmol/L)	0.08	0.04	0.11	0.06	0.08	0.06
% CV	2.4	1.2	2.5	1.2	1.2	0.83
n	26	8	27	8	24	8

Table Q.8 Internal QC for total cholesterol (NDNS RP Year 11)

Total cholesterol	QC 1		QC 3	
	Nov18 to Mar19	Mar19 to Jul19	Nov18 to Mar19	Mar19 to Jul19
Mean (mmol/L)	2.82	2.93	6.93	7.5
SD (mmol/L)	0.09	0.13	0.10	0.40
% CV	3.3	4.4	1.5	5.4
N	56	56	55	58

Table Q.9 Internal QC for HDL cholesterol (NDNS RP Year 10)

HDL cholesterol	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	QC3_2
Mean (mmol/L)	1.75	1.77	1.72	1.56	3.33	2.78
SD (mmol/L)	0.03	0.02	0.04	0.04	0.05	0.10
% CV	1.9	1.4	2.4	2.5	1.6	3.7
N	26	9	26	9	24	8

Table Q.10 Internal QC for HDL cholesterol (NDNS RP Year 11)

HDL cholesterol	QC 1		QC 3	
	Nov18 to Mar19	Mar19 to Jul19	Nov18 to Mar19	Mar19 to Jul19
Mean (mmol/L)	0.58	0.61	1.47	1.47
SD (mmol/L)	0.03	0.02	0.04	0.07
% CV	5.5	3.8	2.6	4.8
N	31	40	31	40

Q.3.5.2 External QC for total and HDL cholesterol

External QC was achieved through NEQAS and also the Randox International QA Scheme (RIQAS); NEQAS pooled samples are unsuitable for the total cholesterol method used by the Siemens instruments. Table Q.11 indicates the percentage deviation of results from the target concentration for Years 10 and 11 for total cholesterol and table Q.12 for HDL cholesterol. These were calculated at MRC EWL (Year 10) and CBAL (Year 11).

Table Q.11 External RIQAS QC results for total cholesterol (NDNS RP Years 10 and 11)

Total cholesterol	Year 10		Year 11	
	RIQAS Cycle 37	RIQAS Cycle 38	RIQAS Cycle 39	RIQAS Cycle 40
Mean % bias	+2.2	+1.2	-4.3	-3.9
SD of % bias	0.71	0.38	2.64	1.92
n	11	11	6	12

Table Q.12 External NEQAS QC results for HDL cholesterol (NDNS RP Years 10 and 11)

HDL cholesterol	Year 10	Year 11
Mean % bias	+4.2	-8.9
SD of % bias	6.91	10.18
n	27	18

Q.3.6 Serum triglycerides (triacylglycerols)

The triglycerides (triacylglycerols) method is based on an enzymatic procedure in which a combination of enzymes are employed for the measurement of serum or plasma triglycerides. The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides into free glycerol and fatty acids. Glycerol kinase (GK) catalyses the phosphorylation of glycerol by adenosine-5-triphosphate (ATP) to

glycerol-3-phosphate. Glycerol-3-phosphate-oxidase oxidises glycerol-3-phosphate to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2). The catalytic action of peroxidase (POD) forms quinoneimine from H_2O_2, aminoantipyrine and 4-chlorophenol.

The change in absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and is measured using a bichromatic (510 nm, 700 nm) endpoint technique.

Triglycerides were measured in Years 9 and 10 at MRC EWL on a Siemens Dimension Xpand and for Year 11 on a Siemens Dimension EXL200 analyser at CBAL.

Q.3.6.1 Internal QC for serum triglycerides (triacylglycerols)

Internal QC material was Liquid Unassayed Multiqual (product code 697) (Bio-Rad). Data are reported for Years 10 (table Q.13) and 11 (table Q.14).

Table Q.13 Internal QC for triglycerides (NDNS RP Year 10)

Triglycerides	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	QC3_2
Mean (mmol/L)	1.50	1.48	2.48	2.53	3.97	4.12
SD (mmol/L)	0.03	0.02	0.03	0.03	0.03	0.04
% CV	2.0	1.2	1.3	1.1	0.9	0.9
n	26	8	26	8	24	8

Table Q.14 Internal QC for triglycerides (NDNS RP Year 11)

Triglycerides	QC 1		QC 3	
	Nov18 to Mar19	Mar19 to Jul19	Nov18 to Mar19	Mar19 to Jul19
Mean (mmol/L)	1.09	0.99	2.48	2.37
SD (mmol/L)	0.04	0.06	0.05	0.14
% CV	3.6	5.6	1.88	6.0
n	55	70	51	73

Q.3.6.2 External QA for serum triglycerides

External QA was achieved through NEQAS. Table Q.15 indicates the percentage deviation of results obtained from the target concentration.

Table Q.15 NEQAS results for triglycerides (NDNS RP Year 10 and 11)

Triglycerides NEQAS	Year 10	Year 11
Mean % bias	-4.9	+5.3
SD of % bias	3.8	17.9
n	27	18

Q.3.7 Plasma glucose

In Years 9 and 10, glucose was measured at MRC EWL on the Siemens Dimension Xpand. The assay uses hexokinase coupled to glucose-6 phosphate dehydrogenase. Quantitation was by measurement of reduced nicotinamide adenine dinucleotide (NADH) at the endpoint using bichromatic spectrophotometry at 340 nm and 383 nm. Glucose was not measured in Year 11.

Q.3.7.1 Internal QC for plasma glucose

Control serum was obtained commercially containing low, medium and high concentrations of glucose and was included in each run. Results were checked to ensure they fell within the manufacturer's target range. The results in table Q.16 indicate good between-batch consistency for glucose results during Year 10.

Table Q.16 Internal QC for glucose (NDNS RP Year 10)

Glucose	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	QC3_2
Mean (nmol/L)	3.16	2.98	7.49	7.69	20.4	19.8
SD (nmol/L)	0.06	0.04	0.10	0.06	0.20	0.14
% CV	1.8	1.3	1.4	0.7	1.0	0.7
n	28	6	28	8	24	7

Q.3.7.2 External QA for plasma glucose

External QA was through the NEQAS scheme. Table Q.17 shows the percentage bias relative to the ALTM concentration in glucose results from MRC EWL during Year 10.

Table Q.17 External QA (NEQAS) results for glucose (NDNS RP Year 10)

Glucose NEQAS[iv]	Year 10
A	252
B (%) (mean bias)	+6.4
C (%) (SD of bias)	7.3

Q.3.8 Erythrocyte transketolase activation coefficient (ETKAC) for thiamine (vitamin B1) status

This assay is based on that of Vuilleumier *et al*[2] and depends on the coupling of pyridine nucleotide oxidation to glycerol phosphate dehydrogenase (GDH) (NADH linked), which produces glycerol-3-phosphate after the transketolase-catalysed conversion of ribose-5-phosphate. The rate of oxidation of NADH is monitored at 340nm, on the Multiskan FC plate-reader, in which instrument temperature equivalence across the plate can be achieved. Thiamine status is assessed using the activation coefficient, which is the ratio of cofactor-stimulated activity to the basal activity without any added cofactor.

There are no available sources of erythrocytes with known ETKAC; therefore unassayed material was prepared in-house. Erythrocytes from the National Blood Transfusion Service (NBTS) or commercial sources were washed to remove the buffy coat and lysed by threefold dilution with water. This lysate was stored at -80^{o}C in single-use aliquots. The lysate was stored and assayed both neat and further diluted x2 with water. No source of thiamin deficient erythrocytes has been identified with which to prepare a lysate giving high ETKAC; similarly none of the participant's samples had resulted in an ETKAC in the deficient range (greater than 1.25).

QC material "K" and "S" are dilute lysates in which the reaction rates are very low, these are included to assess assay performance in similarly dilute samples.

Procedures for Year 10 were as those reported for Year 9.[1] In Year 11 analysis was carried out by NBL.

Q.3.8.1 QC for ETKAC

Descriptive statistics in table Q.18 for internal QCs indicate good batch-to-batch consistency of ETKAC results during Year 10 and 11. Note that 2 of the 3 QCs were different batches in Year 11 compared with Year 10.

[iv] For a description of EQA scheme scores, see section Q.2.

There are no external QA or QC schemes available for ETKAC.

Table Q.18 Internal QC for ETKAC (NDNS RP Years 10 and 11)

	Year 10			Year 11		
ETKAC	QC C	QC K	QC P	QC C	QC S	QC Q
Mean (ratio)	1.10	1.12	1.09	1.07	1.15	1.18
SD	0.03	0.05	0.04	0.03	0.05	0.05
% CV	2.5	4.4	3.4	3.1	3.9	4.1
n	24	18	24	25	25	25

Q.3.9 Erythrocyte glutathione reductase activation coefficient (EGRAC) for riboflavin (vitamin B2) status

The assay was developed from the original manual technique developed by Glatzle *et al*[3] and later adapted modified to an assay carried out on microplates and read on a Thermo iEMS plate reader. The ratio of flavin adenine dinucleotide (FAD) stimulated to unstimulated activity is the EGRAC and is a measure of riboflavin status. The method is a kinetic test with decreasing absorbance and the preincubation with FAD is carried out for a relatively long period, 30 minutes at 37°C, in order to ensure full reactivation of apo-enzyme. The assay is conducted at a low final concentration of FAD (1.5 µM), which is necessary to eliminate activation coefficients (ratios) <1.0; this can result from enzyme inhibition by FAD, or its breakdown products, which may occur if the final concentration of FAD is too high. The generally accepted threshold for riboflavin adequacy is EGRAC below 1.30.

Procedures for Year 10 were as those reported for Year 9.[1] In Year 11 analysis was carried out by NBL.

Q.3.9.1 QC for EGRAC

There is no QC material available with known EGRAC, therefore washed erythrocytes were prepared in-house, aliquoted for single use and stored at -80°C. In addition to the native samples a saturated control was made by incubation with FAD before aliquoting. These 3 controls were run on each assay plate. There is no external QA scheme available for EGRAC.

Descriptive statistics in table Q.19 for internal QCs indicate good batch-to-batch consistency of EGRAC results during Years 10 and 11.

There are no external QA or QC schemes available for ETKAC.

Table Q.19 Internal QC for EGRAC (NDNS RP Years 10 and 11)

EGRAC	Year 10			Year 11		
	QC A	QC C	QC X	QC A	QC C	QC X
Mean (ratio)	2.20	1.54	0.98	2.18	1.51	0.98
SD	0.07	0.03	0.01	0.13	0.06	0.03
% CV	3.1	1.6	1.5	6.1	4.2	3.0
n	24	23	23	28	28	29

Q.3.10 Plasma vitamin B6 (PLP and PA)

The assay for vitamin B6 is based on that published by Rybak & Pfeiffer (2004)[4] uses a metaphosphoric acid protein precipitation followed by filtration of the supernatant to prepare plasma samples for analysis. Detection of pyridoxal-5-phosphate (PLP) and 4-pyridoxic acid (4PA) is accomplished by HPLC with fluorescence detection employing a post-column derivatisation step with sodium chlorite. The analyte peak areas are compared to that of a standard curve to determine concentration.

Analytes are resolved using reversed phase HPLC on a BDS hypersil, C_{18} 3 x 150 mm 5 μm column at 35°C with a gradient mobile phase combining 50 mM sodium dihydrogen phosphate buffer at pH 2.7 plus 0.2% acetonitrile and methanol at 0.7 mL/min.

Procedures for Year 10 were as those reported for Year 9.[1] In Year 11 analysis was carried out by NBL. Several minor changes were made by NBL to improve the efficiency of performing the assay assay for Year 11: (1) extending the standard curve at both the low and high ends to reduce need for repeats (at high end) and extend range of assay (at lower end) (2) diluting the standards with metaphosphoric acid to treat the standards the same as the samples and (3) no adjustment of sample results for spike recovery if the spiked QC gave a recovery of <95 or >105%.

Q.3.10.1 Internal QC for vitamin B6

In Years 10 and 11 three in-house plasma controls containing endogenous vitamin B6 across the assay range for both PLP and 4PA and a spiked plasma sample with a 50 nmol/L nominal concentration were analysed at the beginning and at the end of each batch of samples for assessment of assay precision.

Years 10 and 11 QC data are summarised for PLP and 4PA in tables Q.20 and Q.21.

Table Q.20 Internal QC for PLP (NDNS RP Years 10 and 11)

PLP	Year 10			Year 11			
QC	A02	A07	Spike QC	A02	A07	PA Old QC	Spike QC
Mean (nmol/L)	51.4	26.4	54.2	47.4	24.8	11.6	52.0
SD (nmol/L)	2.6	1.3	3.8	2.3	2.3	1.4	3.7
% CV	5.0	5.1	6.9	4.9	9.2	12.3	7.1
n	27	27	28	24	26	26	26

Table Q.21 Internal QC for 4PA (NDNS RP Years 10 and 11)

4PA	Year 10			Year 11			
QC	A02	PA Old QC	Spike QC	A02	A07	PA Old QC	Spike QC
Mean (nmol/L)	128.5	37.7	52.9	123.7	13.7	37.6	53.0
SD (nmol/L)	7.5	1.8	2.4	5.2	1.6	1.0	1.9
% CV	5.8	4.9	4.5	4.2	11.7	2.7	3.6
n	27	28	28	24	26	26	25

Q.3.10.2 Accuracy for vitamin B6

Standard Reference Material (SRM) 1950 from the National Institute of Standards and Technology (NIST, https://www.nist.gov/srm) was assayed with every run during Year 11 to assess the accuracy of the assay (table Q.22).

Table Q.22 Accuracy for PLP and PA (NDNS RP Year 11)

Vitamin B6 Standard Reference Material	PLP	4PA
Mean (nmol/L)	33.9	27.7
SD (nmol/L)	1.4	0.7
% CV	4.3	2.6
n	13	13
Target Values (nmol/L)	33.17 +/- 1.86	28.7

Q.3.10.3 External QC for PLP

The NBL and the MRC EWL participated in the Royal College of Pathologists of Australasia QA Program (RCPAQAP) external quality assurance scheme during NDNS RP Years 10 and 11. There are only 6 participants in the scheme, the

majority of whom use the Chromsystems complete HPLC kit (Chromsystems, Germany). Due to transition and timing of NBL set up, all Year 11 vitamin B6 samples were run within 6 weeks spanning only 2 distributions of the EQA samples. There is no external QA scheme for 4PA. Table Q.23 provides a summary of EQA data for Years 10 and 11.

Table Q.23 Vitamin B6 RCPQAP summary (NDNS RP Years 10 and 11)

Vitamin B6 (PLP) RCPAQAP	Year 10	Year 11
	Cycles 38/39	Cycle 41
Mean bias (%)[1]	-0.9	-8.6
SD of bias	6.9	9.4
Mean Z-score[2]	n/a	-1.1
n	10	4

[1] Z score = (lab result - all results mean)/all results SD. Z scores were introduced in 2019 so are not available for Year 10
[2] Calculated in-house

Q.3.11 Serum folate

Procedures for Year 10 were as those reported for Year 9.[1] Year 11 analysis was carried out by NBL. The instrument and method for analysis of serum folate remains unchanged with the move from MRC EWL to the NBL.

The assay for serum folates uses solid phase extraction with phenyl columns to isolate the folate forms in serum samples. Analyte matched stable isotope labelled internal standards are added during the extraction step and undergo processing identical to the analytes thereby normalizing for sample preparation and instrument variability. Highly specific detection of the six vitamers; methyltetrahydrofolate (MTHF), tetrahydrofolate (THF), formyltetrahydrofolate (FTHF), folic acid, 5,10 methenyltetrahydrofolate (CH+THF) and pyrazino-s-triazine derivative of 4α-hydroxy-5-methyl tetrahydrofolate (MeFox) and internal standards is accomplished by UPLC-MS/MS analysis and the ratio of analyte to internal standard signal is compared to that of a calibration curve to determine analyte concentration.

Analytes are separated using reversed phase UPLC on a Waters ACQUITY UPLC® HSS T3 C8 2.1 x 100 mm 1.7 µm column at 30°C with a 49.5:40:10:0.5 water:methanol:acetonitrile:acetic acid isocratic mobile phase prior to mass spectrometry analysis. The retention times for all the analytes are very similar and the internal standards are identical to their corresponding analytes but due to their differing masses, there is clear distinction between them in the assay. FTHF and MeFox have the same molecular weights and cannot be chromatographically

separated so transitions unique to each form have to be used. In NDND, serum folate is equal to total folate that is calculated from the sum of the 6 folate vitamers.

Q.3.11.1 Internal QC for serum folate

Three in-house plasma controls containing endogenous folates across the assay range for both MTHF, folic acid and MeFox and a spiked serum sample with a 5 nmol/L nominal concentration for FTHF, THF and CH+THF with endogenous levels of MTHF, folic acid and MeFox were analysed at the beginning and at the end of each batch of samples for assessment of assay precision. The lot numbers of the QCs changed between Year 10 and Year 11 due to the pools for each QC running out. Tables Q.24 to Q.27 provide a summary of QC data for Years 10 and 11.

Table Q.24 Internal QC for MTHF (NDNS RP Years 10 and 11)

MTHF	Year 10			Year 11		
	QC 1 210715	QC 3 210715	Spike QC 220915	QC 1 100118	QC 3 100118	Spike QC 170418
Mean (nmol/L)	24.1	15.5	65.7	27.9	16.7	62.4
SD (nmol/L)	1.8	0.4	3.1	1.1	0.7	2.4
% CV	7.4	2.8	4.8	3.9	4.0	3.8
n	18	18	18	12	12	12

Table Q.25 Internal QC for folic acid (NDNS RP Years 10 and 11)

Folic acid	Year 10			Year 11		
	QC 1 210715	QC 3 210715	Spike QC 220915	QC 1 100118	QC 3 100118	Spike QC 170418
Mean (nmol/L)	9.1	1.1	6.7	7.6	0.5	5.1
SD (nmol/L)	0.9	0.1	0.5	0.3	0.1	0.2
% CV	9.4	6.2	7.1	4.2	13.7	4.2
n	18	18	18	12	12	12

Table Q.26 Internal QC for MeFox (NDNS RP Years 10 and 11)

MeFox	Year 10			Year 11		
	QC 1 210715	QC 3 210715	Spike QC 220915	QC 1 100118	QC 3 100118	Spike QC 170418
Mean (nmol/L)	2.1	2.5	6.1	5.9	1.1	7.9
SD (nmol/L)	0.1	0.2	0.3	0.3	0.1	0.2
% CV	6.2	6.5	4.7	4.6	6.6	2.6
n	18	18	18	12	12	12

Table Q.27 Internal QC for other folates (NDNS RP Years 10 and 11)

Other folates	Year 10 Spike QC 220915			Year 11 Spike QC 170418		
	CH+THF	THF	FTHF	CH+THF	THF	FTHF
Mean (nmol/L)	5.0	5.1	5.6	5.5	5.5	5.5
SD (nmol/L)	0.6	0.5	0.4	0.2	0.3	0.3
% CV	11.2	10.2	7.3	3.1	5.9	5.5
n	18	18	18	12	12	12

Q.3.11.2 Accuracy of serum folate

NIST SRM 1950 was assayed with every run to assess and monitor the accuracy of the assay for MTHF and folic acid. Results are presented in table Q.28.

Table Q.28 Accuracy assessment (SRM 1950) (NDNS RP Years 10 and 11)

Serum folate SRM 1950	MTHF		Folic Acid	
	Year 10	Year 11	Year 10	Year 11
Mean (nmol/L)	28.4	28.3	3.2	3.1
SD (nmol/L)	2.0	0.4	0.2	0.1
% CV	7.1	1.3	4.9	1.8
n	9	6	9	6
SRM target values	26.91 +/- 0.7		3.42 +/- 1.02	

Q.3.11.3 External QA of serum folate

The NBL and MRC EWL participated in NEQAS and CDC run VITAL-EQA quality assurance schemes during NDNS RP Years 10 and 11.

Table Q.29 shows the Years 10 and 11 NEQAS scores for the 6 months previous to the last NDNS RP run for each year, 3 samples are run each month. There are no cumulative scores for Year 11 NEQAS because the assay had not been running in the laboratory for sufficient time to calculate the scores. All NDNS RP Year 11 samples were analysed over a 1-month period, however the B and C scores have been calculated in-house for the single distribution of 3 samples run during this period.

Table Q.29 Serum folate NEQAS (NDNS RP Years 10 and 11)

Total folate NEQAS score[v]	Year 10	Year 11
A	41	Not available
B (%)	-7.5	0.8
C (%)	6.3	1.9

VITAL-EQA samples are sent twice a year and total folate target concentrations are assigned using the 'gold standard' microbiological method run by CDC. The samples are analysed in duplicate on two separate assays, if possible. Round 30 was analysed alongside NDNS RP Year 10 and round 33 was analysed alongside NDNS RP Year 11 (table Q.30).

Table Q.30 Serum folate VITAL EQA (NDNS RP Years 10 and 11)

VITAL EQA Total folate	Year 10 (round 30)			Year 11 (round 33)		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	6.36	9.70	12.5	7.38	12.4	16.6
Imprecision (%)	4.6	3.1	2.8	5.9	2.9	6.2
Bias (%)	5.7	-1.0	4.7	-3.9	2.7	-8.1
Target value (ng/mL)	6.02	9.79	12.0	7.69	12.1	18.1

Q.3.12 Whole blood (WB) folate – analytical method[vi]

Whole blood haemolysate specimens (WB diluted and stabilised with ascorbic acid, 1/11) were analysed for total folate at the Centers for Disease Control and Prevention (CDC, Atlanta, USA) in Years 9 - 11 using the *Lactobacillus rhamnosus* microbiologic growth assay by an adaptation of O'Broin *et al.*[5] and Molloy *et al.*,[6] as described by Pfeiffer *et al.*[7]

Aliquots of whole blood (WB) samples were preserved with 1% ascorbic acid (appendix P) and frozen at -80°C before sending on dry ice to CDC.

Diluted specimen (4 replicates at 2 dilutions) was added to an assay medium containing the microorganism and all of the nutrients, except for folate, necessary for the growth of the microorganism. Since the growth of *L. rhamnosus* is proportional to

[v] For a description of EQA scheme scores, see section Q.2
[vi] QC information provided by CDC.

the amount of total folate present in the specimen, the total folate level was assessed by measuring the turbidity of the inoculated medium at 590 nm in a microplate reader. The assay was calibrated with 5-methyl-tetrahydrofolate (5-methylTHF), using an 11-point calibration curve (0–1.0 nmol/L; 8 replicates/point) with a 3rd degree polynomial curve fit.

Sample dilutions with a concentration below the lowest calibrator or above the highest calibrator were repeated for confirmation, at lower or higher dilution. The standard dilution used for WB haemolysate specimens in this study was 1/70.

Results from 4 replicates at 2 different dilutions were averaged to generate the final result and the CV from the 4 replicates had to be ≤15% (≤10% if only 3 replicates were used). No result was reported from less than 3 replicates. Assays were repeated where necessary.

Samples with a WB folate concentration <127 nmol/L (corresponding to a RBC folate concentration of <305 nmol/L RBC if a haematocrit of 0.4 L/L is assumed) were considered to represent potential folate deficiency and assays were repeated for confirmation. This is the standard practice in the CDC laboratory.

Q.3.12.1 Internal QC for WB folate

QC limits for whole blood folate were established by duplicate analysis of 3 in-house quality control pools. The 3 whole blood bench QC pools were analysed in duplicate in each run (*n* = 15 in Year 10 and n = 10 in Year 11). The between-run imprecision for WB folate and the target concentration are shown in table Q.31.

Table Q.31 Internal QC for WB total folate analysis (NDNS RP Years 10 and 11)

WB folate	LB14810_MA		MB14811_MA		HB14812_MA	
	Y10	Y11	Y10	Y11	Y10	Y11
Mean (nmol/L)	250	244	439	434	744	712
SD (nmol/L)	27	16	33	23	34	33.6
% CV	22%	6.5%	7%	5.3%	5%	4.7%
Target (nmol/L)	232	254	429	442	722	722
Difference from target	8.1%	-3.8%	2.7%	-1.9%	3.0%	-4.5%

Four additional WB QC pools were analysed “blind” (i.e. target concentration unknown to analyst) as part of this study at a rate of 1 blind QC sample in every 20 unknown samples. The between-run imprecision and target concentration are shown in table Q.32.

Table Q.32 WB total folate blind QC pool analysis (NDNS RP Years 10 and 11)

WB folate	936		937		938		939	
Parameter	Y10	Y11	Y10	Y11	Y10	Y11	Y10	Y11
Mean (nmol/L)	516	488	248	262	395	390	188	186
SD (nmol/L)	32	55	11	16	18	24	12	10
% CV	6.2%	11.3%	4.3%	5.9%	4.5%	6.1%	6.4%	5.6%
n	12	7	3	7	14	10	11	6
Target (nmol/L)	527	527	256	256	354	354	187	187
Difference from target	2.0%	-7.3%	-3.0%	2.3%	11.3%	10.1%	0.8%	-0.2%

Accuracy has been established by spiking recovery, by periodic assaying of the 1st International Standard for Whole Blood Folate 95/528, and by successful participation in UK NEQAS Haematinics programme (https://birminghamquality.org.uk/eqa-programmes/hic/).

Q.3.12.2 Calculation of red blood cell (RBC) folate

Red Blood Cell (RBC) folate is calculated from WB folate concentration, serum folate concentration and haematocrit (quantitated as part of the full blood count) using the equation:

RBC folate = (WB folate - (serum folate x (1-haematocrit))) / haematocrit

Where a serum folate concentration was not available a surrogate of 18 nmol/L was used in the calculation. Where haematocrit was not available, a surrogate of 0.4L/L was used.

For the calculation of RBC folate, if the WB folate concentration was less than the limit of detection (44 nmol/L) then a value of 31 nmol/L was assigned to that sample for the calculation of RBC folate. Where there was no result for WB folate then the calculation of RBC was not performed.

Q.3.12.3 Note regarding the effect of postal delays on WB folate

Blood samples for WB folate analysis are initially posted by first class post to the MRC Epidemiology Biorepository. WB folate values are used to calculate RBC folate used in the NDNS RP reports.

During Years 9 to 11 of the NDNS RP, the average time samples have spent in the post has lengthened. Specifically, the percentage of samples taking longer than 2 days to arrive at MRC Epidemiology Biorespository was 9% in Year 9, 19% in Year 10 and 30% in Year 11. Delays in the post may affect both RBC folate concentration directly by decreasing WB folate and indirectly via an effect on haematocrit. Examination of cross-sectional data from NDNS suggests an approximately 5% decrease in RBC folate concentration per additional day in the post. This is consistent with data from the literature; a recent review[8] suggested that "storage of unprocessed whole blood at elevated temperature is unacceptable" with 7%-20% loss after 1-2 days and 20%-25% loss after 3-4 days at 22°C.[9,10]

In Year 10, samples spending 7 or more days in the post were not used to calculate RBC folate. [vii] With the lengthening of the postal times, following discussion with the NDNS RP Scientific Reference Group and the PHE NDNS Project Board, the decision was taken in Year 11 to take a more conservative approach as to which samples to include in the final calculation of RBC folate to include in the data for the Year 9 – 11 report. For Year 11, results from blood samples spending 3 days or more in the post were not included in the calculation of RBC folate for the report but will be included in the data deposited at the UK Data Service.

Q.3.13 Serum vitamin B_{12}

The ADVIA Centaur B_{12} assay is a competitive immunoassay using direct chemiluminescence. Vitamin B_{12} from a participant's sample competes with vitamin B_{12} labelled with acridinium ester for a limited amount of labelled intrinsic factor. The intrinsic factor is covalently bound to paramagnetic particles. The assay uses a releasing agent (sodium hydroxide) and dithiothreitol (DTT) to release the B_{12} from the endogenous binding proteins in the sample.

Q.3.13.1 Internal QC for vitamin B_{12}

The QC results of the instrument measuring Vitamin B_{12} at Addenbrooke's are monitored continually, and analysis is stopped if the results are not satisfactory, ensuring that results are only reported if the analysis is within the QC parameters set by the Laboratory Manager. However, it is not possible to extract these results for NDNS RP reporting. Therefore in order to monitor between-batch precision, control material was aliquoted at MRC EWL (Years 9 and 10) and MRC Epidemiology Biorepository (Year 11) and included in the monthly delivery of NDNS RP samples to Addenbrooke's. Control material was purchased from Bio-Rad and was chosen to reflect the full range of vitamin B_{12} concentrations. Different batches of control material (indicated by underscore) are used across NDNS RP years as batches expire or run out which allows monitoring of continuity between NDNS RP years. QC data are summarised in table Q.33; QC material with underscore "_1" is the same batch as used for Year 9 and thus is directly comparable to Year 9 QC data.

Table Q.33 Internal QC for vitamin B_{12} (NDNS RP Years 10 and 11)

	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	QC3_2	QC4_1	QC4_2	QC4_3	QC5_1	QC5_2	QC5_3
Mean (ng/L)	121	131	197	157	127	116	583	532	581	815	749	1005
SD (ng/L)	13.8	15.9	15.7	20.2	15.7	16.8	33.1	29.2	31.7	57.4	25.6	52.6
% cv	11	12	8	13	12	15	6	5	5	6	3	5
n (Year 10) n (Year 11)	102 -	- 15	102 -	- 15	3 -	- 17	6 -	64 -	- 15	6 -	64 -	- 15

QC1: Lyphochek Anemia Control, single level
QC2: Liquid Assayed Multiqual, Level 2
QC3: Lyphochek Assayed Chemistry Control, Level 2
QC4: Lyphochek Immunoassay Plus Control, Level 2
QC5: Lyphochek Immunoassay Plus Control, Level 3

Q.3.13.2 External QC for vitamin B_{12}

External QC was achieved through the UK NEQAS Haematinics scheme (table Q.34).

Table Q.34 Vitamin B12 NEQAS (NDNS RP Years 10 and 11)

Vitamin B12 NEQAS score[viii]	Year 10	Year 11
A	62	63
B (%)	+4.3	+5.7
C (%)	6.8	13.5

Q.3.14 Holotranscobalamin (holoTC); "active B_{12}"

Vitamin B_{12} (cobalamin) is transported in the circulation bound to transcobalamin (TC) (10-30%) and to haptocorrin (HC) (70-90%). When TC and HC bind vitamin B_{12} the resulting complexes are known as holotranscobalamin (holoTC) and holohaptocorrin (holoHC) to distinguish them from the proteins carrying no vitamin.

HoloTC is the only form of vitamin B_{12} that can be taken up by cells in the body; holoHC is biologically inert. The TC protein alone transports vitamin B_{12} from its site of absorption in the ileum to tissues and cells where it is used as a co-enzyme for essential cellular functions such as DNA synthesis and may be a better marker of metabolic function than serum vitamin B_{12}.[11]

The holoTC assay is an enzyme-linked immunosorbent assay (ELISA) manufactured by Axis Shield. It is conducted in 96-well microplates. HoloTC reacts with a specific antibody immobilised on the plate surface; a second, labelled antibody then reacts to

[viii] For a description of EQA scheme scores, see section Q.2

form a “sandwich”. The enzyme label is quantitated using a coloured substrate and the absorbance read in a microplate spectrophotometer. Concentration is interpolated from a calibration curve. The assays were conducted manually, in Years 9 and 10 at MRC EWL and in Year 11 at NBL.

Q.3.14.1 Internal QC for holoTC

QC samples were supplied by the manufacturer and included in every assay. Tables Q.35 and Q.36 show QC data for the period covering the analysis of Year 10 and Year 11 samples, respectively. In Year 11, 3 levels of pooled serum QC replaced the single QA material used in Year 10 and was run at the beginning of each plate only to allow comparison of performance between reagent lots.

Table Q.35 Internal QC for holoTC (NDNS RP Year 10)

	Lot no. 802910928		Lot no. 902917801		QA (drift control)
HoloTC	QC1	QC2	QC1	QC2	
Mean (pmol/L)	27.0	64.3	21.3	52.5	77.1
SD (pmol/L)	1.5	4.4	1.3	2.2	8.96
% cv	5.5	6.8	6.1	4.2	11.62
n	15	19	21	21	9

Table Q.36 Internal QC for holoTC (NDNS RP Year 11)

	Lot no. 902917801		Pooled serum		
HoloTC	QC1	QC2	Level 1	Level 2	Level 3
Mean (pmol/L)	24.7	65.0	41.4	64.9	92.8
SD (pmol/L)	1.5	2.6	1.0	1.8	2.1
% CV	6.2	4.0	2.4	2.8	2.3
n	27	28	14	14	14

Q.3.14.2 External QA for holoTC

In each survey year, analysis of samples for holoTC was performed as batches over a short time scale and therefore did not allow for “live” participation in the EQA scheme. NEQAS samples received during Years 10 and 11 were stored at -70˚C and assayed retrospectively alongside respective samples for each year, the results being compared with the NEQAS ALTM for results obtained by all participating laboratories (table Q.37), most of whom used automated methods. MRC EWL and NBL data aligned with other ELISA users.

Table Q.37 External QA for holoTC (NDNS RP Years 10 and 11)

HoloTC NEQAS score[ix]	Year 10	Year 11
A	Not available	Not available
B (%)	-12.2	26.3
C (%)	7.8	27.0

Q.3.15 Vitamin C (ascorbic acid)

This assay is based on the procedure described by Vuilleumier & Keck (1989).[12] Samples are stabilised immediately after separation using an equal volume of 10% metaphosphoric acid (appendix P).

Vitamin C (acscorbic acid) in the sample is converted to dehydroascorbic acid by ascorbate oxidase, followed by coupling of the resulting dehydroascorbate with o-phenylene diamine to form a fluorescent derivative quinoxaline. The formation of quinoxaline is linearly related to the amount of vitamin C in the sample. The assay was performed on the BMG Labtech FLUOstar Optima and Omega plate readers at MRC EWL and NBL, respectively.

Procedures for Year 10 were as those reported for Year 9.[1] In Year 11 analysis was carried out by NBL.

Q.3.15.1 Internal QC for vitamin C

QC samples were made in-house by spiking vitamin C-depleted plasma. The results in table Q.38 indicate good between-batch consistency for vitamin C measurements during Years 10 and 11.

Table Q.38 Internal QC for vitamin C (NDNS RP Years 10 and 11)

	Year 10			Year 11		
	QC1	QC2	QC3	QC1	QC2	QC3
Mean (µmol/L)	10.6	34.4	53.0	12.1	34.2	53.1
SD (µmol/L)	1.6	5.3	3.7	1.1	1.4	2.0
% CV	15	15	7	9	4	4
n	19	19	19	41	41	41

[ix] For a description of EQA scheme scores, see section Q.2

Q.3.15.2 External QC for vitamin C

Bias and imprecision are assessed by participation in the Royal College of Pathologists of Australasia QA Program (RCPAQAP). The bias is calculated from the ALTM. The majority of the participating laboratories use the Chromsystems HPLC method and calibrator, which are different from the fluorescence method and calibrator used in the NDNS RP; results from MRC EWL and NBL show a bias relative to the Chromsystems method (table Q.39). The MRC EWL calibrators, which are still in use at NBL for continuity, are made up gravimetrically from the AnalaR ascorbic acid, the purest grade available. This methodological difference shows as a deviation of our results from the Chromsystems consensus. The consistency of the bias indicates consistency in MRC EWL / NBL results, as does the low "% imprecision" result.

Table Q.39 External QC for vitamin C (NDNS RP Years 10 and 11)

Vitamin C	Year 10		Year 11
RCPAQAP	Cycle 38	cycle 39	Cycle 40
Mean imprecision (%)	3.4	2.6	6.8
%CV of imprecision	4.9	4.0	10.5
Mean Bias (%)	17.5	22.2	19.8

Q.3.16 Retinol, α– and γ–tocopherol, and individual carotenoids (fat-soluble vitamins (FSVs)

Procedures for Year 10 were as those reported for Year 9.[1] In Year 11 analysis was carried out by NBL. The fat-soluble vitamin (FSV) method remains unchanged with the move from MRC EWL to NBL.

The assay for FSVs is based on that published by Sowell *et al*. (1994)[13] and uses a methanol protein precipitation step followed by liquid-liquid extraction in hexane to separate fat soluble vitamins from other analytes in plasma samples. Internal standards are added during the extraction step and undergo processing identical to the analytes thereby normalizing for sample preparation and instrument variability. Detection of the 9 analytes; retinol, α-tocopherol, γ-tocopherol, lutein and zeaxanthin (combined due to co-elution), lycopene, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene and internal standards; tocopherol acetate and apo-8'-carotenal is accomplished by HPLC analysis with PDA (UV, photo diode array) detection. The ratio of analyte to internal standard signal is compared to that of a calibration curve to determine analyte concentration.

Analytes are resolved using reversed phase HPLC on a YMC-pack pro C_{18} 4.6 x 150 mm 3 µm column at 40°C with a 25:75 ethanol + 0.1% triethylamine: acetonitrile + 0.1% triethylamine isocratic mobile phase at 1.2 mL/min.

Q.3.16.1 Internal QC for plasma retinol, α– and γ–tocopherol and individual carotenoids

The FSV results for Year 10 and 11 were reported as plasma retinol, α– and γ–tocopherol and individual carotenoids (lutein and zeaxanthin co-elute and therefore are measured as a sum). Internal controls were selected containing physiologically relevant concentrations of each analyte; these were aliquoted for use in each analytical run. Between-batch precision was calculated from these values, as for all other analytes measured in the NDNS RP. Tables Q.40 to Q.48 provide a summary of QC data for Years 10 and 11.

Table Q.40 Internal QC for retinol (NDNS RP Years 10 and 11)

Retinol	Year 10		Year 11	
	NIST 257	QC 7	NIST 257	QC 7
Mean (µmol/L)	1.73	2.34	2.00	2.67
SD (µmol/L)	0.10	0.10	0.07	0.22
% CV	5.6	4.1	3.7	8.2
n	30	30	18	18

Table Q.41 Internal QC for α-tocopherol (NDNS RP Years 10 and 11)

α Tocopherol	Year 10		Year 11	
	NIST 170	QC 7	NIST 170	QC 7
Mean (µmol/L)	13.95	34.7	14.97	37.21
SD (µmol/L)	0.62	1.26	0.47	1.71
% CV	4.5	3.6	3.2	4.6
n	30	30	17	18

Table Q.42 Internal QC for γ-tocopherol (NDNS RP Years 10 and 11)

γ Tocopherol	Year 10	Year 11	
	QC 7	NIST 170	QC 7
Mean (µmol/L)	1.73	4.95	1.83
SD (µmol/L)	0.12	0.21	0.19
% CV	6.7	4.3	10.6
n	30	17	18

Table Q.43 Internal QC for lutein and zeaxanthin (co-eluting compounds) (NDNS RP Years 10 and 11)

Lutein and Zeaxanthin	Year 10			Year 11		
	NIST 170	NIST 257	QC 7	NIST 170	NIST 257	QC 7
Mean (µmol/L)	0.27	0.12	0.40	0.23	0.10	0.35

SD (µmol/L)	0.03	0.01	0.02	0.01	0.01	0.03
% CV	10.3	11.1	5.7	4.7	8.5	8.3
n	30	30	30	17	18	18

Table Q.44 Internal QC for α-cryptoxanthin (NDNS RP Years 10 and 11)

α cryptoxanthin	Year 10		Year 11		
	NIST 265	QC 7	NIST 265	QC 7	QC 1
Mean (µmol/L)	0.08	0.06	0.06	0.05	0.06
SD (µmol/L)	0.01	0.01	0.003	0.003	0.003
% CV	13.4	16.0	5.9	7.7	5.2
n	30	30	6	18	10

Table Q.45 Internal QC for β-cryptoxanthin (NDNS RP Years 10 and 11)

β Cryptoxanthin	Year 10	Year 11	
	QC 7	QC 7	High QC
Mean (µmol/L)	0.13	0.07	0.17
SD (µmol/L)	0.01	0.01	0.01
% CV	5.7	10.2	8.3
n	30	17	18

Table Q.46 Internal QC for lycopene (NDNS RP Years 10 and 11)

Lycopene	Year 10			Year 11			
	NIST 170	NIST 265	QC 7	NIST 170	NIST 265	QC 7	QC 1
Mean (µmol/L)	0.48	0.71	1.88	0.45	0.56	1.93	0.54
SD (µmol/L)	0.04	0.06	0.15	0.04	0.03	0.12	0.03
% CV	7.5	9.1	8.0	8.6	5.0	6.5	5.9
n	30	30	30	17	6	18	10

Table Q.47 Internal QC for α-carotene (NDNS RP Years 10 and 11)

α carotene	Year 10		Year 11		
	NIST 265	QC 7	NIST 265	QC 7	QC 1
Mean (µmol/L)	0.17	0.11	0.16	0.10	0.13
SD (µmol/L)	0.01	0.01	0.01	0.01	0.01
% CV	6.9	7.4	5.3	13.8	10.4
n	30	30	6	18	10

Table Q.48 Internal QC for β-carotene (NDNS RP Years 10 and 11)

β carotene	Year 10		Year 11		
	NIST 170	NIST 265	NIST 170	NIST 265	QC 1
Mean (µmol/L)	1.12	0.58	1.13	0.54	0.77
SD (µmol/L)	0.06	0.04	0.04	0.02	0.04
% CV	5.6	7.0	3.2	4.1	4.5
n	30	30	17	6	10

Q.3.16.2 Accuracy for plasma retinol, α– and γ–tocopherol and individual carotenoids

NIST SRM 1950 was assayed with every run during Year 11 to assess the accuracy of the assay. Results are presented in table Q.49.

Table Q.49 Accuracy for FSVs assessed with NIST SRM 1950

NIST SRM 1950	Retinol	α-tocopherol	γ-tocopherol	Lutein and zeaxanthin	Lycopene	β-carotene
Mean (µmol/L)	1.43	19.71	4.40	0.13	0.71	0.15
SD (µmol/L)	0.08	0.88	0.30	0.01	0.05	0.01
% CV	5.4	4.5	6.8	10.8	7.6	5.1
n	7	7	7	8	8	8
NIST Targets (µmol/L)	1.41 +/- 0.122	19.0 +/- 0.511	4.1 +/- 0.408	0.16 +/- 0.049	0.615 +/- 0.037	0.15 +/- 0.07

Q.3.16.3 External QC for plasma retinol, α– and γ–tocopherol and individual carotenoids

NBL and MRC EWL participated in NEQAS quality assurance scheme during NDNS RP Years 9 to 11. Table Q.50 shows the Year 10 and 11 NEQAS scores for the 6 months previous to the final NDNS RP run for each year, 3 NEQAS samples were run each month. It should be noted that there were 14 laboratories returning results for β-carotene and only 4 laboratories returning results for lutein + zeaxanthin and lycopene. NBL and MRC EWL also returned results for α-carotene and α-cryptoxanthin when possible but these are commonly below the limit of detection so there are no cumulative scores available for these assays.

Table Q.50 External QA (NEQAS) for FSVs (NDNS RP Years 10 and 11)

NEQAS	Retinol		α-tocopherol		Lutein and zeaxanthin		Lycopene		β-carotene	
Score[x]	Y10	Y11	Y10	Y11	Y10	Y11	Y10	Y11	Y10	Y11
A	98	113	48	41	54	83	22	20	98	82
B (%)	-5.9	8.4	0.1	1.8	-10	-15.2	5.6	3.7	16.6	13.3
C (%)	7.1	7.8	4.7	3.7	7.5	12.3	11.2	10.5	9.1	10.9

Q.3.17 25-hydroxyvitamin D (25OHD)

Procedures for Year 10 were as those reported for Year 9.[1] Year 11 analysis was carried out by NBL. The method and instrument for analysis of serum 25-hydroxyvitamin D (25OHD) remain unchanged with the move from the MRC EWL to the NBL.

The assay for serum 25OHD uses a methanol protein precipitation step followed by liquid-liquid extraction in hexane to separate 25OHD from other analytes in serum samples. Stable isotope labelled internal standards are added during the extraction step and undergo processing identical to the analytes thereby normalizing for sample preparation and instrument variability. Highly specific detection of the analytes and internal standards is accomplished by UPLC-MS/MS analysis using a Waters ACQUITY UPLC system coupled to an AB Sciex QTrap mass spectrometer and the ratio of analyte to internal standard signal is compared to that of a calibration curve to determine analyte concentration.

Analytes are resolved using reversed phase UPLC on a Thermo Scientific Hypersil GOLD PFP 2.1 x 100 mm 1.9 µm column at 40°C with a 72% methanol +0.1% formic acid isocratic mobile phase at 0.25 mL/min prior to mass spectrometry analysis.

Total 25OHD is calculated from the sum of $25OHD_2$ and $25OHD_3$. The epimer of 25 hydroxyvitamin D_3 is not reported in NDNS but is chromatographically separated using this method and so is not included in the total 25OHD.

Q.3.17.1 Internal QC for 25OHD

In Year 10 Chromsystems MassCheck® 3-epi-25OH-vitamin D3/D2 and 25OH-vitamin D3/D2 serum controls and an in-house serum control were used to monitor assay precision. In Year 11, the QC material was changed to Bio-Rad Lyphochek Specialist Immunoassay Control material. The concentrations of $25OHD_2$ and $25OHD_3$ controls covered the concentration range observed in NDNS; QC material was analysed at the beginning and at the end of each batch of samples. Tables Q.51

[x] For a description of EQA scheme scores, see section Q.2.

and Q.52 provide a summary of QC data for Years 10 and 11 for $25OHD_2$ and $25OHD_3$.

Table Q.51 Internal QC for $25OHD_2$ (NDNS RP Years 10 and 11)

$25OHD_2$	Year 10					Year 11		
	Chromsystems QC				In-house QC	Bio-Rad QC		
Lot	0716 L1	0716 L2	4517 L1	4517 L2	151014	25251	25252	25283
Mean (nmol/L)	38.2	85.2	41.8	98.9	12.8	12.9	22.0	57.7
SD (nmol/L)	1.4	5.0	2.0	7.7	1.1	1.3	1.4	3.7
% CV	3.8	5.8	4.9	7.8	9.0	10.1	6.2	6.4
n	19	19	4	4	23	17	17	17

Table Q.52 Internal QC for $25OHD_3$ (NDNS RP Years 10 and 11)

$25OHD_3$	Year 10					Year 11		
	Chromsystems QC				In-house QC	Bio-Rad QC		
Lot	0716 L1	0716 L2	4517 L1	4517 L2	151014	25251	25252	25283
Mean (nmol/L)	40.2	90.8	43.1	108.5	19.1	15.8	29.0	79.8
SD (nmol/L)	2.1	3.0	2.5	1.7	1.1	1.7	1.8	5.8
% CV	5.1	3.3	5.7	1.6	5.6	10.4	6.4	7.3
n	19	19	4	4	23	17	17	17

Q.3.17.2 Accuracy for 25-OHD

NIST SRM 972a was assayed during the set-up of the assay at NBL and samples from the Vitamin D External Quality Assessment Scheme (DEQAS, http://deqas.org/) are analysed every quarter, which have concentrations assigned by the reference measurement procedure at CDC. The bias for the samples run during Years 10 and 11 are shown in table Q.53.

Table Q.53 % bias for DEQAS samples analysed during NDNS RP Years 10 and 11

25OHD (nmol/L) DEQAS		
	Year 10	Year 11
Mean bias (%)	-0.76	-1.80
SD of % bias	6.3	4.5
n	15	14

Q.3.17.3 External QA for 25OHD

The NBL and MRC EWL participated in DEQAS and NEQAS QA schemes during NDNS RP Years 10 and 11.

Table Q.54 shows the NEQAS scores for the 6 months previous to the final NDNS RP run for each year, 3 samples were run each month. NEQAS performance is assessed against the mass spectrometry mean. The DEQAS scheme is accuracy-based and bias data are summarised in Table Q.51.

Table Q.54 NEQAS scores for 25OHD during NDNS RP Years 10 and 11

25OHD		
NEQAS score[xi]	Year 10	Year 11
A[xii]	n/a	n/a
B (%) (mean bias)	-6.1	-8.2
C (%) (SD of bias)	5.8	7.4

Q.3.18 Plasma creatinine

Creatinine was measured up to and including NDNS RP Year 10 but not from Year 11 onwards. Creatinine was measured with an enzymatic method which uses creatininase coupled to creatinase, sarcosine oxidase and peroxidase. The coloured end product is measured bichromatically (540 nm, 700 nm) at the endpoint of the reaction. Enzymatic creatinine methods are reported to be less susceptible to non-creatinine interfering substances.

[xi] For a description of EQA scheme scores, see section Q.2

[xii] There is no A score for the vitamin D scheme as this is a new scheme, introduced in 2018 and there are insufficient data points for UK NEQAS to set degree of difficulty scores to transform the accuracy of the measurements into the A score.

Q.3.18.1 Internal QC for plasma creatinine

QCs containing low, moderate and high concentrations of creatinine were run with each sample set. Table Q.55 shows internal QC results for creatinine, covering the period of Year 10 sample analysis.

Table Q.55 Internal QC for plasma creatinine (NDNS RP Year 10)

Creatinine	QC1_1	QC1_2	QC2_1	QC2_2	QC3_1	QC3_2
Mean (µmol/L)	51.8	47.7	126.4	131.8	398.1	418.2
SD (µmol/L)	2.13	1.79	2.29	2.35	5.48	2.44
% CV	4.1	3.8	1.8	1.8	1.4	0.6
n	15	8	15	8	13	8

Q.3.18.2 External QA for plasma creatinine

MRC EWL subscribed to the NEQAS clinical chemistry. Table Q.56 shows that during Year 10 the Dimension assay at MRC EWL gave results acceptably close to the consensus of all laboratories using the same method.

Table Q.56 External QA for creatinine performance relative to method mean (enzymatic method) (NDNS RP Year 10)

Creatinine NEQAS	Year 10
A[xiii]	121
B (%) (mean bias)	+0.5
C (%) (SD of bias)	6.3

Q.3.19 Serum selenium and serum zinc

Procedures for Year 10 were performed by MRC EWL and were the same as those reported for Year 9.[1] In Year 11, analysis of selenium and zinc concentrations was sub-contracted to Trace Element Laboratory, University Hospital Southampton.

Selenium (Se) and zinc (Zn) concentrations in serum were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer NEXION 300D, equipped with a dynamic reaction cell (DRC).

[xiii] For a description of EQA scheme scores, see section Q.2

Prior to analysis the ICP-MS instrument was tuned for optimum signal sensitivity and minimum oxide species and doubly charged ion formation.

Serum was diluted 1 in 50 in 0.01% nitric acid for Zn and 0.5% butanol for Se with rhodium (Rh) as internal standard and run against matrix matched calibration solutions prepared with bovine serum (Sigma-Aldrich). ^{66}Zn and ^{78}Se were determined and the analyte isotope signals were compared against the internal standard, to compensate for matrix related effects and any signal fluctuation due to instrument drift. Se analysis employed DRC to overcome argon-based interferences, with methane as the DRC gas.

Trial samples, blanks, calibration standards and QCs were analysed in each batch and the signal data generated was converted to concentrations via the calibration plot.

Q.3.19.1 QC for selenium and zinc

In order to establish quality assurance of each analytical batch and inter-batch variation across the year's cohort as a whole, QC material was analysed in conjunction with the blanks, calibration standards and samples. In Year 10, Recipe ClicChek Serum Control for Trace Elements Level 1 and 2 (Recipe Chemicals and Instruments GmbH) were analysed and in Year 11, Seronorm Serum Trace Elements Level 1 and 2 certified reference material (Sero, Norway) and in-house material.

Table Q.57 summarises the measured concentration of Se and Zn following analysis of these QC samples for Year 10. The mean measured concentration of the QC was within the target concentration range defined by the manufacturer and CV was ≤ 6%, demonstrating acceptable analytical accuracy and precision.

Table Q.57 QC for selenium and zinc analysis (NDNS RP Year 10)

	Selenium		Zinc	
Recipe ClinChek Lot 1286	L1	L2	L1	L2
Target (µg/L)	73.8	120	1760	2130
Acceptable range (µg/L)	59.0 – 88.5	96 – 144	1500 – 2020	1810 - 2450
Mean measured (µg/L)	71.4	114.6	1775.3	2158.7
Standard deviation (SD)	4.3	6.4	100	91.1
% CV	6.0	5.6	5.6	4.2
Agreement with Target (%)	96.7	95.5	100.9	101.3
n	28	27	28	27

Tables Q.58 and Q.59 summarise the measured concentration of Se and Zn following analysis of these QC samples for Year 11. The mean measured concentration of the QC was within the target concentration range defined by the manufacturer and CV was ≤11%, demonstrating acceptable analytical accuracy and precision.

Table Q.58 Selenium QC analysis (NDNS RP Year 11)

Selenium	Seronorm Serum L1	Seronorm Serum L2	AS 1	AS 2	AS 3
Lot number	1309438	1309416	In-house QC		
Target (µmol/L)	1.10	1.75	0.55	1.15	2.10
Acceptable range (µmol/L)	0.96-1.25	1.52-1.98	0.50-0.74	0.95-1.35	1.85-2.35
Mean measured (µmol/L)	1.04	1.69	0.62	1.15	2.10
Standard deviation (SD)	0.05	0.11	0.05	0.11	0.11
% CV	5.6	2.4	5.2	1.9	1.9
Agreement with Target (%)	94.5	96.6	112.7	100	100
n	10	10	42	37	34

Table Q.59 Zinc QC analysis (NDNS RP Year 11)

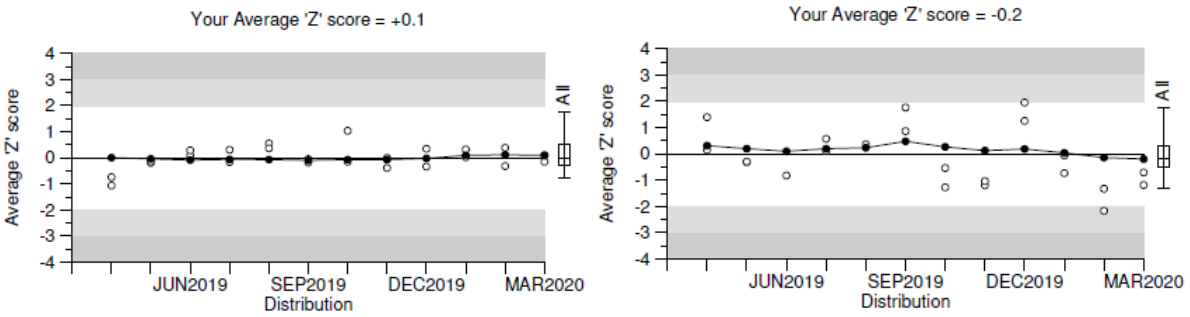
Zinc	Seronorm Serum L1	Seronorm Serum L2	QC 171	QC 172	QC 173
Lot number	1309438	1309416	In-house QC		
Target (µmol/L)	16.8	24.7	5.2	10.8	21.1
Acceptable range (µmol/L)	14.6-19.0	21.5-28.0	3.4-7.0	8.8-12.8	16.7-25.5
Mean measured (µmol/L)	17.6	24.8	5.0	10.7	20.3
Standard deviation (SD)	0.9	1.7	0.5	1.1	1.7
% CV	5.2	3.3	10.6	10.1	8.6
Agreement with Target (%)	104.8	100.4	96.2	99.1	96.2
n	10	10	38	34	34

Q.3.19.2 External QC for selenium and zinc in Serum

MRC EWL and Southampton Trace Elements participated in the Inter-Laboratory Comparison Program for Metals in Biological Matrices (PCI) operated by Centre de Toxicologie du Québec at the Institut National de Santé Publique du Québec (INSPQ, https://www.inspq.qc.ca/en). In addition, Southampton Trace Elements also participated in TEQAS (NEQAS for Trace Elements, Guildford, http://www.surreyeqas.org.uk/trace-elements-teqas/).

Good EQA Zn and Se performance throughout the period of analysis can be seen in all schemes, with reported results within the criteria defined in the multi-laboratory programme. For the Quebec scheme, laboratory scores of 100% for Zn and 100% for Se were achieved for the 3 rounds analysed in 2018 by MRC EWL and laboratory scores of 96% for Zn and 84% for Se were achieved for 2019 by Southampton Trace Elements Laboratory. Performance in TEQAS scheme is shown in Figure Q.3.

Figure Q.3.TEQAS serum Zn (left panel) and Se (right panel) performance October 2019 – March 2020



Q.3.20 Urinary iodine

Procedures for Year 10 were performed by MRC EWL and were the same as those reported for Year 9.[1] In Year 11, analysis of urinary iodine concentration was sub-contracted to the Trace Element Laboratory, University Hospital Southampton.

Urine iodine concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS), using a Perkin Elmer NEXION 300D.

Urine samples and QC materials were diluted 1 in 50 in 0.3% ammonia with rhodium (Rh) as internal standard. ^{127}I isotope signals were measured and compared against the internal standard, to compensate for matrix related effects and any signal fluctuation due to instrument drift. Matrix matched external calibration standards were prepared in human urine for each analytical batch.

Prior to analysis the ICP-MS instrument was tuned for optimum signal sensitivity and minimum oxide species and doubly charged ion formation. Trial samples, blanks, calibration standards and QCs were analysed in each batch and the signal data generated converted to concentrations via the calibration plot.

Q.3.20.1 QC for Iodine

In order to establish quality assurance of each analytical batch and inter-batch variation across the fieldwork year's cohort as a whole, QC material was analysed in conjunction with blanks, calibration standards and samples. In Year 10 Recipe

ClinChek Urine Control for Trace Elements level 1 and 2 were analysed. In Year 11, Seronorm Urine Trace Elements Level 1 and 2 controls (Sero, Norway) certified reference material were analysed.

Table Q.60 summarises the measured concentration of iodine following analysis of the QC samples for Year 10. The mean measured concentration of the QC was within the target concentration range defined by the manufacturer and CV was ≤5% for this survey year, showing that there was acceptable analytical accuracy and precision for samples.

Table Q.60 QC analysis for urinary iodine (NDNS RP Year 10)

Urinary iodine	Clinchek urine	
	Level 1	Level 2
Lot number	432	432
Target Concentration /Range (µg/L)	120 (89.9 - 150)	497 (373 - 622)
Mean measured concentration (µg/L)	114	484
n (QC samples)	95	97
SD	3.4	11.6
% CV	3.0	2.4

Table Q.61 summarises the measured concentration of iodine following analysis of these QC samples for Year 11. The mean measured concentration of the QC was within the target concentration range defined by the manufacturer and CV was ≤10% for this survey year, showing that there was acceptable analytical accuracy and precision for samples.

Table Q.61 QC analysis urinary iodine (NDNS RP Year 11)

Urinary iodine	Seronorm urine	
	Level 1	Level 2
Lot number	1403080	1403081
Target concentration / range (µmol/L)	0.83 (0.66-0.99)	2.30 (1.9-2.8)
Mean measured concentration (µmol/L)	0.76	2.17
n (QC samples)	104	99
SD	0.06	0.15
% CV	7.6	7.1

Q.3.20.2 External QC for urinary iodine

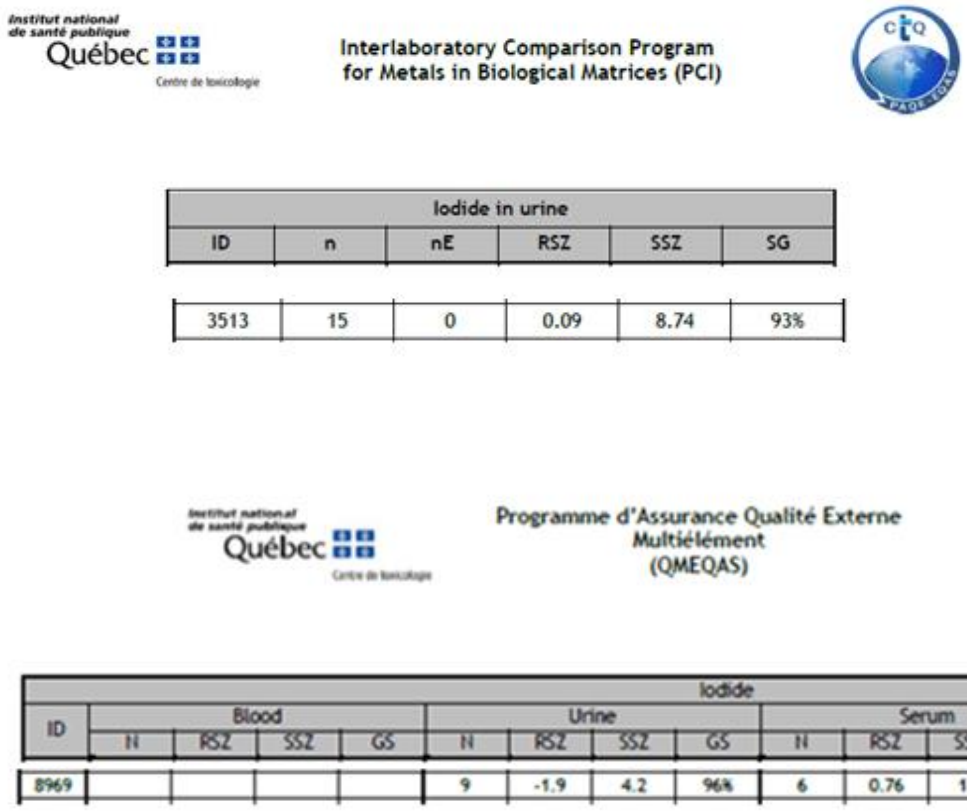
MRC EWL participated in the Ensuring Quality of Iodine Procedures (EQUIP) operated by CDC (table Q.62). Southampton Trace Elements participates in the Interlaboratory Comparison Program for Metals in Biological Matrices (PCI), and the Multi-Element External Quality Assurance Scheme (QMEQAS) operated by Centre de Toxicologie du Québec at the Institut National de Santé Publique du Québec (INSPQ).

Table Q.62 External QC analysis for urinary iodine (NDNS RP Year 10)

Distribution	Sample ID	CDC target value (µg/L)	Reported results (µg/L)	Bias from target (%)
Round 49	UI 150627	467.2	453.9	-2.8
	UI 150630	46.9	43.9	-6.5
	UI 150645	107.3	104.6	-2.5
	UI 150652	173.6	166.8	-3.9
Round 50	UI 150617	11.7	8.9	-24.4
	UI 150640	190.9	185.9	-2.6
	UI 150641	65.3	59.5	-8.8
	UI 150697	343.9	328.1	-4.6

For Year 11, good EQA urine iodine performance can be seen in both schemes throughout 2019, with reported results within the criteria defined in these multi-laboratory programmes. Laboratory scores of 93% for PCI and 96% for the QMEQAS scheme were achieved, further details are in figure Q.4.

Figure Q.4 QUEBEC PCI (top panel) and QMEQAS (bottom panel) scheme for urine iodine analysis (NDNS RP Year 11)



Interlaboratory Comparison Program for Metals in Biological Matrices (PCI)

Iodide in urine					
ID	n	nE	RSZ	SSZ	SG
3513	15	0	0.09	8.74	93%

Programme d'Assurance Qualité Externe Multiélément (QMEQAS)

Iodide													
ID	Blood				Urine				Serum				
	N	RSZ	SSZ	GS	N	RSZ	SSZ	GS	N	RSZ	SSZ	GS	N
8969					9	-1.9	4.2	96%	6	0.76	1.3	100%	

Q.4 Acknowledgements

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Basildon and Thurrock University Hospitals NHS Foundation Trust, Belfast Health and Social Care Trust, Betsi Cadwaladr University Health Board, Brighton & Sussex University Hospitals NHS Trust, Calderdale & Huddersfield NHS Trust, Cardiff & Vale University Local Health Board, Countess of Chester NHS Foundation Trust, Cwm Taf University Health Board, Great Western Hospitals NHS Foundation Trust, Hillingdon Hospital NHS Foundation Trust, Hywel Dda University Health Board, James Cook University Hospital, Keele University, Lewisham and Greenwich NHS Trust, Maidstone & Tunbridge Wells NHS Trust, Middlesex University London, Midland Pathology Services, National Veterinary Services, NHS Grampian, NHS Lothian, Norfolk and Norwich University Hospital NHS Trust, Northern Lincolnshire and Goole Hospitals NHS Trust, Nuffield Health Glasgow, Nuffield Health Newcastle, Portsmouth Hospitals NHS Trust, Royal Cornwall Hospitals NHS Trust, Royal Devon and Exeter NHS Foundation Trust, Salisbury NHS Foundation Trust, Sheffield Hallam University Enterprises Ltd, Sherwood Forest Hospitals NHS Foundation Trust, Shrewsbury and Telford Hospital NHS Trust, Spire Healthcare Bristol, Spire

Healthcare Gatwick Park, The Pennine Acute Hospitals NHS Trust, The Royal Bournemouth and Christchurch Hospitals NHS Trust, The University of Manchester, Torbay and South Devon NHS Foundation Trust, UEA Consulting Ltd, UK Biocentre Ltd, University Hospitals Bristol NHS Foundation Trust, University Hospitals Coventry & Warwickshire NHS Trust, University Hospitals of Leicester NHS Trust, University of Cambridge, University of Dundee, University of Leeds, University of Surrey, University of the Highlands and Islands, York Teaching Hospitals NHS Foundation Trust.

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