Appendix N Methods of blood analysis and quality control

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N.1 Introduction

Samples of coagulated and ethylenediaminetetraacetate (EDTA) anticoagulated blood were sent directly by post to the Department of Haematology and Department of Clinical Biochemistry and Immunology, Addenbrooke's Hospital, Cambridge (Addenbrooke's) after their collection. Serum samples were obtained by centrifugation of the coagulated blood sample.

The following assays were conducted at Addenbrooke's:

- Full blood count including haemoglobin and haematocrit (see section N.2.1)
- Serum C-reactive protein (see section N.2.2)
- Serum vitamin B_{12} (see section N.2.3)
- Serum Total, HDL and LDL cholesterol (see section N.2.4)
- Serum triglycerides (triacylglycerols) (see section N.2.5)

The assays described in Sections N.2.6 to N.2.16 (and listed below) were conducted at HNR:

- Plasma ferritin (see section N.2.6)
- Plasma transferrin receptors (see section N.2.7)
- Plasma vitamin C (see section N.2.8)
- ETKAC for thiamin status (see section N.2.9)
- EGRAC for riboflavin status (see section N.2.10)
- Plasma vitamin B_6 (PLP and PA) (see section N.2.11)
- Plasma total homocysteine (see section N.2.12)
- Plasma retinol (see section N.2.13)
- Plasma retinyl palmitate (see section N.2.13)
- Plasma α-tocopherol (see section N.2.13)

- Plasma γ-tocopherol (see section N.2.13)
- Plasma individual carotenoids; α–carotene, β–carotene, α–cryptoxanthin, β–cryptoxanthin, lycopene, lutein and zeaxanthin (see section N.2.13)
- Plasma 25-hydroxyvitamin D (see section N.2.14)
- Plasma creatinine (see section N.2.15)
- Plasma selenium (see section N.2.16)
- Plasma zinc (see section N.2.16)

The following analytes were measured in the survey blood samples but are not included in the present report. However, their data will be deposited at the UK Data Archive[1] together with data for the other analytes presented in this report and listed above:

- Full blood count excluding haemoglobin and haematocrit (see section N.2.1)
- Serum triglycerides (triacylglycerols) (see section N.2.5)
- Plasma transferrin receptors (see section N.2.7)
- Plasma pyridoxic acid (see section N.2.11)
- Plasma retinyl palmitate (see section N.2.13)
- Plasma γ-tocopherol (see section N.2.13)
- Plasma creatinine (see section N.2.15)

Samples of lithium heparin anticoagulated blood were collected and stored in a cool box, at approximately 4°C, and delivered to a local processing field laboratory within two hours of collection. The field laboratories processed blood samples into whole blood, red cells, plasma, serum and metaphosphoric acid stabilised plasma portions. The metaphosphoric acid had been previously prepared and aliquotted at HNR and delivered by courier on dry ice to each field laboratory. Blood sample sub-fractions were stored frozen at a maximum of -20°C (typically at -40°C) at field laboratories for a period of six to eight weeks, before the samples were transported to HNR on dry ice, where they were stored frozen, at -80°C, until further subdivided and analysed.

N.2 Analysis of blood samples

Details of the method of analysis and the associated quality control (QC) procedures for each analyte are given in sections N.2.1 to N.2.13. Where appropriate, the results of these procedures are also shown. Internal quality control samples were run in every batch to assess assay precision for each analyte; results are tabulated below. Accuracy was assessed by comparisons with target values (determined by the manufacturer using appropriate reference materials) and/or results obtained by other laboratories by taking part in EQAS (external quality assessment) schemes for those analytes where such schemes were available.

N.2.1 Full blood count including haemoglobin and haematocrit

Full Blood Count was analysed on a Beckman Coulter LH700 series analyser which mainly uses the Coulter Principle[2,3] to count the red blood cells, mean cell volume (MCV), white blood cells 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.

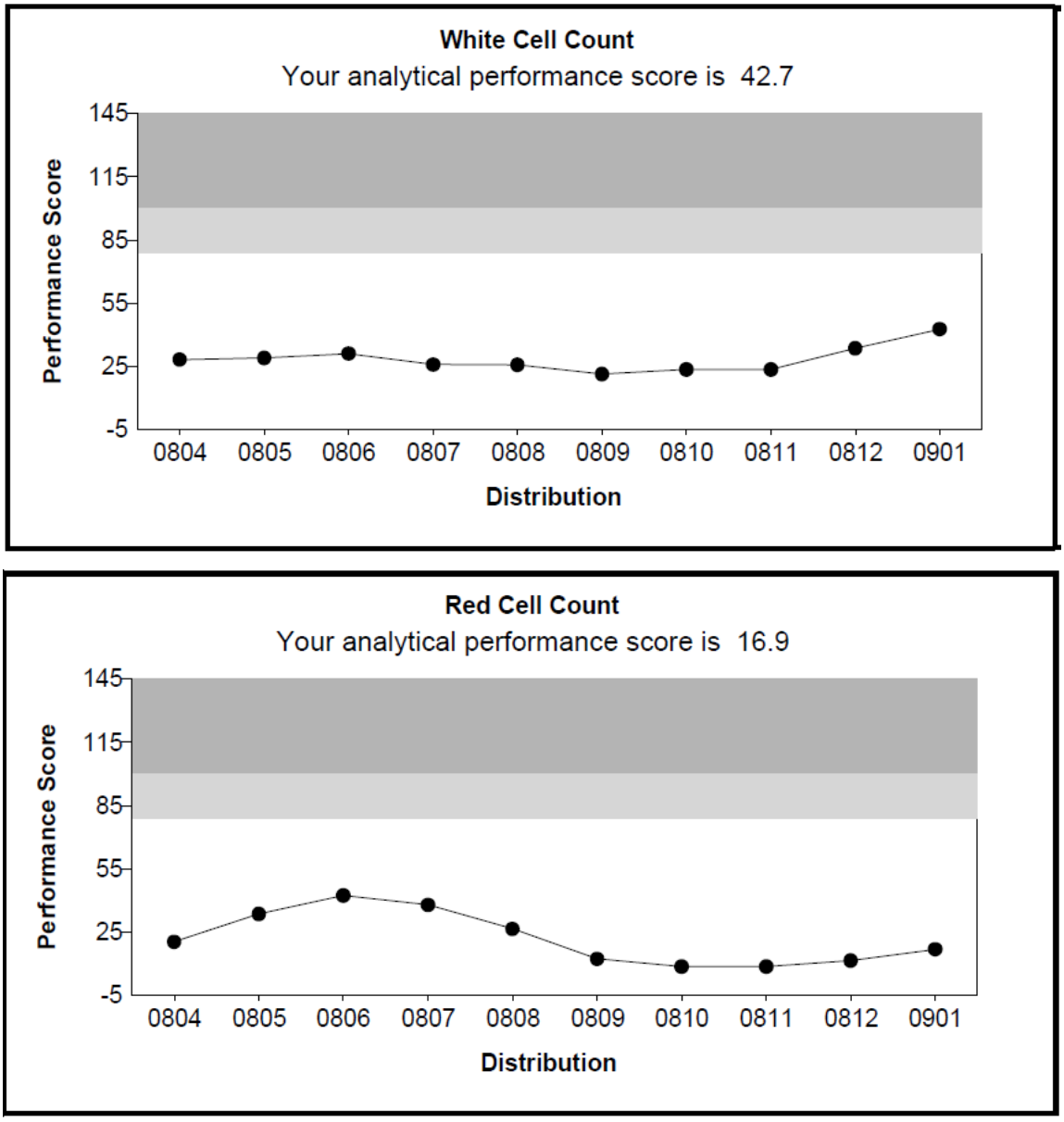
Haemoglobin was measured spectrophotomically at 525nm by a photocell in a sample that was diluted 1:256 (final) with isotonic diluent and lysing solution. The red cells were destroyed with a lysing agent releasing the haemoglobin into solution, which enabled the white blood cell count to be estimated using the Coulter Principle (impedance counting of the red blood cells)[2,3] without interference by red cells. The same lysing reagent also converted the haemoglobin to cyanmethaemoglobin.

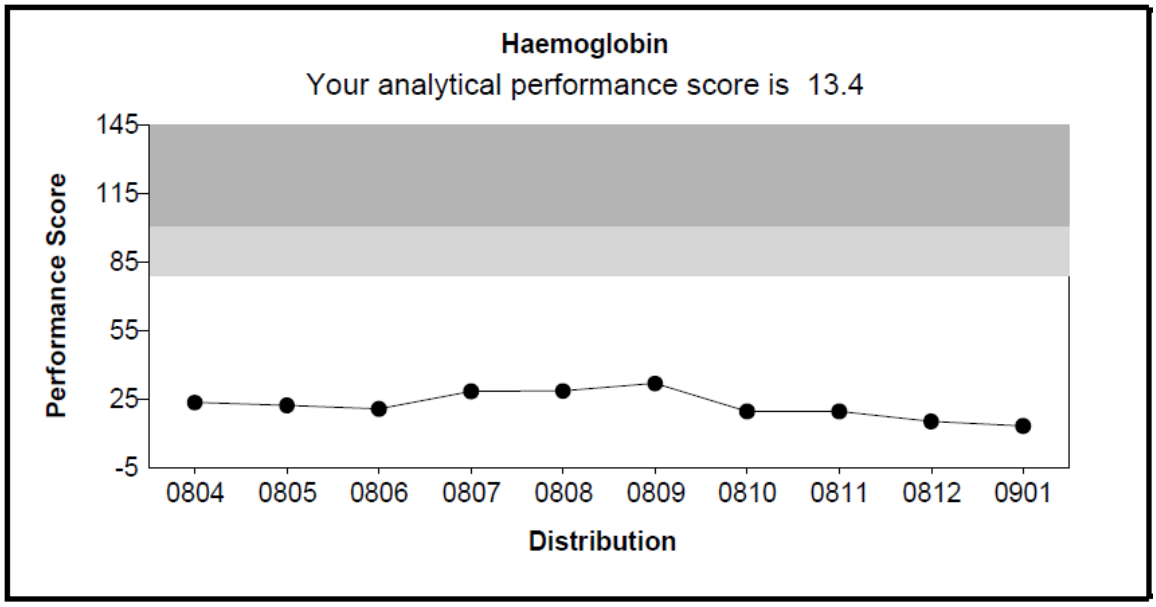
N.2.1.1 Quality controls for full blood count including haemoglobin and haematocrit

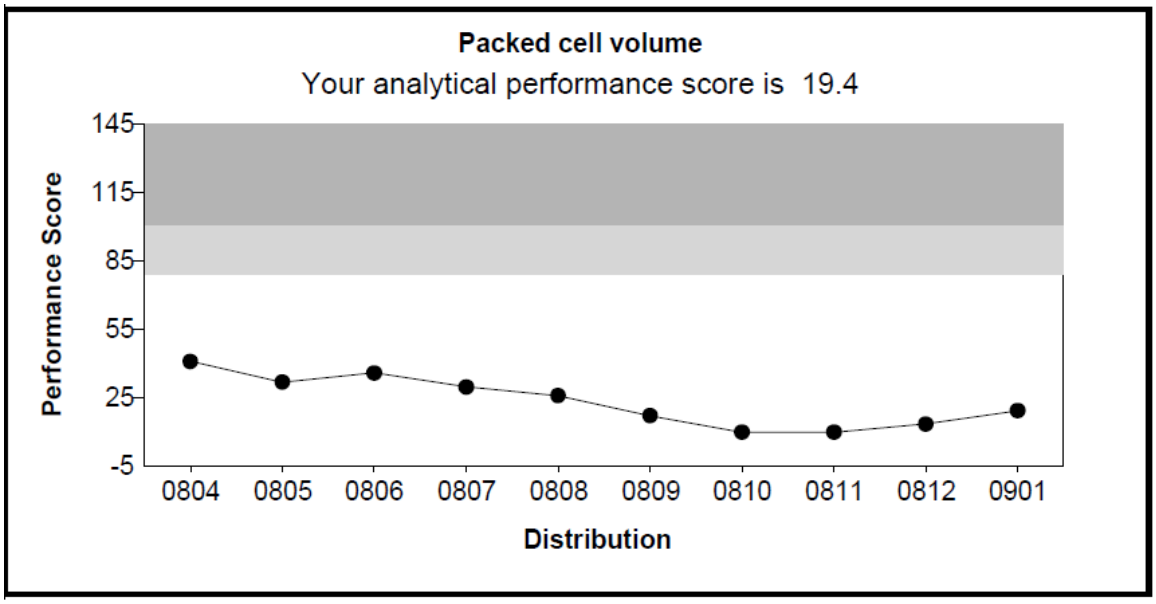
QC was achieved through the UK National External Quality Assessment Service (UKNEQAS) for haematology and Addenbrooke's EQAS scheme. Results are compared against the all laboratories trimmed mean (ALTM) that is given for laboratories in the NEQAS scheme that are using the same analyser and method as that used by Addenbrooke's. Figure N.1 shows illustrative UKNEQAS overall performance results for full blood counts including haemoglobin and haematocrit for specimen 0901FB between the periods of April 2008 and January 2009. This

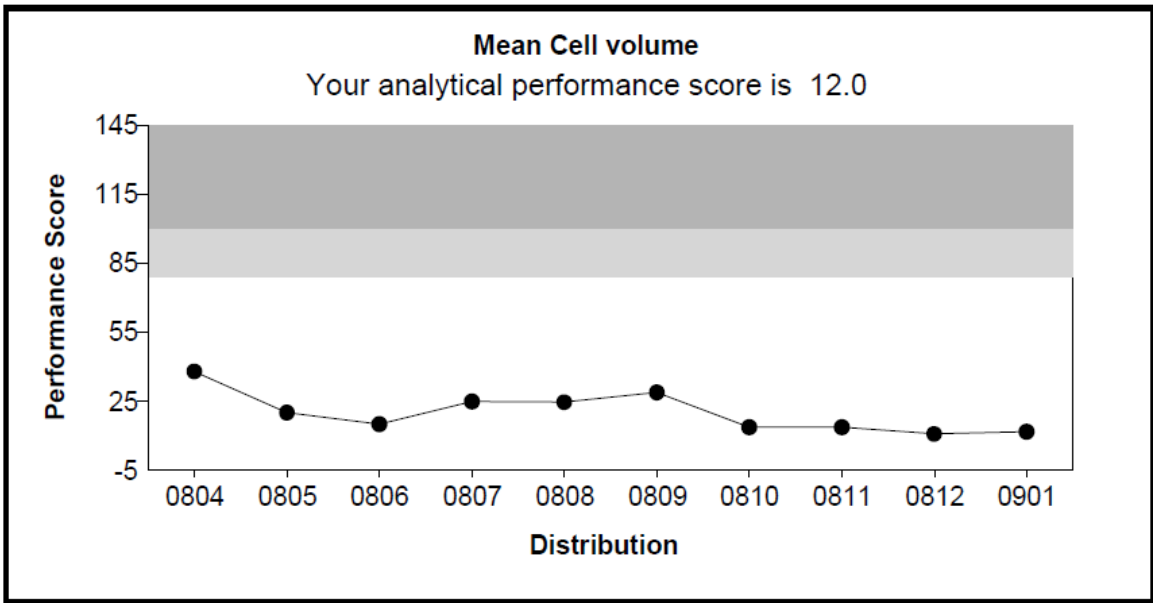
includes a proportion of the period when Year 1 full blood counts including haemoglobin and haematocrit were measured. Figure N.2 shows illustrative UKNEQAS overall performance results for full blood counts including haemoglobin and haematocrit for specimen 1001FB between the periods of April 2009 and January 2010. This includes a proportion of the period when Year 2 full blood counts, including haemoglobin and haematocrit, were measured. Results within the white area of the charts indicate acceptable performance as determined by UKNEQAS. Figure N.3 shows illustrative UKNEQAS overall performance results for full blood counts including haemoglobin and haematocrit for specimen 1001FB between the periods of April 2010 and January 2011. This includes a proportion of the period when Year 3 full blood counts, including haemoglobin and haematocrit, were measured. Results within the white area of the charts indicate acceptable performance as determined by UKNEQAS.

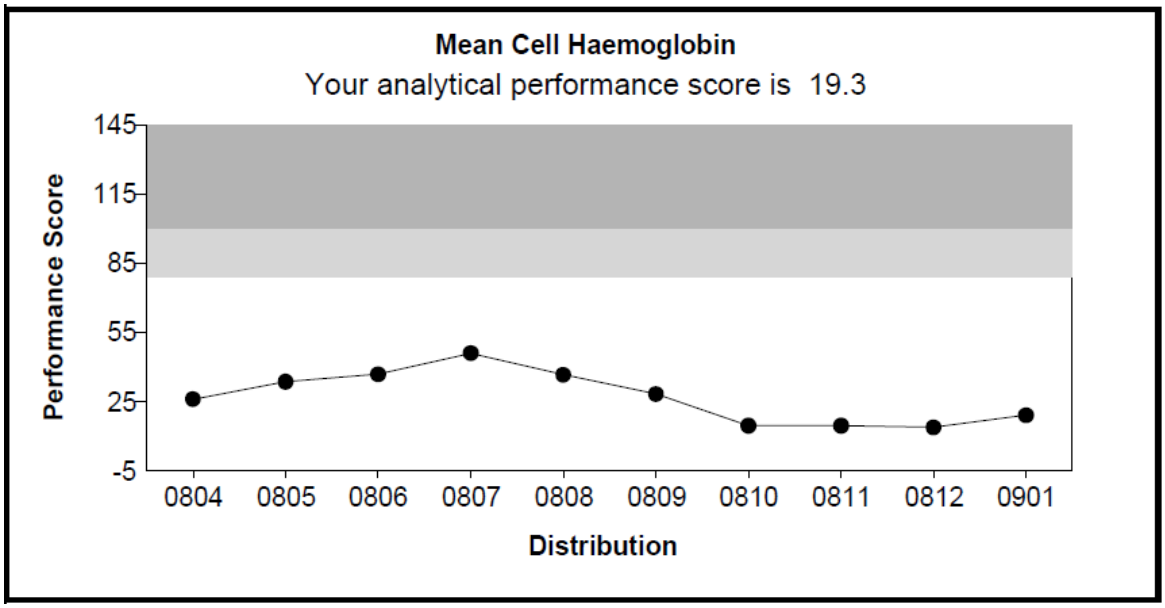
Figure N.1 Illustrative overall performance charts for UKNEQAS for Year 1 of the NDNS rolling programme




Haemoglobin
Your analytical performance score is 13.4
Performance Score
145
115
85
55
25
-5
0804 0805 0806 0807 0808 0809 0810 0811 0812 0901
Distribution


Packed cell volume
Your analytical performance score is 19.4
Performance Score
145
115
85
55
25
-5
0804 0805 0806 0807 0808 0809 0810 0811 0812 0901
Distribution


Mean Cell volume
Your analytical performance score is 12.0
Performance Score
145
115
85
55
25
-5
0804 0805 0806 0807 0808 0809 0810 0811 0812 0901
Distribution


Mean Cell Haemoglobin
Your analytical performance score is 19.3
Performance Score
145
115
85
55
25
-5
0804
0805
0806
0807
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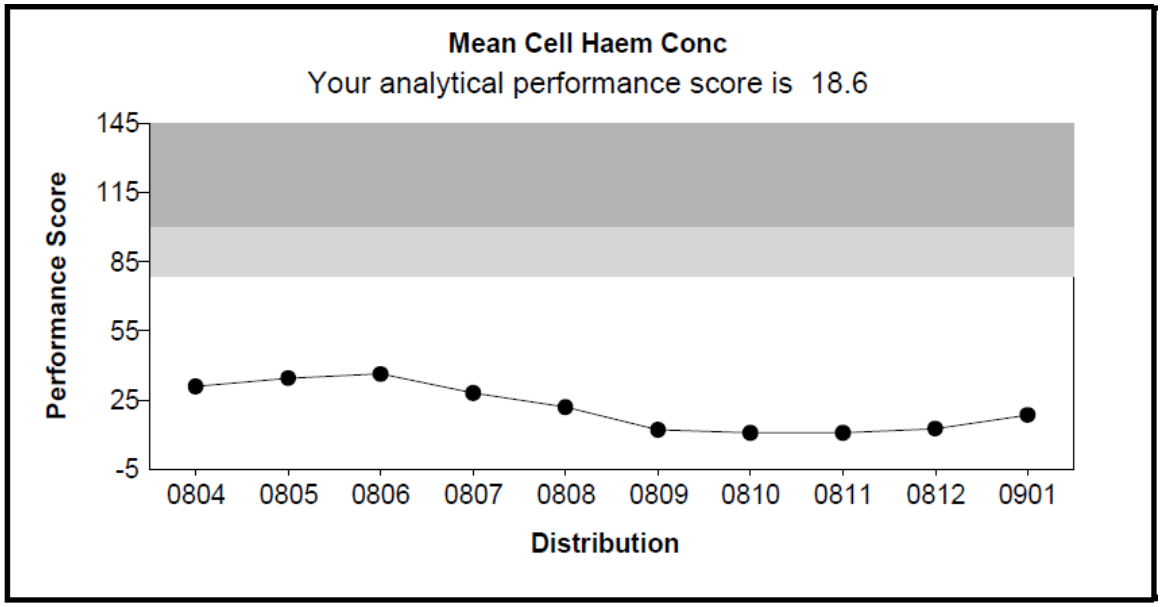
0810
0811
0812
0901
Distribution


Mean Cell Haem Conc
Your analytical performance score is 18.6
Performance Score
145
115
85
55
25
-5
0804
0805
0806
0807
0808
0809
0810
0811
0812
0901
Distribution

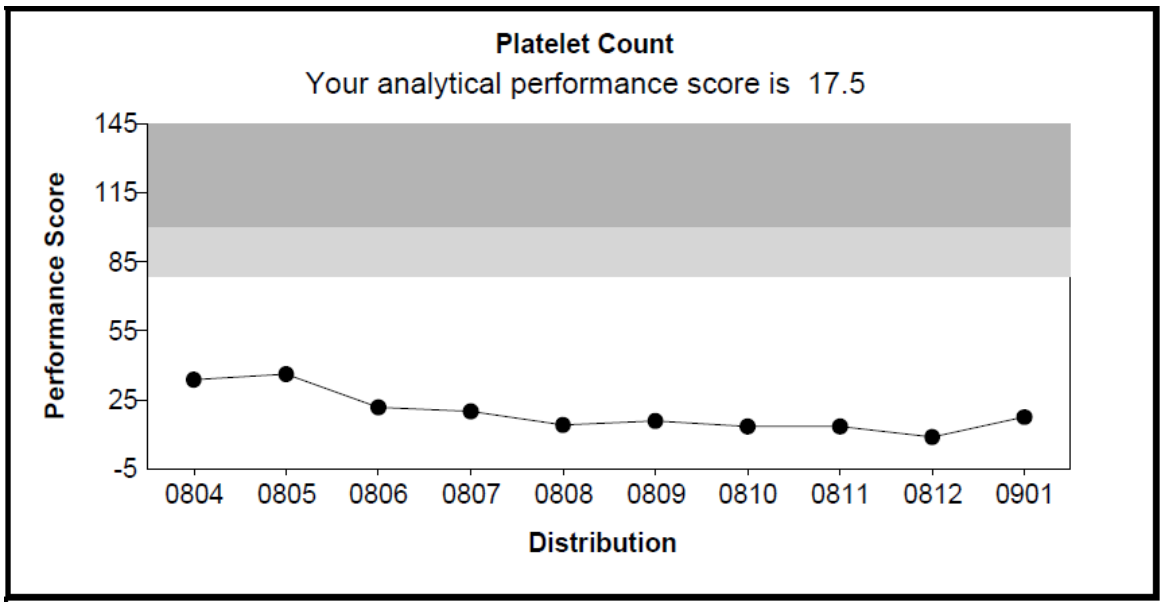
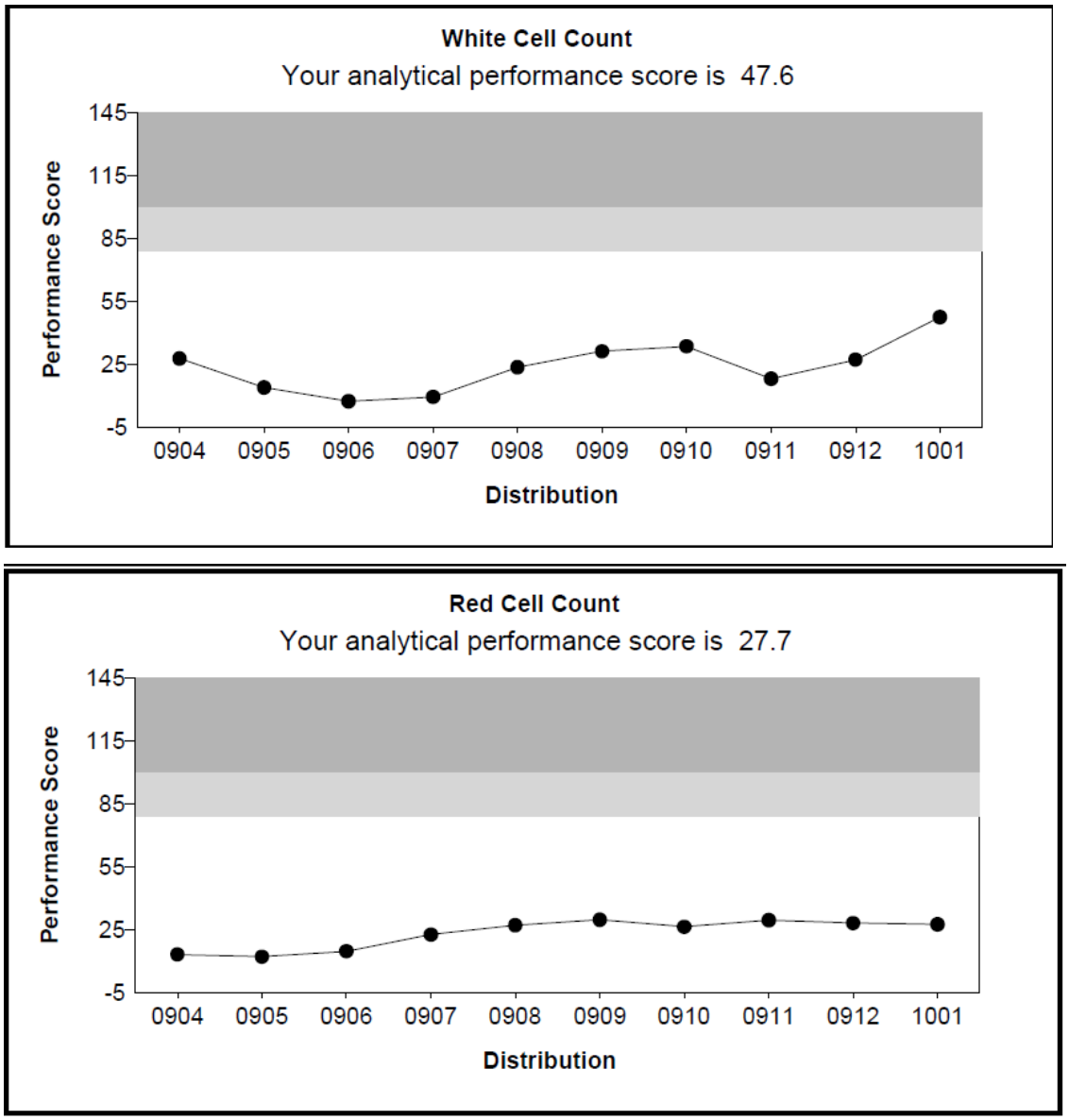
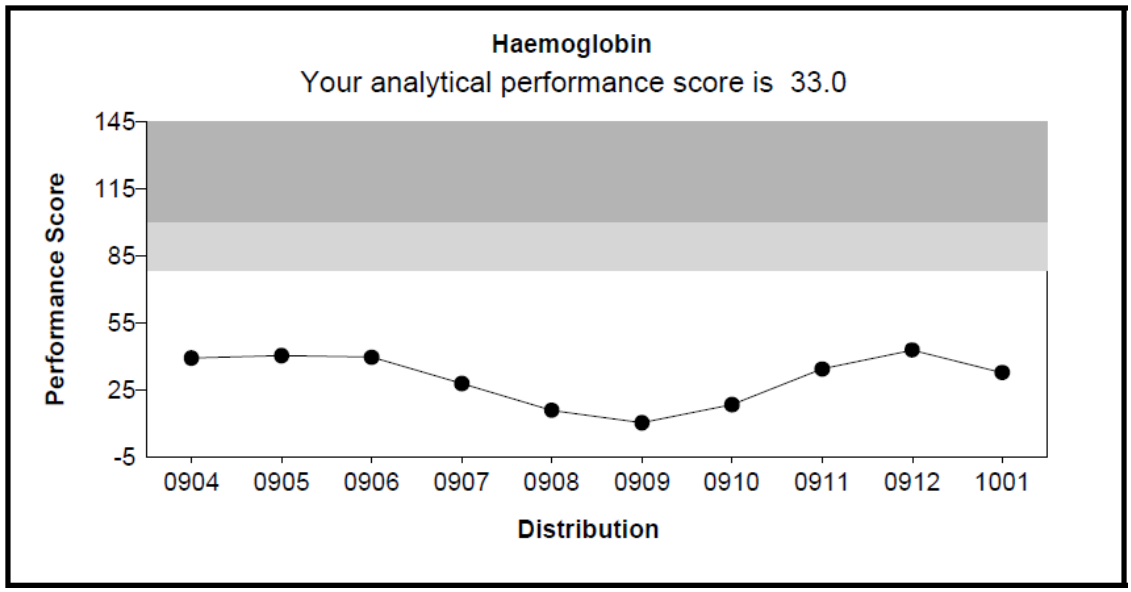
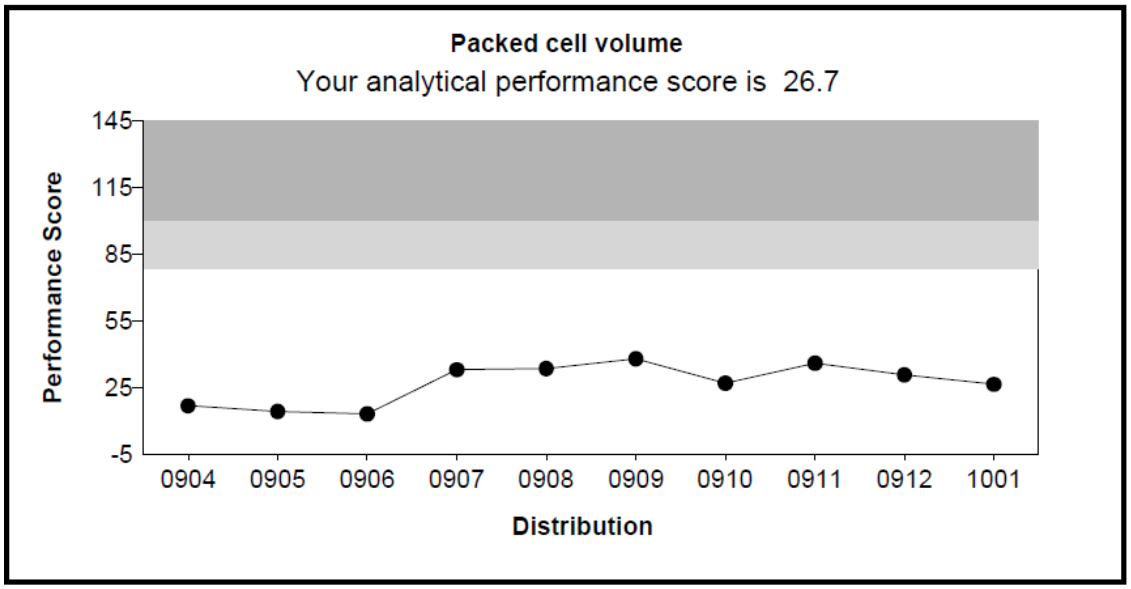
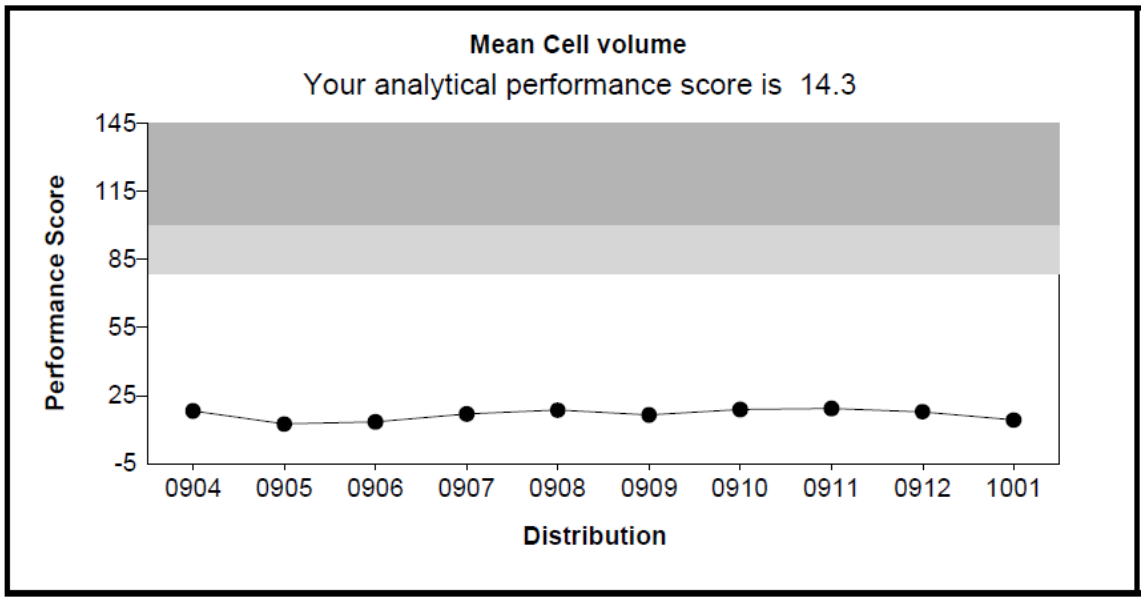

Platelet Count
Your analytical performance score is 17.5
Performance Score
145
115
85
55
25
-5
0804
0805
0806
0807
0808
0809
0810
0811
0812
0901
Distribution

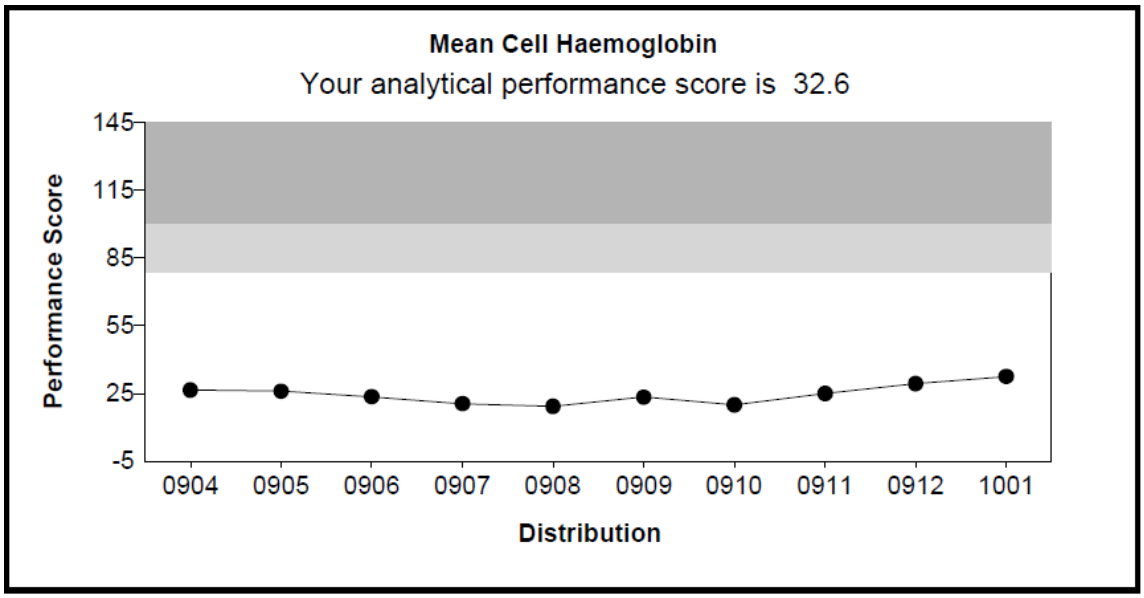
Figure N.2 Illustrative overall performance charts for UKNEQAS for Year 2 of the NDNS rolling programme

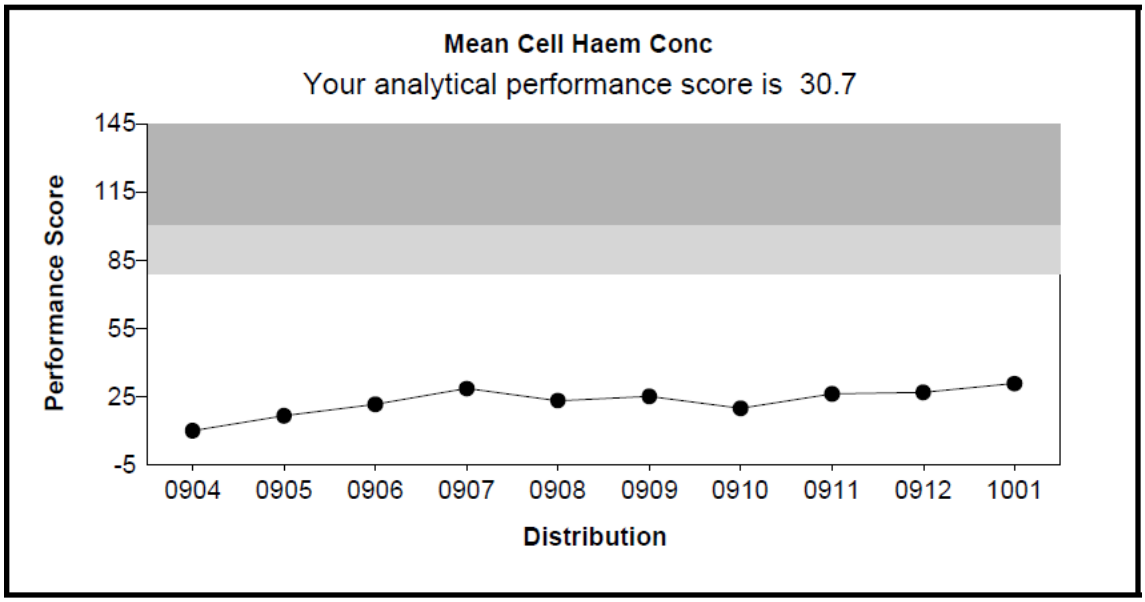



Haemoglobin
Your analytical performance score is 33.0
Performance Score
145
115
85
55
25
-5
0904
0905
0906
0907
0908
0909
0910
0911
0912
1001
Distribution


Packed cell volume
Your analytical performance score is 26.7
Performance Score
145
115
85
55
25
-5
0904
0905
0906
0907
0908
0909
0910
0911
0912
1001
Distribution


Mean Cell volume
Your analytical performance score is 14.3
Performance Score
145
115
85
55
25
-5
0904
0905
0906
0907
0908
0909
0910
0911
0912
1001
Distribution


Mean Cell Haemoglobin
Your analytical performance score is 32.6
Performance Score
145
115
85
55
25
-5
0904
0905
0906
0907
0908
0909
0910
0911
0912
1001
Distribution


Mean Cell Haem Conc
Your analytical performance score is 30.7
Performance Score
145
115
85
55
25
-5
0904
0905
0906
0907
0908
0909
0910
0911
0912
1001
Distribution

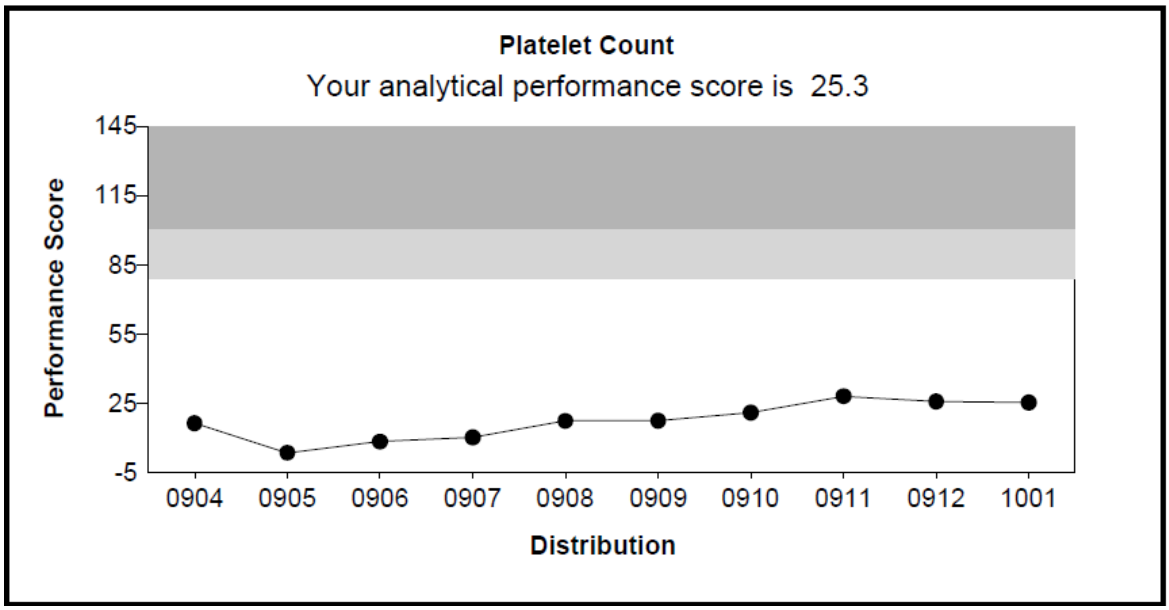
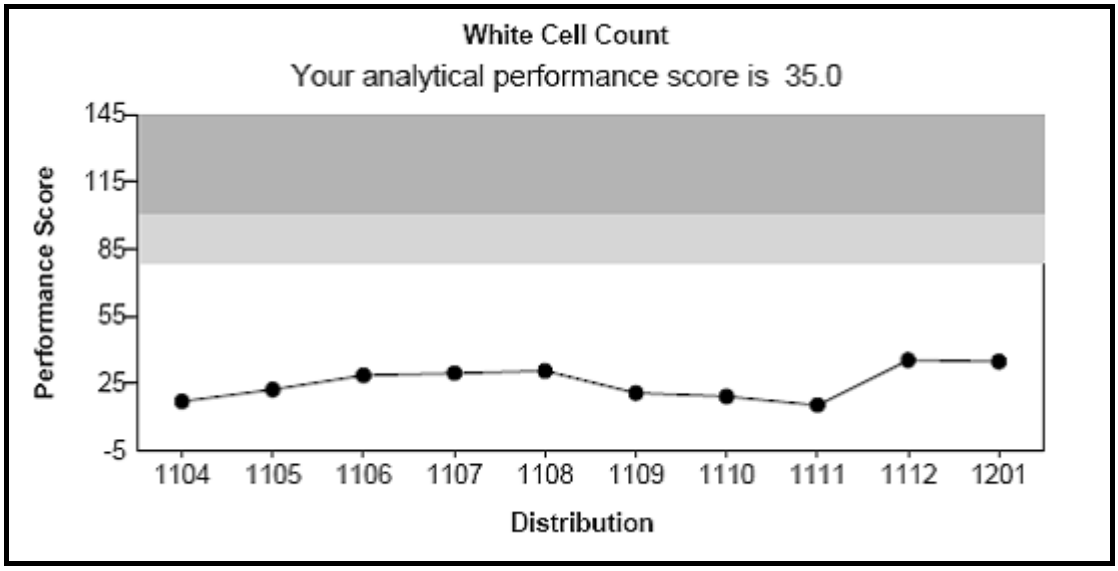
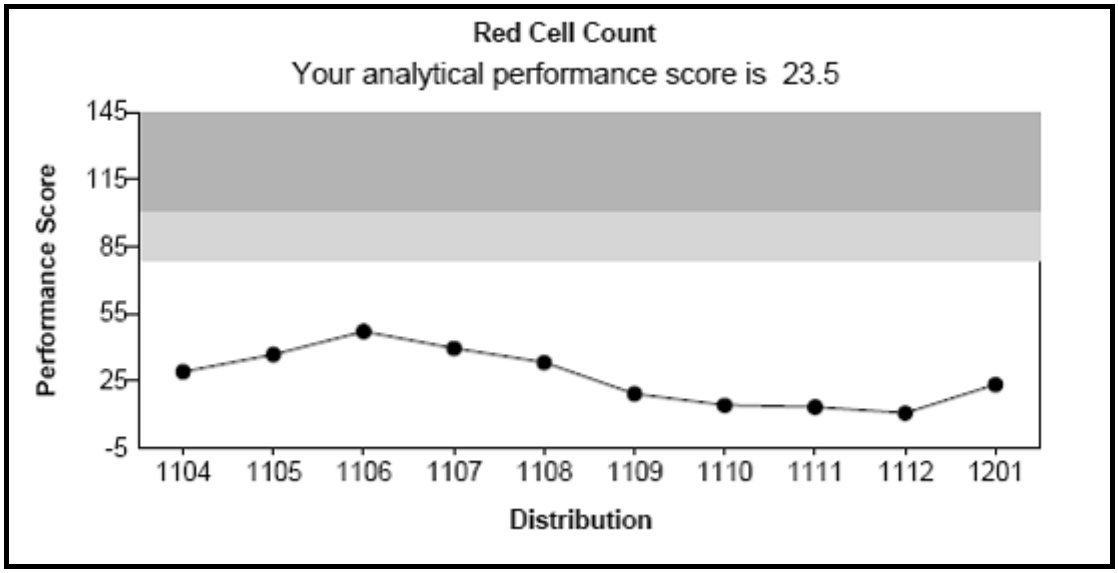
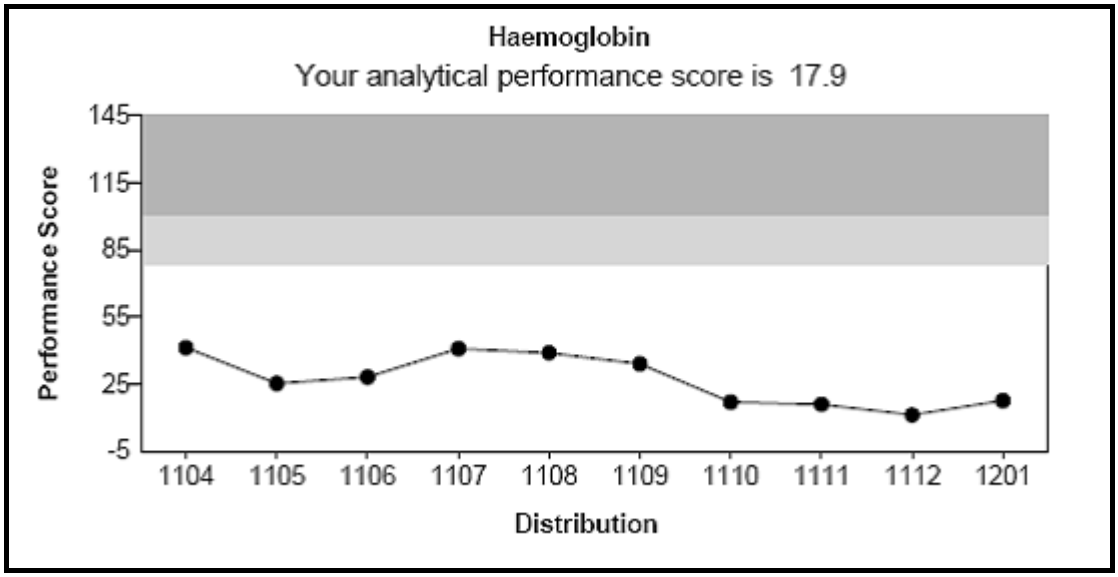
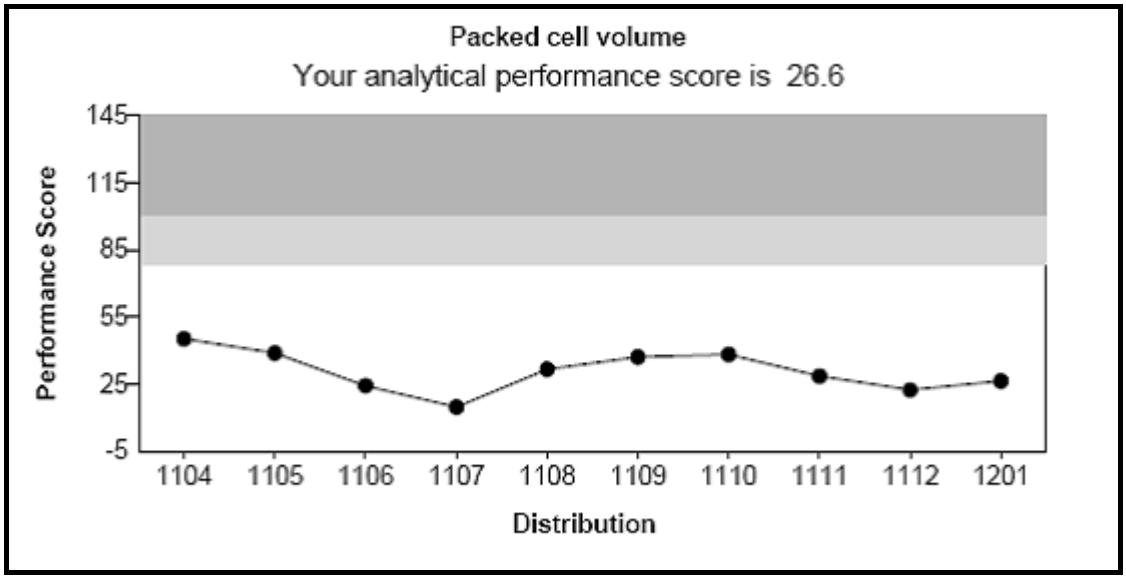

Platelet Count
Your analytical performance score is 25.3
Performance Score
145
115
85
55
25
-5
0904
0905
0906
0907
0908
0909
0910
0911
0912
1001
Distribution

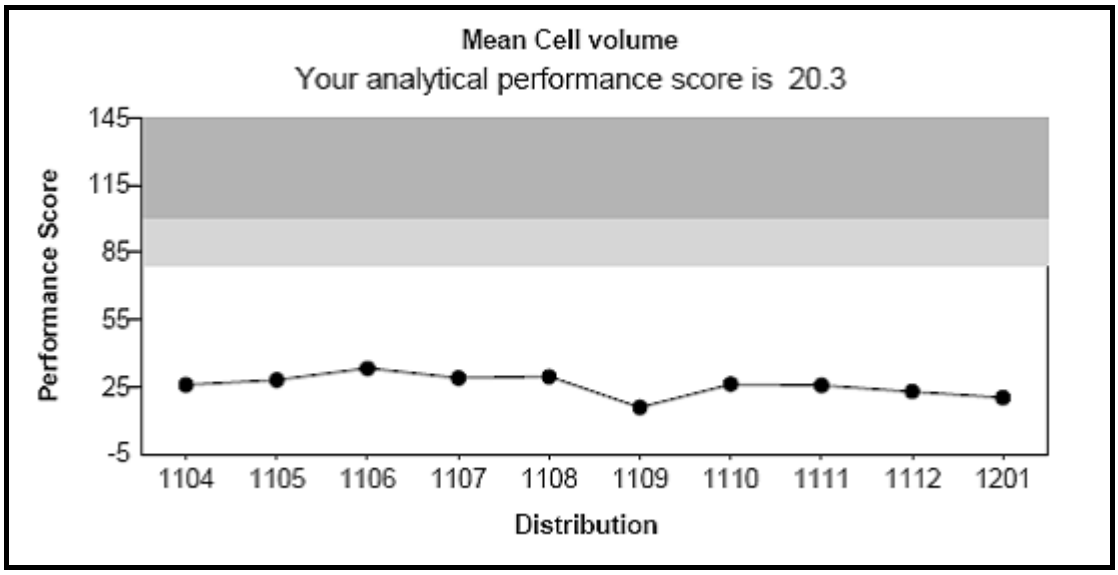
Figure N.3 Illustrative overall performance charts for UKNEQAS for Year 3 of the NDNS rolling programme

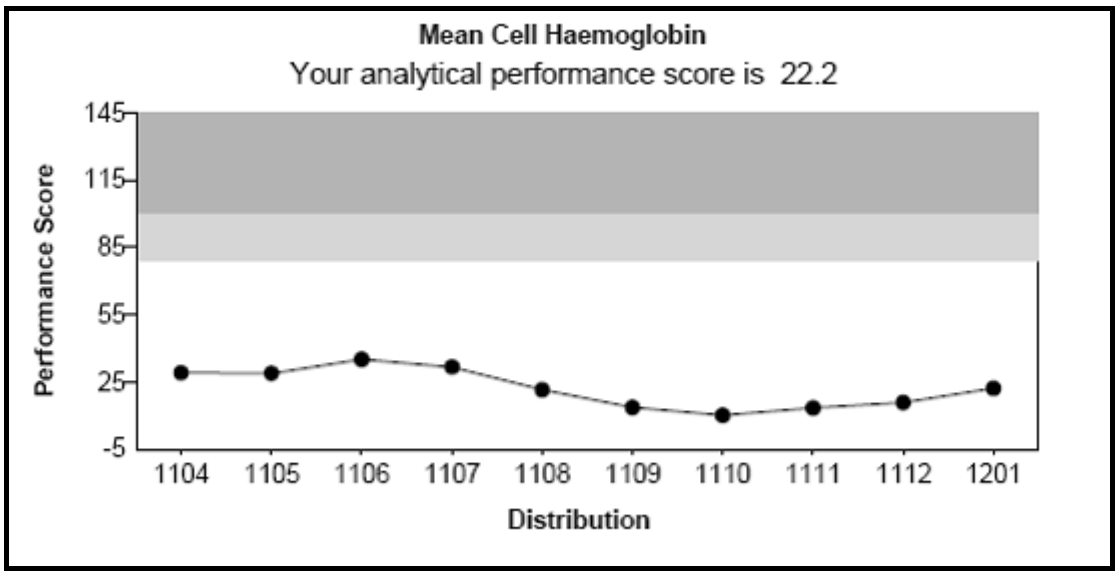


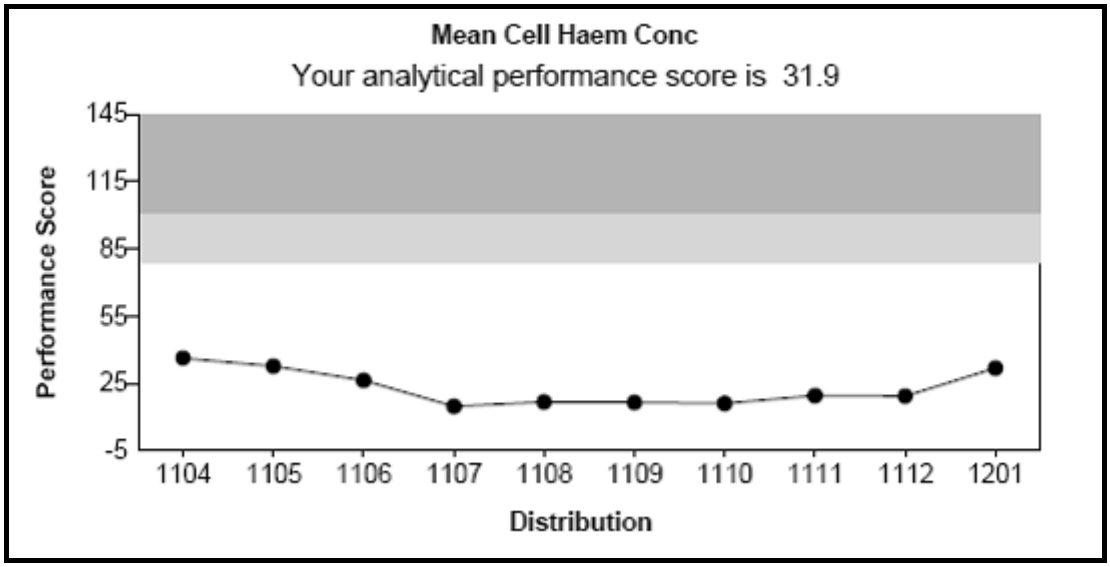


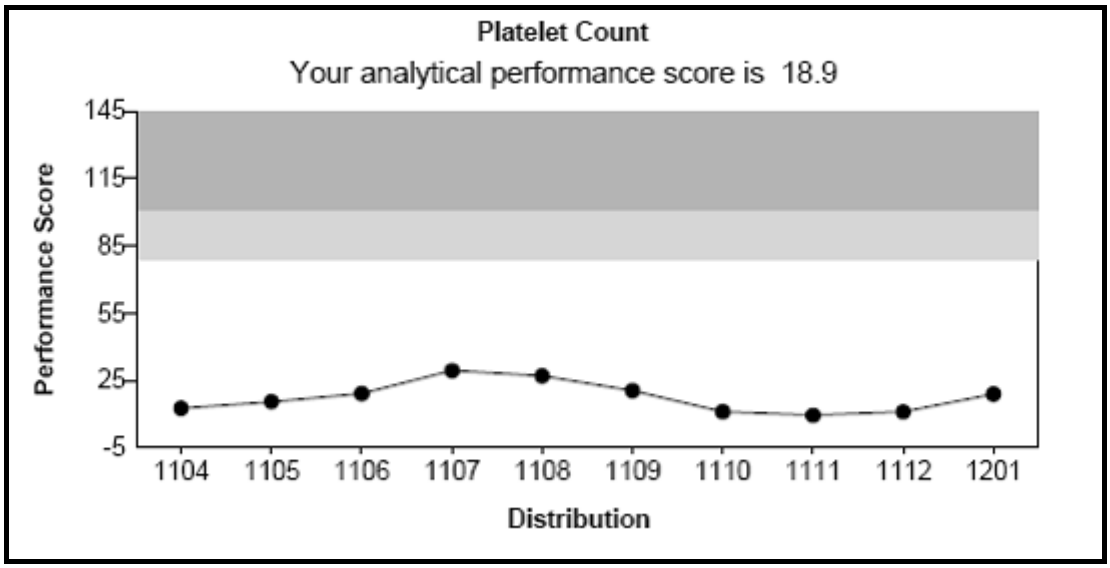



Packed cell volume
Your analytical performance score is 26.6
Performance Score
145
115
85
55
25
-5
1104
1105
1106
1107
1108
1109
1110
1111
1112
1201
Distribution


Mean Cell volume
Your analytical performance score is 20.3
Performance Score
145
115
85
55
25
-5
1104
1105
1106
1107
1108
1109
1110
1111
1112
1201
Distribution


Mean Cell Haemoglobin
Your analytical performance score is 22.2
Performance Score
145
115
85
55
25
-5
1104
1105
1106
1107
1108
1109
1110
1111
1112
1201
Distribution





N.2.2 Serum C-reactive protein (CRP)

C-reactive protein (CRP) was assayed using a Dade Behring Dimension RXL Clinical Chemistry Analyser. The CRP method is based on a particle enhanced turbidimetric immunoassay (PETIA) technique, giving high sensitivity by extending the detection range down to 1.0mg/L. Latex particles coated with antibody to CRP (AbPR) aggregate in the presence of CRP in the sample. The increase in turbidity that accompanies aggregation is proportional to the CRP concentration.

N.2.2.1 Internal quality controls for CRP

The performance statistics in Tables N.1-N.3 were calculated using data from two or three different reagent lots in order to include batch to batch variation. Table N.1

shows imprecision data produced from a combination of two typical months in Year 1 (01/05/2008-30/06/2008) over six instruments using two reagents; data greater than 3x interquartile range (IQR) from the 25th or 75th percentile was omitted. Table N.2 shows imprecision data produced from a typical month in Year 2 (January 2010) using three reagents; data greater than 3x IQR from the 25th or 75th percentile was omitted. Table N.3 shows imprecision data produced from three typical months in Year 3 (01/09/2010 – 30/11/2010) using three reagents; data greater than 3x IQR from the 25th or 75th percentile was omitted. The QC data were omitted for a number of reasons including the wrong level of QC being put on the analyser or insufficient QC sample being presented to the instrument. At the point that a QC sample failed, the operator would determine why the QC result was different from the expected result and would act accordingly to rectify the problem and repeat the QC. If the QC failure was found to be caused by the reagent the samples were re-assayed before reporting to HNR.

Table N.1 Internal quality controls for CRP for Year 1 of the NDNS rolling programme

QC Lot No	**0506314**	**0704214**
Mean (mg/L)	15.56	71.30
SD	0.58	2.28
% CV	3.73	3.20
Data points included	141	151
Data points omitted	0	0

Table N.2 Internal quality controls for CRP for Year 2 of the NDNS rolling programme

QC Lot No	**29743**	**991179**
Mean (mg/L)	7.55	87.30
SD	0.40	3.50
% CV	5.30	4.01
Data points included	360	341
Data points omitted	12	20

Table N.3 Internal quality controls for CRP for Year 3 of the NDNS rolling programme

QC Lot No	29753	35282
Mean (mg/L)	7.88	81.80
SD	0.46	2.87
% CV	5.80	3.51
Data points included	1098	1120
Data points omitted	13	1

N.2.2.2 External quality controls for CRP

External quality control was achieved through the UKNEQAS CRP scheme.

N.2.3 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.

N.2.3.1 Internal quality controls for vitamin B_{12}

The performance statistics in Tables N.4-N.6 were calculated using data from two or three different reagent lots in order to include batch to batch variation. Table N.4 shows two lots of Lyphochek QC data produced for Year 1. Data in the upper section of the table is for the period 18/02/2008-16/07/2008 and data in the lower section of the table is for the period 16/07/2008-22/05/2009. Table N.5 shows two lots of Lyphochek QC data produced for Year 2. Data in the upper section of the table is for the period 08/07/2009-01/05/2010 and data in the lower section of the table is for the period 01/05/2010-09/08/2010. Table N.6 shows imprecision data produced from a combination of five typical months in Year 3 (01/08/2010-31/12/2010) over two instruments using two reagents; data greater than 3x IQR from the 25th or 75th percentile was omitted.

Table N.4 Internal quality controls for vitamin B_{12} for Year 1 of the NDNS rolling programme

QC Lot No	40181	40182	40183
Mean	332	577	898
SD	25.9	54.4	68.6
% CV	7.8	9.4	7.6
Data points included	115	112	124
Data points omitted	0	0	0
QC Lot No	**40201**	**40202**	**40203**
Mean	398	665	1127
SD	39.9	51.6	80.0
% CV	10.0	7.8	7.1
Data points included	433	422	467
Data points omitted	0	0	0

Table N.5 Internal quality controls for vitamin B_{12} for Year 2 of the NDNS rolling programme

QC Lot No	40201	40202	40203
Mean	396	658	1120
SD	35.2	48.1	82.9
% CV	8.9	7.3	7.4
Data points included	559	555	556
Data points omitted	0	0	0
QC Lot No	**40231**	**40232**	**40233**
Mean	354	651	1373
SD	30.0	46.9	82.9
% CV	8.4	7.2	6.0
Data points included	149	156	125
Data points omitted	0	0	0

Table N.6 Internal quality controls for vitamin B_{12} for Year 3 of the NDNS rolling programme

QC Lot No	40231	40232	40233	43170
Mean (µg/L)	345	677	1403	131
SD	24.4	38.0	105.4	19.9
% CV	7.06	5.86	7.51	15.17
Data points included	347	338	336	291
Data points omitted	1	0	0	0

N.2.3.2 External quality controls for vitamin B_{12}

Quality control was achieved through the UK NEQAS Haematinics scheme.

N.2.4 Serum total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol

The CHOL method is based on the principle first described by Stadtman[4] and later adapted by other workers, including Rautela and Liedtke.[5] Cholesterol esterase (CE) catalyses 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 (CO) to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxidase (HPO), the hydrogen peroxide thus formed is used to oxidize N,N-diethylaniline-HCl/4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs at 540 nm.

The AHDL cholesterol assay is a homogeneous method for directly measuring HDL cholesterol levels without the need for off-line pre-treatment or centrifugation steps. The method is in a two reagent format and depends on the properties of a unique detergent. It is based on accelerating the reaction of cholesterol oxidase (CO) 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 (CE) and chromagenic coupler to develop colour for the quantitative determination of HDL-C.

N.2.4.1 Internal quality controls for total cholesterol

The performance statistics in Tables N.7-N.9 were calculated using data from two or three different reagent lots in order to include batch to batch variation. Table N.7 shows imprecision data produced from a combination of two typical months in Year 1 (01/12/2008-10/02/2009) over six instruments; data greater than 3x IQR from the 25th or 75th percentile were omitted. Table N.8 shows imprecision data produced from a typical month in Year 2 (January 2010); data greater than 3x IQR from the 25th or 75th percentile were omitted. Table N.9 shows imprecision data produced from a combination of three typical months in Year 3 (01/09/2010-30/11/2010) over six instruments using two reagents; data greater than 3x IQR from the 25th or 75th percentile were omitted. At the point that a QC sample failed, the operator would

determine why the QC result was different to the expected result and would act accordingly to rectify the problem and repeat the QC. Results of samples were not provided to HNR until the QC failure had been addressed and where the problem was found to be related to the reagent the samples were repeated to ensure accuracy of results.

Table N.7 Internal quality controls for total cholesterol for Year 1 of the NDNS rolling programme

QC Lot No	46381	46383
Mean (mmol/L)	2.34	6.58
SD	0.069	0.167
% CV	2.9	2.5
Data points included	869	943
Data points omitted	12	4

Table N.8 Internal quality controls for total cholesterol for Year 2 of the NDNS rolling programme

QC Lot No	46381	46383
Mean (mmol/L)	2.35	6.53
SD	0.105	0.126
% CV	4.5	1.9
Data points included	389	147
Data points omitted	11	4

Table N.9 Internal quality controls for total cholesterol for Year 3 of the NDNS rolling programme

QC Lot No	46401	46403
Mean (mmol/L)	2.63	6.75
SD	0.088	0.159
% CV	3.3	2.4
Data points included	1115	1209
Data points omitted	2	0

N.2.4.2 Internal quality controls for HDL cholesterol

The performance statistics in Tables N.10-N.12 were calculated using data from two or three different reagent lots in order to include batch to batch variation. Table N.10 shows imprecision data produced from a combination of two typical months in Year 1 (01/05/2008-30/06/2008) over four instruments; data greater than 3x IQR from the 25th or 75th percentile were omitted. Table N.11 shows imprecision data produced from a typical month in Year 2 (January 2010); data greater than 3x IQR from the 25th or 75th percentile were omitted. Table N.12 shows imprecision data produced from a combination of three typical months in Year 3 (01/09/2010-30/11/2010) over four instruments using three reagents; data greater than 3x IQR from the 25th or 75th percentile were omitted. The QC data were omitted for a number of reasons including the wrong level of QC being put on the analyser or QC samples short sampling from a sample cup. At the point that a QC sample failed, the operator would determine why the QC result was different to the expected result and would act accordingly to rectify the problem and repeat the QC. Results of samples were not provided to HNR until the QC failure had been addressed and where the problem was found to be related to the reagent the samples were repeated to ensure accuracy of results.

Table N.10 Internal quality controls for HDL cholesterol for Year 1 of the NDNS rolling programme

QC Lot No	46381	46383
Mean (mmol/L)	0.91	2.03
SD	0.04	0.11
% CV	4.4	4.8
Data points included	140	149
Data points omitted	0	0

Table N.11 Internal quality controls for HDL cholesterol for Year 2 of the NDNS rolling programme

QC Lot No	46381	46383
Mean (mmol/L)	0.93	2.15
SD	0.03	0.09
% CV	3.2	4.2
Data points included	232	237
Data points omitted	8	8

Table N.12 Internal quality controls for HDL cholesterol for Year 3 of the NDNS rolling programme

QC Lot No	46401	46403
Mean (mmol/L)	0.95	1.91
SD	0.05	0.08
% CV	4.9	4.0
Data points included	738	862
Data points omitted	2	0

N.2.4.3 External quality controls for total and HDL cholesterol

External quality control was achieved through the Randox International Quality Assessment Scheme (RIQAS).

N.2.5 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 (triacylglycerols). The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides (triacylglycerols) 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 (510nm, 700 nm) endpoint technique.

N.2.5.1 Internal quality controls for serum triglycerides (triacylglycerols)

The performance statistics in Tables N.13-N.15 were calculated using data from two or three different reagent lots in order to include batch to batch variation. Table N.13 shows imprecision data produced from a combination of two typical months in Year 1 (01/05/2008-30/06/2008). Table N.14 shows imprecision data produced from a typical month in Year 2 (January 2010); data greater than 3x IQR from the 25th or 75th percentile were omitted. Table N.15 shows imprecision data produced from a combination of three typical months in Year 3 (01/09/2010-30/11/2010) over four instruments using three reagents; data greater than 3x IQR from the 25th or 75th percentile were omitted. Results of samples were not provided to HNR until the QC failure had been addressed and where the problem was found to be related to the reagent the samples were repeated to ensure accuracy of results.

Table N.13 Internal quality controls for serum triglycerides (triacylglycerols) for Year 1 of the NDNS rolling programme

QC Lot No	46381	46383
Mean (mmol/L)	0.89	2.22
SD	0.08	0.13
% CV	9.0	5.9
Data points included	150	152
Data points omitted	0	0

Table N.14 Internal quality controls for serum triglycerides (triacylglycerols) for Year 2 of the NDNS rolling programme

QC Lot No	46381	46383
Mean (mmol/L)	0.73	2.28
SD	0.05	0.07
% CV	6.8	3.1
Data points included	304	297
Data points omitted	31	42

Table N.15 Internal quality controls for serum triglycerides (triacylglycerols) for Year 3 of the NDNS rolling programme

QC Lot No	46401	46403
Mean (mmol/L)	0.88	2.42
SD	0.04	0.06
% CV	4.3	2.6
Data points included	807	820
Data points omitted	32	20

N.2.5.2 External quality controls for serum triglycerides (triacylglycerols)

External quality control was achieved through the UKNEQAS General Chemistry and RIQAS.

N.2.6 Plasma ferritin

This assay was performed using the Siemens BN ProSpec® system which uses particle-enhanced immunonephelometry for the quantitative determination of ferritin in heparinised human plasma. Polystyrene particles coated with specific antibodies to human ferritin are agglutinated when mixed with samples containing human

ferritin. The intensity of the scattered light in the nephelometer is proportional to the ferritin content of the sample; therefore, the ferritin concentration can be quantitated by comparison to dilutions of a calibrant of known concentration.

N.2.6.1 Internal quality controls for plasma ferritin

Control serum was obtained commercially containing low, medium and high concentrations of ferritin and was included in each run. Results were checked to ensure they fell within the manufacturer's target range. The results in Tables N.16, N.17 and N.18 indicate good between-batch consistency for ferritin results during Years 1 to 3.

Table N.16 Internal quality controls for ferritin for Year 1 of the NDNS rolling programme

Year 1	Low			Medium			High			
Mean	38.9	40.9	38.5	99.0	97.5	98.9	143.4	152.8	150.8	164.6
SD	2.3	3.0	3.3	4.5	4.7	3.1	7.1	10.2	6.3	5.7
% CV	6.0	7.3	8.6	4.5	4.8	3.1	5.0	6.6	4.2	3.5
N	38	21	21	13	42	21	14	24	17	15

Table N.17 Internal quality controls for ferritin for Year 2 of the NDNS rolling programme

Year 2	Low	Medium	High
Mean	37.5	114.1	153.7
SD	3.0	10.2	12.3
% CV	8.1	8.9	8.0
N	15	14	15

Table N.18 Internal quality controls for ferritin for Year 3 of the NDNS rolling programme

Year 3	Low		Medium		High	
Mean (µg/l)	37.6	38.9	98.6	92.6	155.2	140.9
SD (µg/l)	3.7	3.9	9.1	6.6	15.0	12.6
CV (%)	9.8	10.1	9.2	7.2	9.6	8.9
N	11	17	15	14	13	17

N.2.6.2 External quality controls for plasma ferritin

External quality assessment was through the UKNEQAS Haematinics scheme.

Figures N.4, N.5 and N.6 show the bias relative to the target concentration during the years when NDNS samples were being analysed. Closed circles represent HNR results; open circles represent results from other laboratories which use the same method as HNR. DI (Deviation Index) relates to the distribution of results from all laboratories and indicates by how many standard deviations a HNR result differs from the All Laboratory Trimmed Mean.

A small DI (+ or -) indicates close agreement. Mean Running Bias Index Score (MRBIS) is the mean of the 10 most recent bias estimates; results of 0.51 (Year 1), 0.22 (Year 2) and -0.44 (Year 3) indicate that there was good overall agreement between HNR results and the target concentrations.

Figure N.4 External quality controls for ferritin for Year 1 of the NDNS Rolling Programme

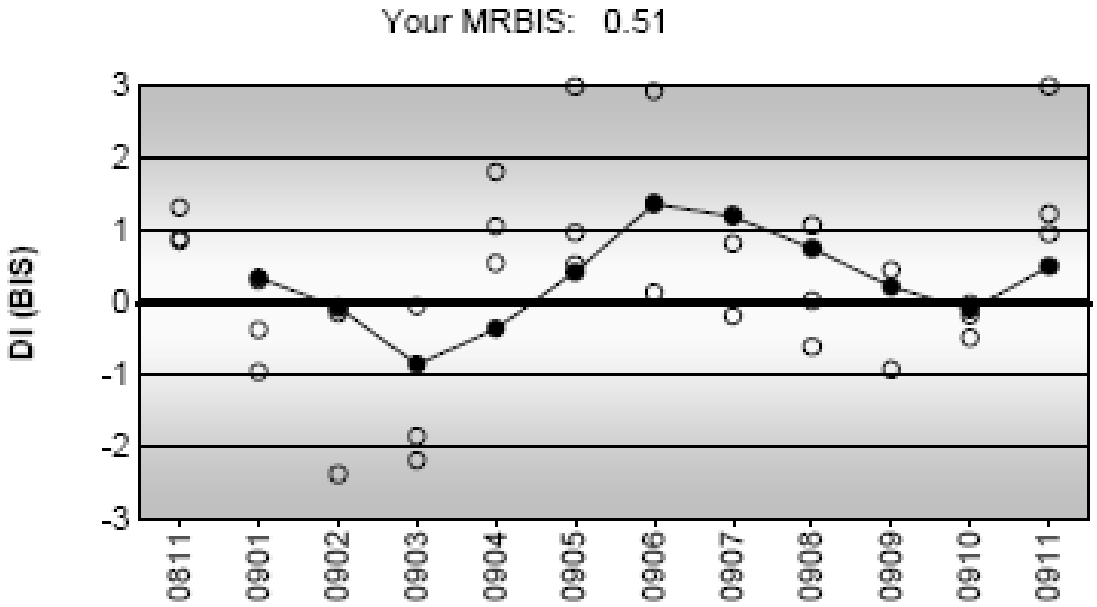


Figure N.5 External quality controls for ferritin for Year 2 of the NDNS Rolling Programme

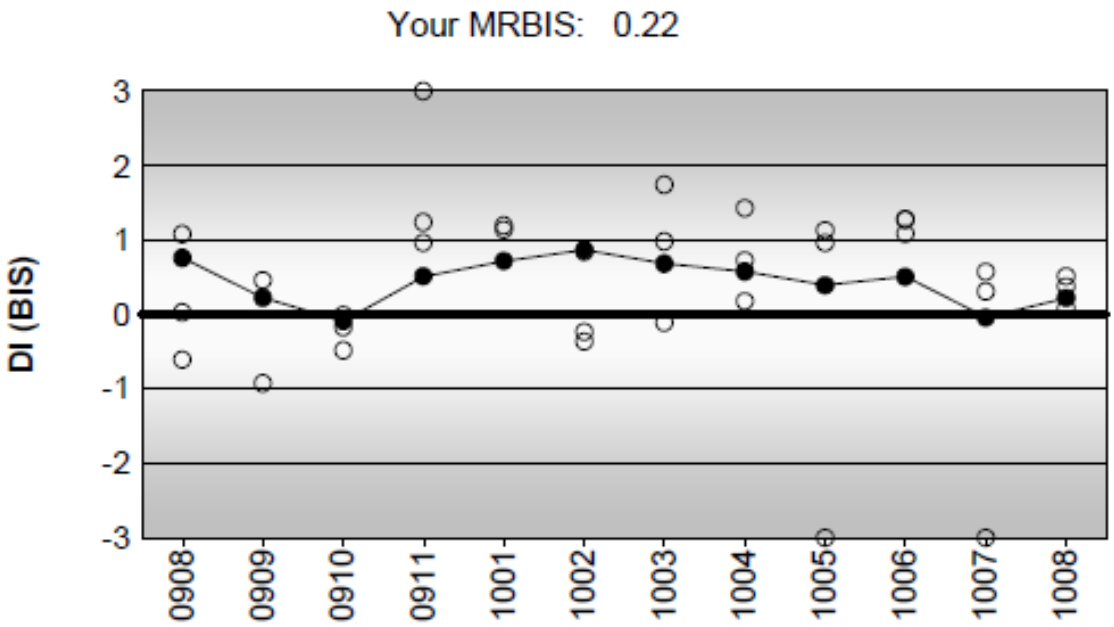
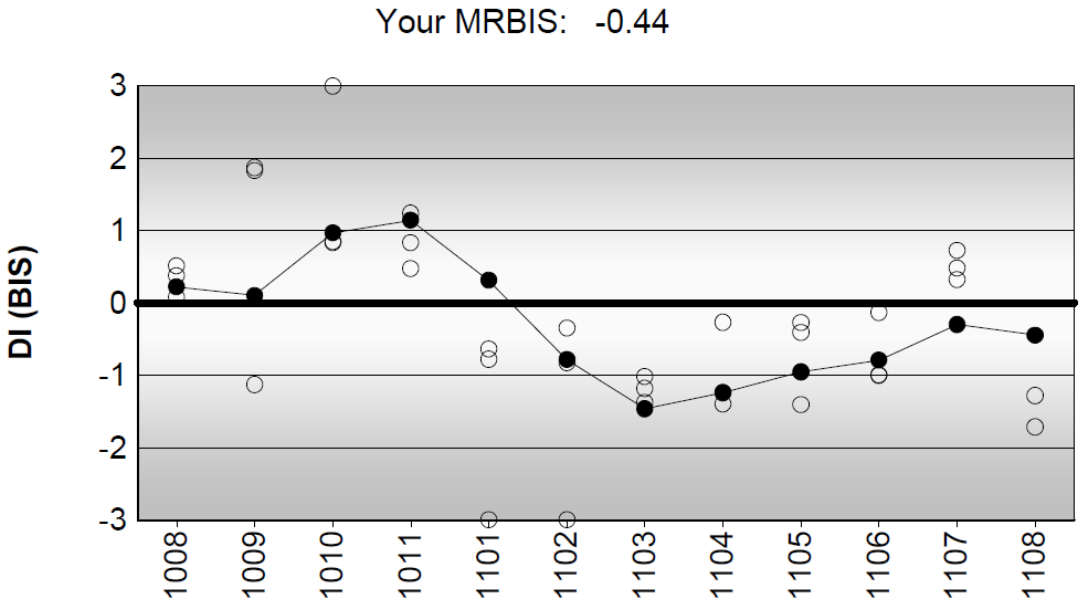


Figure N.6 External quality controls for ferritin for Year 3 of the NDNS Rolling Programme



N.2.7 Plasma transferrin receptors

The transferrin receptor (TfR) assay is an enzyme immunoassay (EIA) based upon the double antibody sandwich method (Ramco Laboratories Inc, Texas, USA). Plasma samples are diluted in buffer and pipetted into microwells pre-coated with a polyclonal antibody to TfR. Horseradish peroxidase (HRP) conjugated murine monoclonal antibody specific for TfR is added to the wells and incubated for two hours at room temperature. During this incubation, the TfR binds to the polyclonal antibodies adsorbed to the wells and the HRP-conjugated second antibodies bind to the captured TfR. Any unbound TfR and excess HRP conjugate are removed from the wells by washing. Enzyme substrate (tetramethylbenzidine, TMB) is added to the wells and through the action of HRP forms a blue product. Upon the addition of an acid stop solution the blue product is converted to a yellow colour, the intensity of which is measured in a plate reader set at 450nm. A standard curve is generated by plotting the absorbance versus concentration of the TfR standards provided in the kit. The concentration of the TfR in the sample is then determined by comparing the sample's absorbance with the standard curve.

The manufacturers' estimate of limit of detection is 0.07µg/mL. In order to optimise sample ID tracking and to minimise analyst-to analyst variation HNR has automated this assay using the BEST 2000 (Launch Diagnostics). Results are not compromised by haemolysis; there is slight positive interference by high concentrations of bilirubin.

N.2.7.1 Quality controls for plasma transferrin receptors

QC samples (low, high) supplied by the kit manufacturer were run in each batch. Because batch changes for these controls will preclude comparisons over the rolling programme, unassayed human plasma was also included. The controls were assayed at the beginning and end of each batch and therefore these statistics represent a combination of intra- and inter-assay precision.

Results for each run were checked to ensure they fell within the manufacturer's target range. The results in the tables below indicate good between-batch consistency for transferrin receptor results during Year 1, 2 and 3.

There is no external quality assessment scheme for plasma transferrin receptor measurement.

Table N.19 Internal quality controls for transferrin receptors for Year 1 of the NDNS rolling programme

	Kit low control	Kit high control	Dade low control	Dade medium control	Dade high control	Unassayed serum
Mean	5.24	14.95	2.21	3.47	5.43	8.47
SD	0.50	1.02	0.33	0.31	0.46	0.93
% CV	9.5	6.8	14.9	8.9	8.5	11.0
N	28	28	25	22	24	28

Table N.20 Internal quality controls for transferrin receptors for Year 2 of the NDNS rolling programme

	Kit low control	Kit high control	Dade low control	Dade medium control	Dade high control	Unassayed serum
Mean	5.0	15.9	2.80	4.41	5.52	8.7
SD	0.22	1.11	0.16	0.21	0.27	0.42
% CV	4.4	7.0	5.7	4.9	4.9	4.8
N	35	35	30	30	31	35

Table N.21 Internal quality controls for transferrin receptors for Year 3 of the NDNS rolling programme

	Kit low control	Kit high control	Kit low control	Kit high control	Kit low control	Kit high control	In-house QA
Mean	5.03	16.68	4.50	18.26	4.59	20.70	8.05
SD	0.26	1.42	0.40	1.45	0.49	2.96	0.56
% CV	5.12	8.52	8.81	7.95	10.63	14.31	6.95
N	27	28	26	26	18	18	76

Dade controls were discontinued during the year.

N.2.8 Plasma vitamin C

This assay is based on the procedure described by Vuilleumier and Keck.[6] Samples are stabilised immediately after separation using an equal volume of 10% metaphosphoric acid.

Ascorbic 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 plate reader, which measures the fluorescence.

N.2.8.1 Internal quality controls for plasma vitamin C

QC samples were made in-house by spiking ascorbic acid-depleted plasma. The results in Tables N.22, N.23 and N.24 indicate good between-batch consistency for vitamin C (ascorbic acid) measurements during Year 1, 2 and 3.

Table N.22 Internal quality controls for vitamin C for Year 1 of the NDNS rolling programme

Vitamin C	µmol/L	
	QC2	QC3
Mean	28.0	51.0
SD	2.8	4.5
% CV	11.1	9.5
N	189	188

Table N.23 Internal quality controls for vitamin C for Year 2 of the NDNS rolling programme

Vitamin C	µmol/L	
	QC 2	QC 3
Mean	28.0	51.0
SD	3.8	6.2
% CV	13.4	12.0
N	80	80

Table N.24 Internal quality controls for vitamin C for Year 3 of the NDNS rolling programme

Vitamin C	µmol/L	
	QC2	QC3
mean (µmol/L)	28.1	52.6
sd (µmol/L)	2.5	2.9
cv (%)	9.0	5.6
N	68	68

N.2.8.2 External quality controls for vitamin C

HNR subscribes to the NIST External Quality Assessment scheme for vitamin C. Samples were distributed quarterly and results were always within the target range. The close agreement of the results in Figures N.7, N.8 and N.9 (open and closed circles) with the consensus (blue line) indicates excellent accuracy of our results.

Figure N.7 External quality controls for vitamin C for Year 1 of the NDNS rolling programme

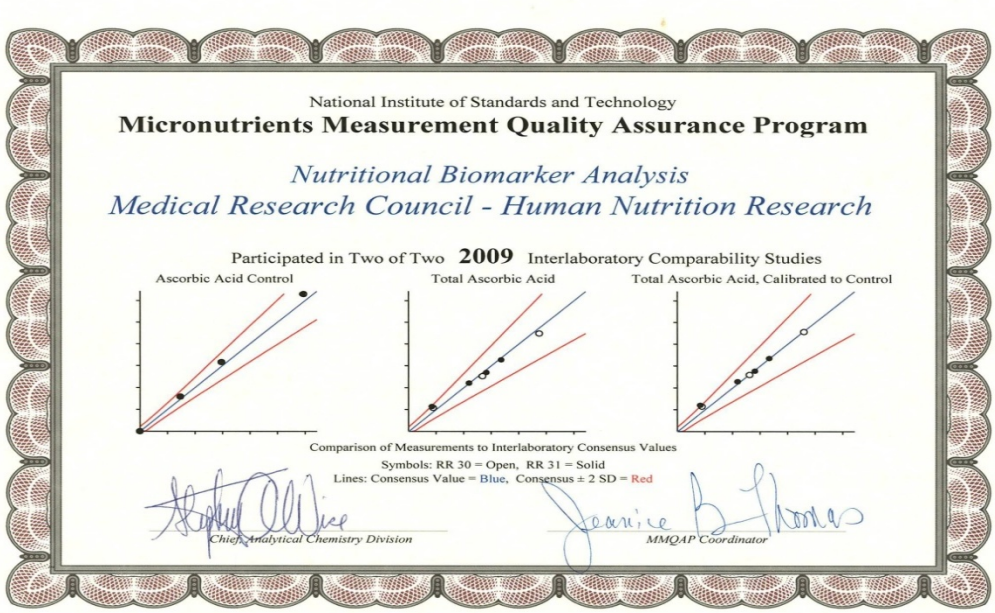


Figure N.8 External quality controls for vitamin C for Year 2 of the NDNS rolling programme

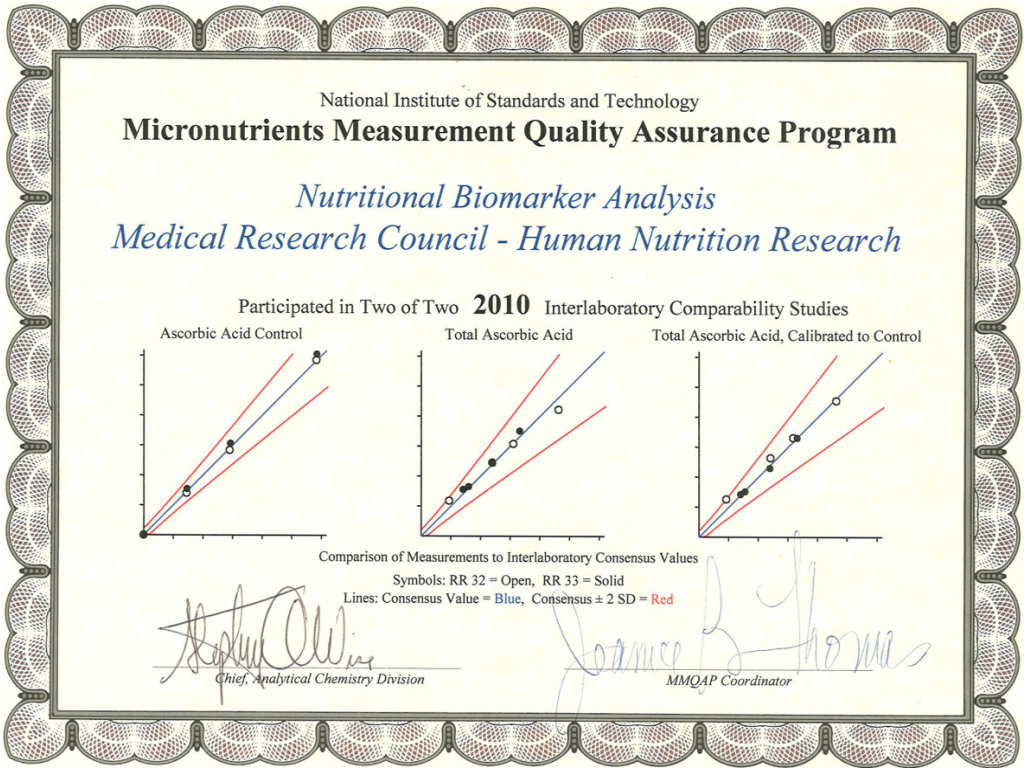
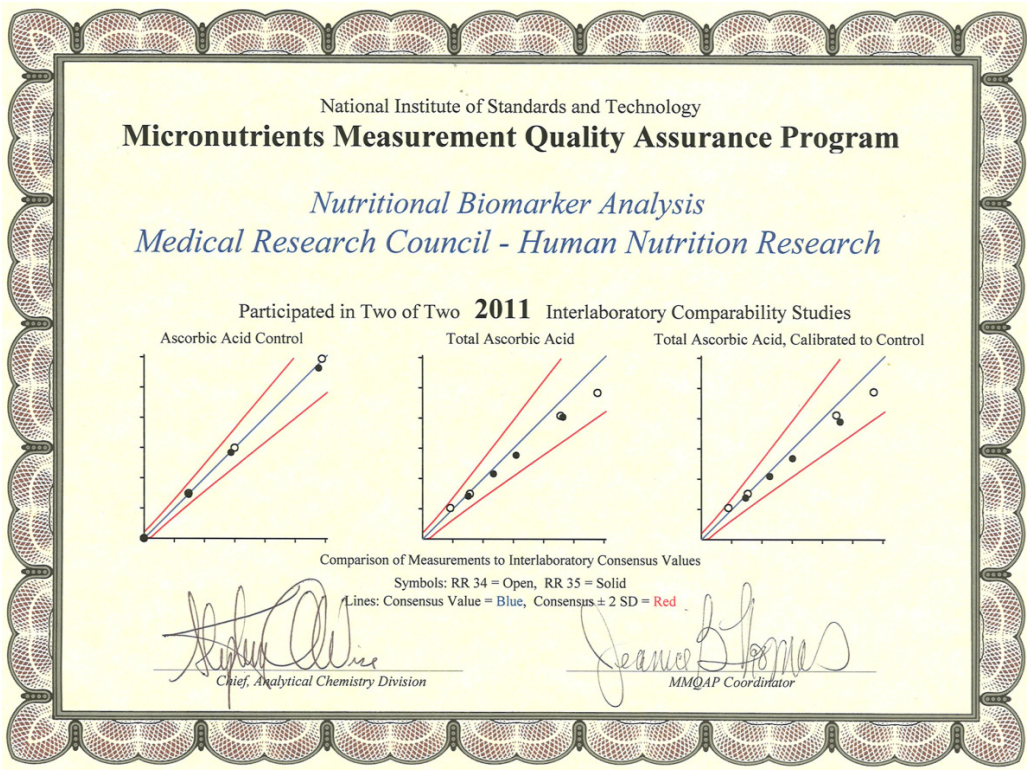


Figure N.9 External quality controls for vitamin C for Year 3 of the NDNS rolling programme



N.2.9 Erythrocyte transketolase activation coefficient (ETKAC) for thiamin status

This assay is based on that of Vuilleumier *et al*[7] 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. Thiamin status is assessed using the activation coefficient, which is the ratio of cofactor-stimulated activity to the basal activity without any added cofactor.

This method is identical in principle with its predecessor on the Cobas Fara platform. An analysis of bias between the results determined on the two platforms was performed ahead of the rolling programme.

There are no available sources of erythrocytes with known ETKAC; therefore unassayed material was prepared in-house. Erythrocytes from 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 B_1 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).

N.2.9.1 Quality control results for ETKAC

Descriptive statistics in Tables N.25, N.26 and N.27 for internal QCs indicate good batch-to-batch consistency of ETKAC results during Years 1, 2 and 3

There are no external Quality Assurance or QC schemes available for ETKAC.

Table N.25 Internal quality controls for ETKAC for Year 1 of the NDNS rolling programme

Control ID	Scipac B	UK NBTS neat*	UK NBTS diluted x2*	UK NBTS neat**	UK NBTS diluted x2**
Mean	1.06	1.08	1.07	1.05	1.05
SD	0.04	0.03	0.04	0.04	0.05
% CV	3.6	2.3	3.8	3.6	4.7
N	35	28	27	15	15

* old QC material, now finished
** new QC material being run in parallel

Table N.26 Internal quality controls for ETKAC for Year 2 of the NDNS rolling programme

Control ID	Scipac B	UK NBTS neat	UK NBTS, diluted x 2
Mean	1.07	1.02	1.03
SD	0.06	0.07	0.04
% CV	5.4	6.8	3.6
N	29	29	29

Table N.27 Internal quality controls for ETKAC for Year 3 of the NDNS rolling programme

Control ID	Scipac B	UK NBTS neat	UK NBTS, diluted x 2	Scipac A (purple)	Scipac C (red)	Scipac A diluted x2
Mean	1.05	1.03	1.06	1.11	1.08	1.11
SD	0.04	0.03	0.03	0.07	0.03	0.07
% CV	3.66	2.86	2.37	6.20	3.12	6.12
N	22	30	30	126	126	125

N.2.10 Erythrocyte glutathione reductase activation coefficient (EGRAC) for riboflavin status

This assay was developed from the original manual technique developed by Glatzle *et al*[8] and was adapted to the 'in-house' method using a Cobas Fara centrifugal analyser, which in turn has been 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 (1.5μM), 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 assay is in principle identical to its predecessor which used the Cobas Fara. A comparison of results obtained on the two platforms was performed using NDNS Year 1 Quarter 1 samples, which showed good agreement.

N.2.10.1 Quality controls for EGRAC

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

N.2.10.1.1 Internal quality control results during NDNS Year 1 to 3

Descriptive statistics in Tables N.28, N.29 and N.30 for internal QCs indicate good batch-to-batch consistency of EGRAC results during Years 1, 2 and 3.

Table N.28 Internal quality controls for EGRAC for Year 1 of the NDNS rolling programme

Control ID	A	C	X
Mean	2.14	1.52	0.99
SD	0.16	0.07	0.02
% CV	7.3	4.9	1.8
N	30	32	32

Table N.29 Internal quality controls for EGRAC for Year 2 of the NDNS rolling programme

Control ID	A	C	X
Mean	2.28	1.60	0.99
SD	0.15	0.06	0.02
% CV	6.4	3.6	2.3
N	33	34	34

Table N.30 Internal quality controls for EGRAC for Year 3 of the NDNS rolling programme

Control ID	A	C	X
Mean	2.27	1.63	1.00
SD	0.10	0.03	0.02
% CV	4.23	2.14	1.95
N	71	69	72

N.2.11 Plasma vitamin B_6 (PLP and PA)

A reverse-phase high performance liquid chromatography (HPLC) method with post column derivatisation and fluorimetric detection was used to determine pyridoxal-5-phosphate (PLP) and 4-pyridoxic acid (PA) in plasma.[9]

N.2.11.1 Quality controls for vitamin B_6

QC was achieved through internal procedures. QC material was produced by spiking human plasma with aqueous solutions of PLP and PA. The final QC concentration was designed to match typical mid-range human samples and previously provided CDC 'mid bench' quality controls. The QC material was spiked so that the additional aqueous content represented only 0.02% of the total medium. Duplicate analysis of the QC material was performed with each analytical run. When the mean percentage recovery was outside of the range 95 to 105% of nominal the analytical results for that run were corrected accordingly. There were no external quality schemes for the vitamin B_6 HPLC method.

N.2.11.1.1 Internal quality controls for vitamin B_6

The good agreement between the obtained values for PLP and PA in the quality control and the expected values in Table N.31 indicates a high degree of accuracy for this method.

Table N.31 Internal quality controls for PLP for Years 1, 2 and 3 of the NDNS rolling programme

PLP	Year 1	Year 2	Year 3
Mean (nmol/L)	43.9	44.1	46.0
SD	2.0	2.4	3.8
% CV	4.6	5.4	8.4
n	16	18	19
PA	**Year 1**	**Year 2**	**Year 3**
Mean (nmol/L)	52.0	52.6	54.0
SD	1.4	1.6	3.0
% CV	2.6	3.1	5.5
n	16	18	19

Note the expected PLP concentration of the spiked plasma was 43nmol/L (the sum of the basal level in the plasma plus the spike concentration). The expected PA concentration of the spiked plasma was 51nmol/L (the sum of the basal level in the plasma plus the spike concentration).

N.2.12 Plasma total homocysteine

This assay was performed using the Siemens BN ProSpec® system which uses particle-enhanced immunonephelometry for the quantitative determination of homocysteine in heparinised human plasma. In the competitive assay, bound homocysteine in the sample is reduced to free homocysteine by the action of dithiothreitol, and then converted enzymatically to S-adenosyl-homocysteine (SAH) in the next step. Conjugated S-adenosylcysteine (SAC), added at the onset of the reaction, competes with the SAH in the sample for bonding by anti-SAH antibodies bound to polystyrene particles. In the presence of SAH, there is either no aggregation or a weaker aggregation of particles. In the absence of SAH in the sample, an aggregation of the polystyrene particles by the conjugated SAC occurs. The higher the SAH content of the reaction mixture, the smaller the scattered light signal. The concentration is determined by comparison with a calibrant of known concentration.

N.2.12.1 Quality controls for plasma total homocysteine

QC was achieved through internal and external procedures. Control serum was obtained commercially containing low medium and high concentrations of

homocysteine and was included in each run. Results were checked to ensure they fell within the manufacturer's target range. Because the manufacturer's "acceptable" results range was very wide, HNR determined more stringent site-specific precision requirements within the manufacturer's published range and QC results for each analytical run were judged against these more demanding criteria, using JMPIN QC software.

In order to confirm analytical accuracy, HNR participated in an international external quality assessment scheme for homocysteine. Four samples were distributed by the scheme per year to a large number of laboratories, and the results analysed overall and for each analytical method. HNR results compared well with others in the same method group and performance was rated within consensus relative to the overall spread of results, indicating acceptable accuracy of the reported homocysteine results.

N.2.12.1.1 Internal quality controls for plasma total homocysteine

The results in Tables N.32, N.33 and N.34 indicate good between-batch consistency for homocysteine results during Years 1, 2 and 3

Table N.32 Internal quality controls for homocysteine for Year 1 of the NDNS rolling programme

Year 1	Low		Medium		High	
Mean	7.2	12.2	11.1	12.1	23.8	24.4
SD	0.6	0.9	0.8	0.9	2.2	1.6
% CV	7.8	7.2	7.5	7.5	9.1	6.6
N	19	30	20	30	18	23

Table N.33 Internal quality controls for homocysteine for Year 2 of the NDNS rolling programme

Year 2	Low	Medium	High
Mean	7.6	11.9	24.3
SD	0.5	1.0	1.5
% CV	6.9	8.8	6.0
N	11	12	13

Table N.34 Internal quality controls for homocysteine for Year 3 of the NDNS rolling programme

Year 3	Low			Medium		High		
Mean	7.2	6.5	7.0	10.8	12.0	25.2	23.8	24.1
SD	0.8	0.5	0.7	1.1	1.1	2.0	1.7	2.6
% CV	11.0	7.5	9.6	9.7	8.8	7.8	7.2	10.7
N	11	7	12	9	10	11	7	12

N.2.13 Plasma retinol, retinyl palmitate, α– and γ–tocopherol, and individual carotenoids

Fat soluble micronutrients were determined by HPLC coupled with a photodiode array detector, capable of multi-wavelength detection. The analytical method used was derived from Thurnham *et al.*[10] Plasma concentrations of vitamin A (retinol), retinyl palmitate, α-, and γ-tocopherol, and seven carotenoids (α- and β-carotene, α- and β-cryptoxanthin, lycopene and lutein and zeaxanthin [xanthophyll]) were determined. The analytical method (based upon Thurnham *et al*[10]) in the current survey was essentially the same as that used in all previous NDNS surveys. However it should be noted that differences in calibration techniques and in the application of extraction efficiency corrections may mean that it is not possible to directly compare the results of this survey with previous NDNS surveys.

An internal standard of tocopherol acetate was used to monitor losses during the extraction period and to account for any changes in volumes.

N.2.13.1 Quality controls for plasma retinol, retinyl palmitate, α– and γ–tocopherol and individual carotenoids

Heparinised human plasma from a commercial source (e.g. Seralab International, UK) was used to monitor long-term drift control and to provide an early warning of any changes in sensitivity of the assay and was run in duplicate for every batch of samples. The data from the pooled QCs was used with inter- and intra-analysis precision measurements. A commercial statistical package (JMP, SAS Institute, USA) was used to check the analysis was within the control limits. To check the extraction efficiency of the method a sample of human plasma was spiked with a known concentration of each fat soluble vitamin analyte. Following extraction, the

concentration of each fat soluble vitamin component was determined in both un-spiked and fortified plasma and the percentage recovery for each spike determined. Any batch with less than 100% extraction efficiency was corrected. Acceptable extraction efficiency was based on established extraction efficiencies. Mean extraction efficiencies for Years 1 and 2 were 95.2% (retinol), 87.5% (retinyl palmitate), 96.7% (α-tocopherol), 93.6% (γ-tocopherol), 79.5% (lutein), 86.6% (α-cryptoxanthin), 82.5% (β-cryptoxanthin), 85.2% (lycopene), 77.7% (α-carotene) and 70.8% (β-carotene). Mean extraction efficiencies for NDNS year 3 were 107% (retinol), 93% (retinyl palmitate), 91% (α-tocopherol), 93% (γ-tocopherol), 104% (lutein), 84% (α-cryptoxanthin), 85% (β-cryptoxanthin), 77% (lycopene), 68% (α-carotene), 68% (β-carotene).

Participation in round-robin studies conducted by National Institute of Standards and Technology (NIST), Centres of Disease Control VITAL-External Quality Assurance (CDC VITAL EQA) and UKNEQAS allowed inter-laboratory comparison of results. HNR participated in a twice yearly round robin with both NIST and VITAL EQA as well as a bi-monthly round robin with UKNEQAS.

N.2.13.1.1 Internal quality controls for plasma retinol, retinyl palmitate, α– and γ–tocopherol and individual carotenoids

The Fat soluble vitamin (FSV) results for Years 1, 2 and 3 were reported as plasma retinol, retinyl palmitate, α– and γ–tocopherol and individual carotenoids.

Table N.35 Internal quality controls (unspiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 1 of the NDNS rolling programme

	Retinol (µmol/L)	Retinyl palmitate (µmol/L)[1]	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	1.41	-	6.05	3.35	0.040
SD	0.06	-	0.30	0.18	0.01
% CV	4.5	-	4.9	5.4	31.2
N	16	-	16	16	16

[1] No value is given for retinyl palmitate in unspiked plasma because the levels are too low to yield reliable results.

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L) [1]	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.182	-	0.048	0.163	0.22
SD	0.03	-	0.01	0.03	0.02
% CV	14.3	-	23.9	21.3	8.7
N	16	-	16	16	16

[1] No value is given for α-cryptoxanthin in unspiked plasma because the levels are too low to yield reliable results.

Table N.36 Internal quality controls (unspiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 2 of the NDNS rolling programme (same QC plasma as Year 1)

	Retinol (µmol/L)	Retinyl palmitate (µmol/L) [1]	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	1.31	-	12.10	3.16	0.07
SD	0.36	-	5.34	0.51	0.03
% CV	27.3	-	44.2	16.2	45.5
N	6	-	6	6	6

[1] No value is given for retinyl palmitate in unspiked plasma because the levels are too low to yield reliable results.

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L) [1]	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.28	-	0.08	0.41	0.33
SD	0.13	-	0.02	0.14	0.10
% CV	46.1	-	24.0	34.6	30.9
N	6	-	6	6	6

[1] No value is given for α-cryptoxanthin in unspiked plasma because the levels are too low to yield reliable results.

Table N.37 Internal quality controls for plasma (unspiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 2 of the NDNS rolling programme (new QC plasma batch)

	Retinol (µmol/L)	Retinyl palmitate (µmol/L) [1]	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	1.44	-	20.57	3.62	0.08
SD	0.14	-	1.69	0.76	0.02
% CV	9.7	-	8.2	20.9	23.3
N	9	-	8	8	7

[1] No value is given for retinyl palmitate in unspiked plasma because the levels are too low to yield reliable results.

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L) [1]	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.27	-	0.09	0.46	0.30
SD	0.09	-	0.02	0.07	0.03
% CV	33.1	-	17.8	16.1	8.6
N	7	-	7	7	7

[1] No value is given for α-cryptoxanthin in unspiked plasma because the levels are too low to yield reliable results.

Table N.38 Internal quality controls (unspiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 3 of the NDNS rolling programme

	Retinol (µmol/L)	Retinyl palmitate (µmol/L) [1]	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	1.57	-	17.1	4.82	0.05
SD	0.19	-	2.25	1.42	0.02
% CV	12.2	-	13.1	29.4	47.4
N	15	-	15	15	15

[1] No value is given for retinyl palmitate in unspiked plasma because the levels are too low to yield reliable results.

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L) [1]	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.17	-	0.06	0.22	0.23
SD	0.08	-	0.01	0.12	0.07
% CV	45.5	-	21.0	53.4	32.3
N	15	-	15	15	15

[1] No value is given for α-cryptoxanthin in unspiked plasma because the levels are too low to yield reliable results.

Table N.39 Internal quality controls (spiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 1 of the NDNS rolling programme

	Retinol (µmol/L)	Retinyl palmitate (µmol/L)	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	3.67	0.28	20.02	5.69	0.14
SD	0.17	0.06	0.75	0.26	0.01
% CV	4.7	19.7	3.8	4.5	6.6
N	16	11	9	9	13

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L)	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.50	0.13	0.12	0.26	0.24
SD	0.11	0.02	0.02	0.05	0.04
% CV	22.0	15.4	14.1	18.8	18.2
N	15	15	14	13	14

Table N.40 Internal quality controls (spiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 2 of the NDNS rolling programme

	Retinol (µmol/L)	Retinyl palmitate (µmol/L)	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	2.50	0.48	16.64	4.11	0.15
SD	0.05	0.02	0.85	0.17	0.04
% CV	2.0	4.6	5.1	4.2	23.7
N	8	8	6	6	15

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L)	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.59	0.12	0.12	0.37	0.31
SD	0.07	0.03	0.01	0.07	0.05
% CV	12.4	22.9	8.9	17.9	16.1
N	15	13	15	15	15

Table N.41 Internal quality controls (spiked plasma) for plasma retinol, plasma retinyl palmitate, α– and γ– tocopherol and individual carotenoids for Year 3 of the NDNS rolling programme

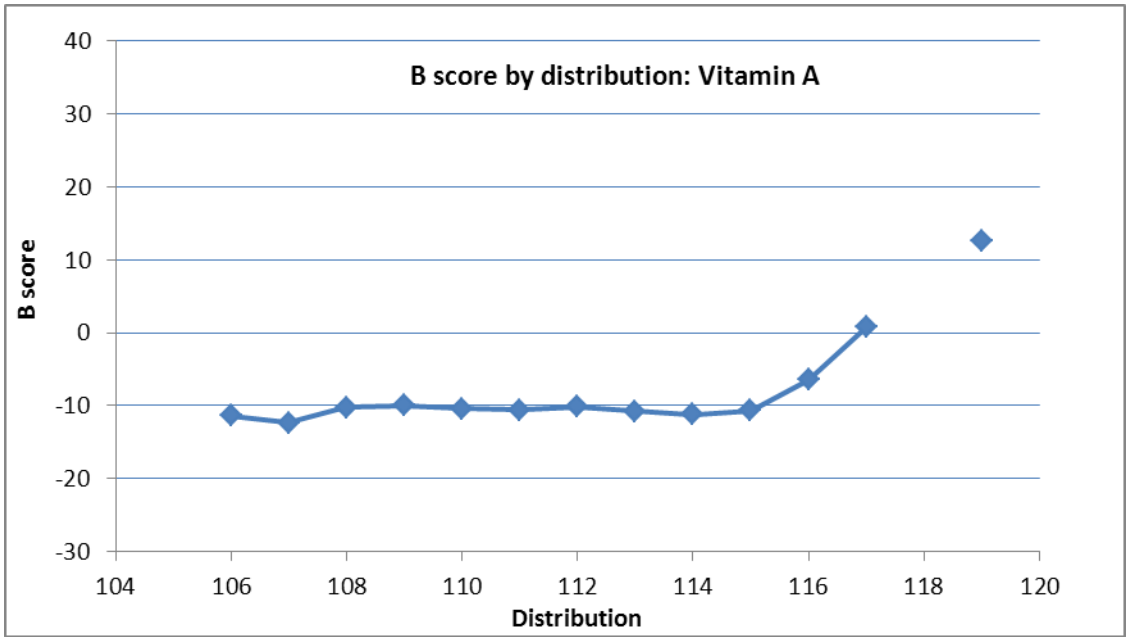
	Retinol (µmol/L)	Retinyl palmitate (µmol/L)	α-tocopherol (µmol/L)	γ-tocopherol (µmol/L)	α-carotene (µmol/L)
Mean	3.48	0.60	16.74	4.6	0.13
SD	0.49	0.06	1.66	0.45	0.02
% CV	14.1	9.2	9.9	9.7	17.8
N	12	13	13	13	14

	β-carotene (µmol/L)	α-cryptoxanthin (µmol/L)	β-cryptoxanthin (µmol/L)	Lycopene (µmol/L)	Lutein (µmol/L)
Mean	0.5	0.12	0.12	0.28	0.39
SD	0.07	0.01	0.01	0.08	0.10
% CV	14.1	11.0	6.8	28.7	25.4
N	14	14	14	14	14

N.2.13.1.2 External quality controls for plasma retinol, retinyl palmitate, α– and γ–tocopherol and individual carotenoids

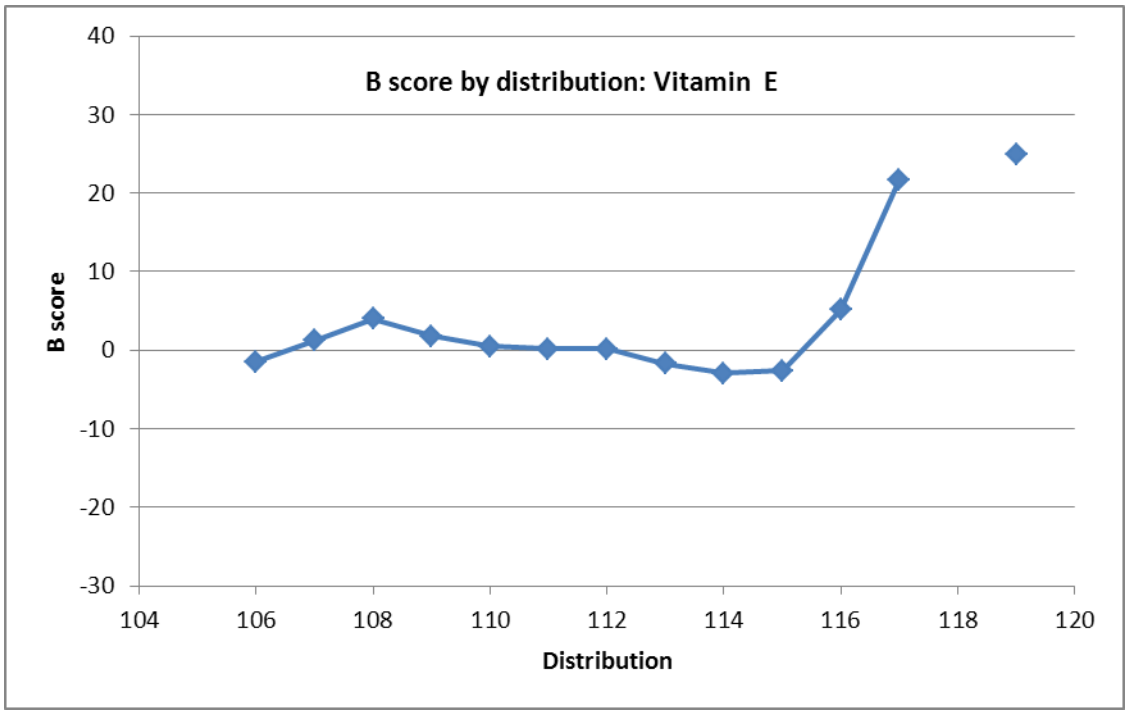
Figure N.10 shows bias scores for UKNEQAS returns during the period covering Years 1 to 3 of the NDNS rolling programme. Percentage bias was calculated as (result-target)/target*100 and was calculated as a rolling average by UKNEQAS. The results from the external quality schemes shown in Figure N.10 suggest that HNR is typically within the range for each fat soluble vitamin analyte. Values for α-cryptoxanthin are not given. For the purpose of this report retinol and retinyl palmitate, and α- and γ-tocopherol are represented by the vitamin A and vitamin E UKNEQAS returns respectively. The target values for many of the carotenoids are based upon results from a small number of participating laboratories and should be interpreted with care.

Figure N.10 Bias score charts for UKNEQAS returns during the period of Years 1-3 of the NDNS rolling programme



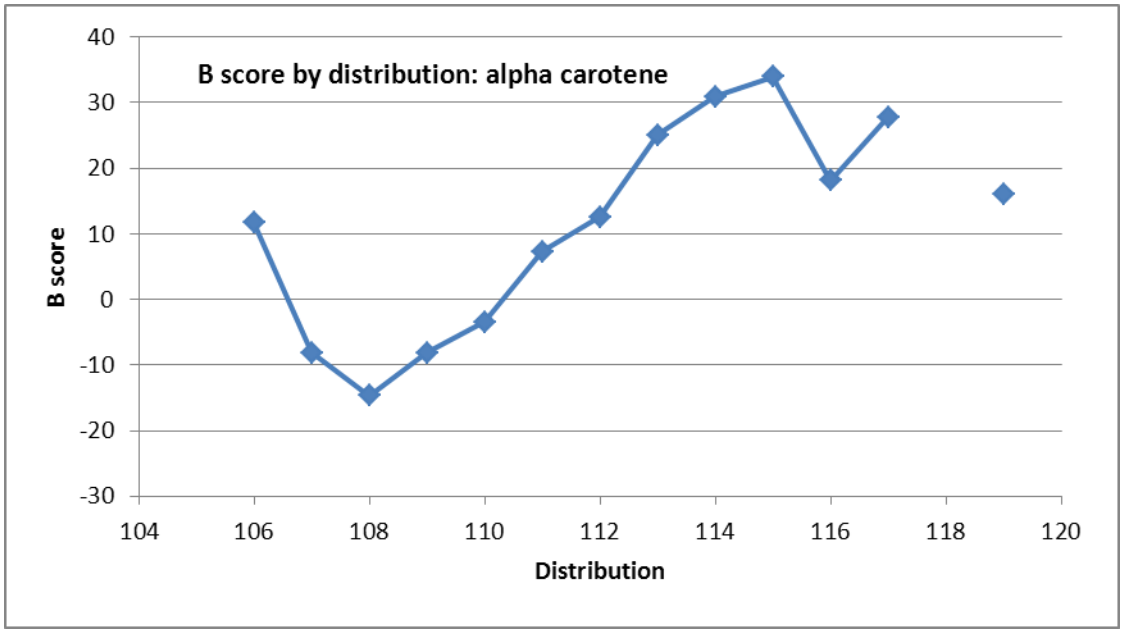
NEQAS B score limit = +/- 15

number of participating labs = 44



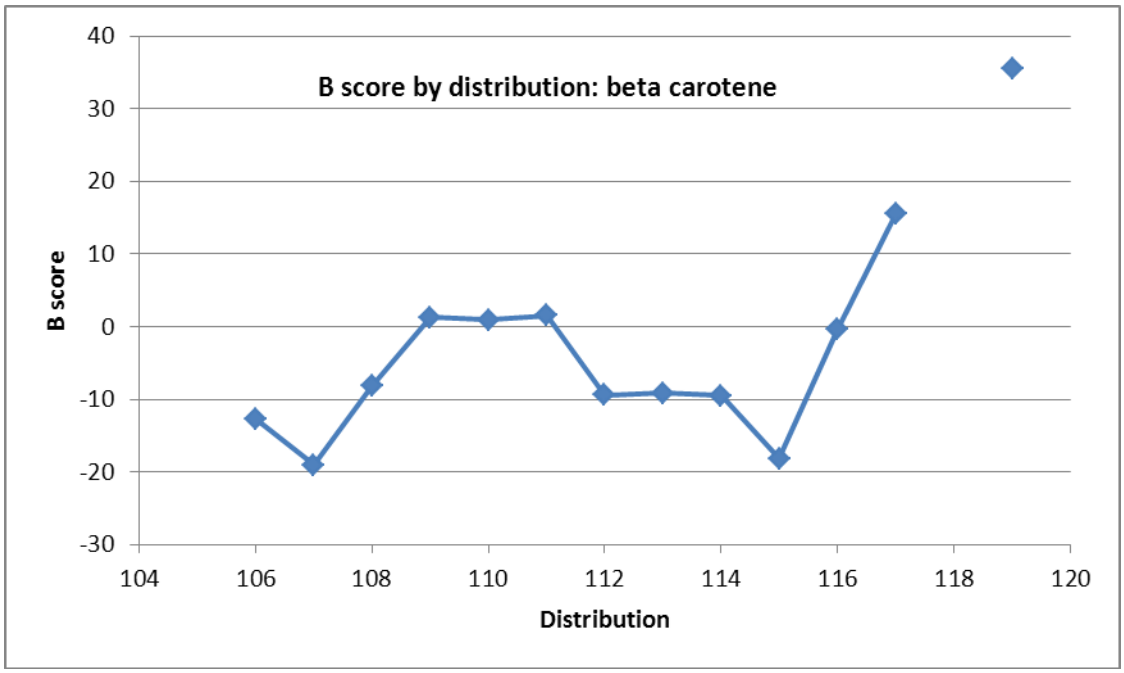
NEQAS B score limit = +/- 15

number of participating labs = 44



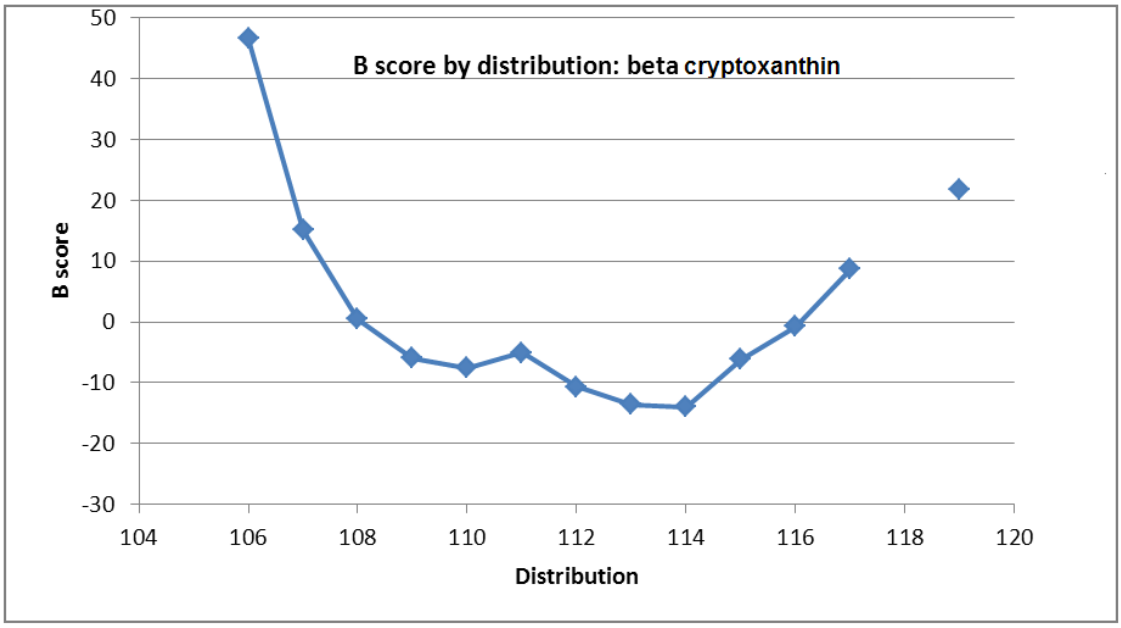
NEQAS B score limit = +/- 25

number of participating labs = 3



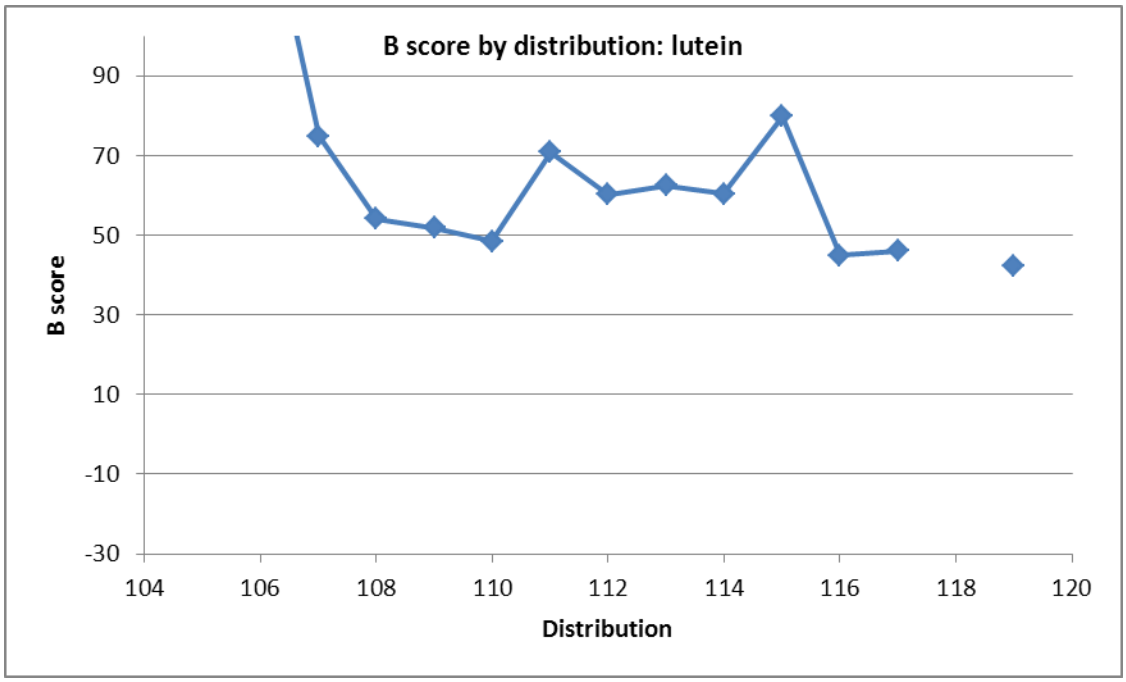
NEQAS B score limit = +/- 20

number of participating labs = 18



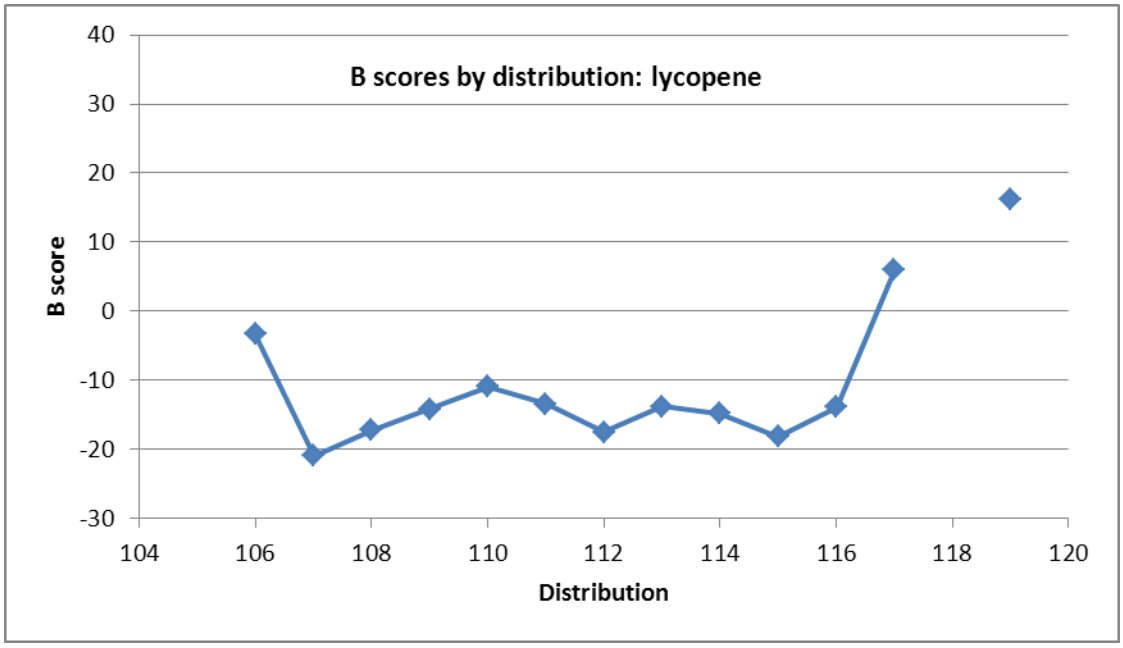
NEQAS B score limit = +/- 25

number of participating labs = 2



NEQAS B score limit = +/- 20

number of participating labs = 3



NEQAS B score limit = +/- 25

number of participating labs = 3

N.2.14 Plasma 25-hydroxyvitamin D (25-OHD)

The DiaSorin Liaison method for quantitative determination of 25-OHD is a direct, competitive chemiluminescence immunoassay (CLIA). A specific antibody to vitamin D is used for coating magnetic particles (solid phase), and vitamin D is linked to an isoluminol derivative. During the incubation, 25-OHD is dissociated from its binding protein, and competes with labeled vitamin D for binding sites on the antibody. After the incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25-OHD present in calibrators, controls, or samples.

The DiaSorin radio-immunoassay method was used in previous NDNS[11,12,13,14] to measure 25-OHD in plasma. A comparison study was carried out between the DiaSorin RIA method and the new DiaSorin Liaison method, which showed good agreement.

Samples for Year 1 to Year 3 were analysed in lithium heparin plasma, using DiaSorin Liaison reagents prior to the company's reformulation of the reagent kit during late 2011.

N.2.14.1 Quality controls for 25-OHD

Internal QCs were run with every batch, and HNR NBA also subscribed to the DEQAS external quality assessment scheme .

N.2.14.1.1 Internal quality controls for 25-OHD

Manufacturer's controls were run with each kit. These allow an instant assessment of whether the results obtained for respondents' samples are within limits. However as each batch of these is only issued for a short period they do not assess longer-term stability of the assay. For Year 1, the numbers for each lot were too small to be meaningful and so Lyphochek control was also run at intervals within each run to check for assay drift and consistency over a longer period,. For Year 2 and 3 more data were available for manufacturer controls and these are also included below.

Table N.42 Internal quality controls for 25-OHD for Year 1 of the NDNS rolling programme

	Lyphochek (nmol/L)
Mean	48.8
SD	6.1
%CV	12.6

Table N.43 Internal quality controls for 25-OHD for Year 2 of the NDNS rolling programme

	Control 1 (123521D) (nmol/L)	Control 2 (123521D) (nmol/L)	Lyphochek (nmol/L)
Mean	40.1	134	51.4
SD	3.8	10.3	6.0
% CV	9.4	7.7	11.7
N	23	22	21

Table N.44 Internal quality controls for 25-OHD for Year 3 of the NDNS rolling programme

Manufacturer's controls:							Lyphochek
Mean	41.6	146.2	41.0	133.2	40.6	130.4	55.9
SD	3.4	7.8	3.2	9.3	4.4	8.8	6.6
% CV	8.3	5.3	7.7	7.0	10.9	6.7	11.9
N	16	16	23	23	18	18	42

N.2.14.1.2 External quality controls for 25-OHD

HNR subscribed to the DEQAS external quality assessment scheme and the NBA laboratory's performance was assessed by the scheme organisers as meeting the performance target set by the DEQAS Advisory Panel (i.e. 80% or more of results were within +- 30% of the All Laboratory Trimmed Mean).DEQAS do not issue cumulative performance data as do NEQAS.

Figures N.11 and N.12 show the relationship between 25-OHD as reported on individual DEQAS samples by the NBA laboratory at HNR and the mean value obtained internationally using the Diasorin Liaison (n = approx. 400 laboratories), and the relationship between the results obtained in the NBA lab at HNR and the international All Laboratory Trimmed Mean (ALTM, n=approx.1100 laboratories).

Figure N.11 External quality controls for plasma 25OH vitamin D – Years 1-3 of rolling programme (DEQAS; HNR result versus Liaison method mean)

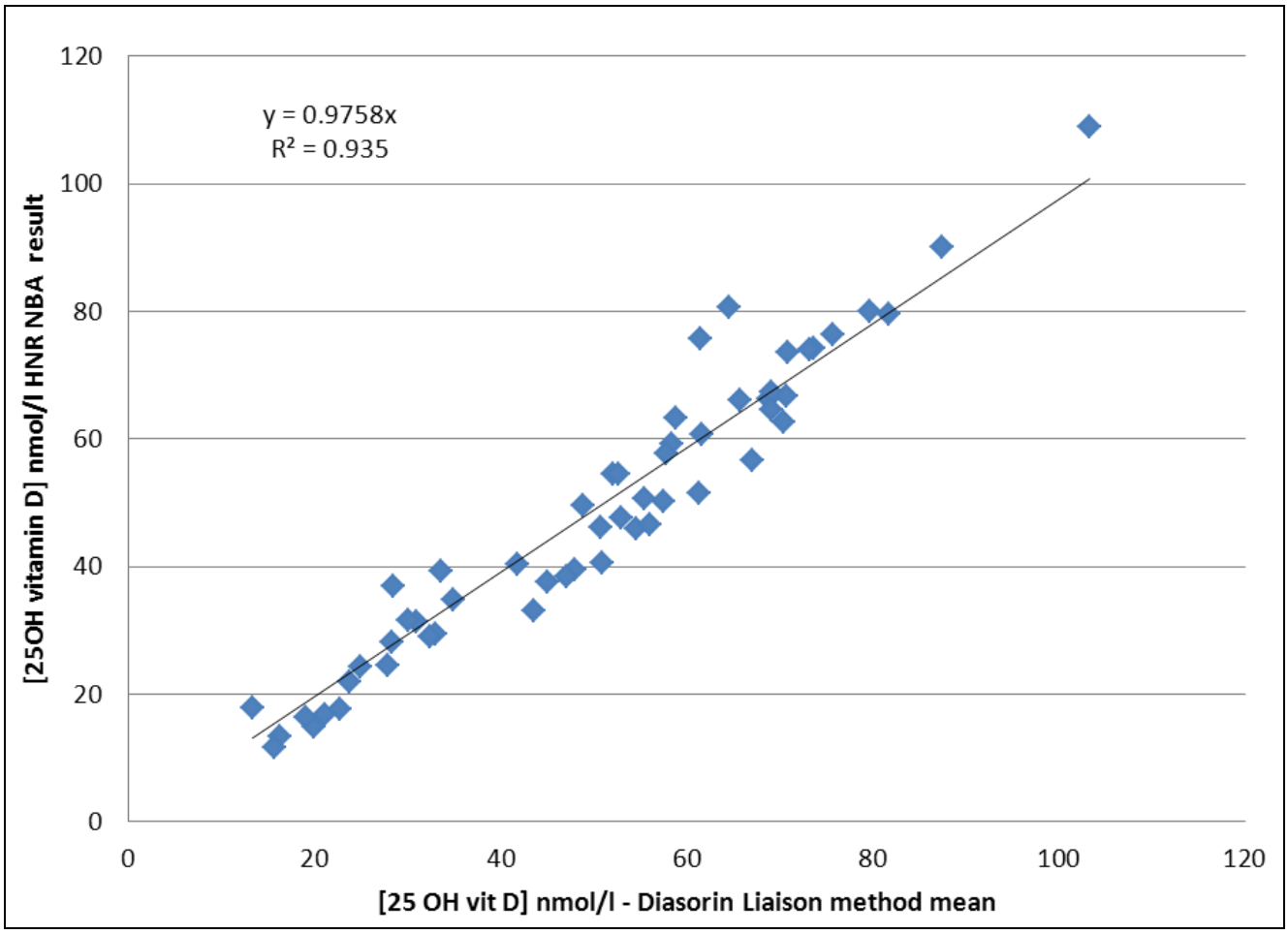
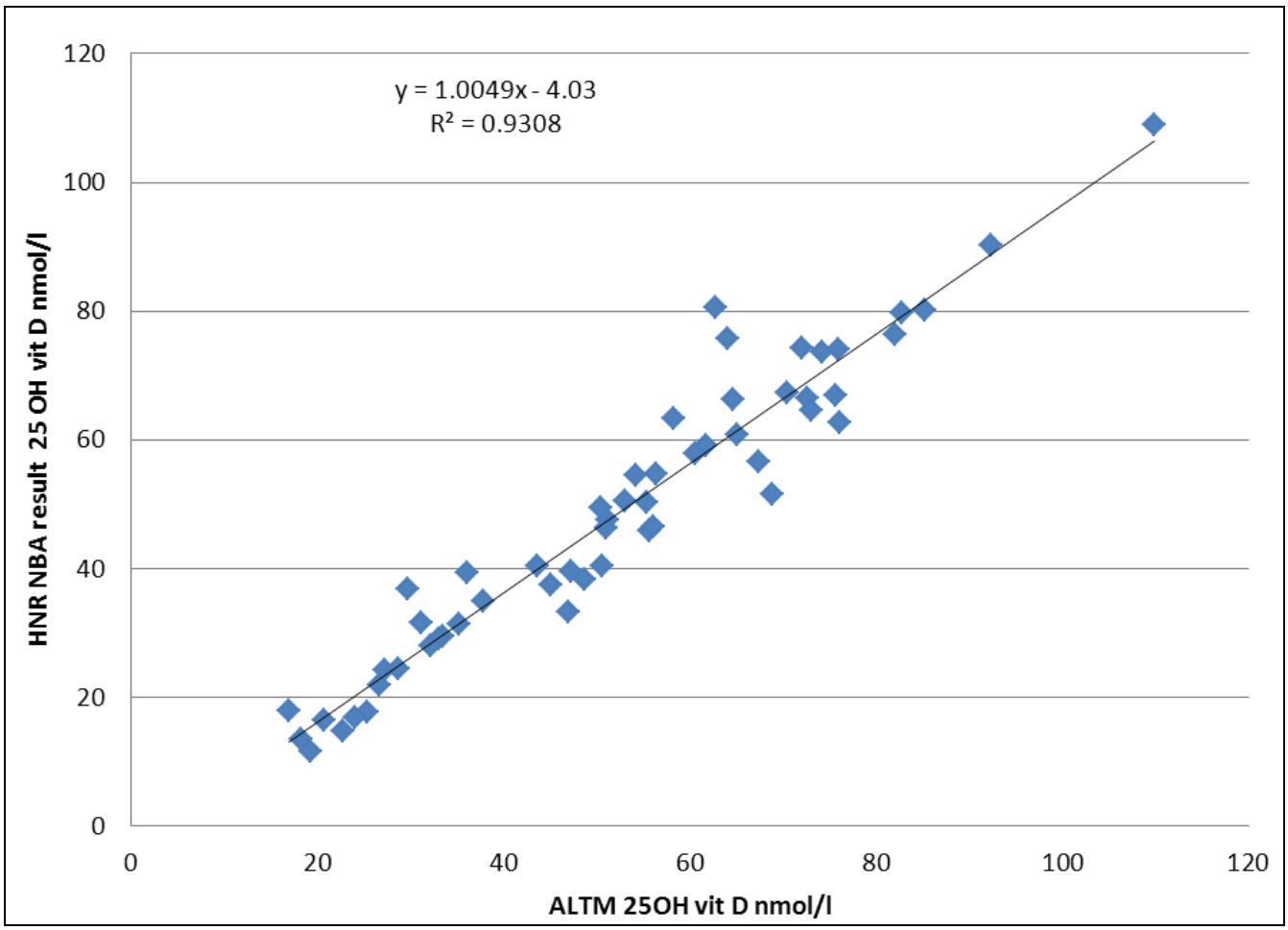


Figure N.12 External quality controls for plasma 25OH vitamin D– Years 1-3 of rolling programme (DEQAS; HNR results versus ALTM)



N.2.15 Plasma creatinine

The creatinine method used in the rolling programme employs a modification of the kinetic Jaffe reaction reported by Larsen.

Under alkaline conditions, creatinine reacts with picrate to form a red chromophore. The rate of increasing absorbance at 510nm due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured using a bichromatic (510nm, 600nm) rate technique. Bilirubin is oxidised by potassium ferricyanide to prevent interference. Plasma which has been in contact with blood cells for more than 8 hours before separation is not suitable for analysis. Therefore this method has been reported to be less susceptible than conventional methods to interference from non-creatinine, Jaffe-positive compounds.

N.2.15.1 Internal quality controls for plasma creatinine

Multiqual QCs containing low, moderate and high concentrations of creatinine are run with each sample set. If the results obtained are not within manufacturer's range and also within the range determined within our laboratory, the run is rejected. Table N.45 shows internal QC results for creatinine, covering the period when NDNS Year 1 and Year 2 samples were analysed and Table N.46 shows internal QC results for creatinine, covering the period when NDNS Year 3 samples were analysed.

Table N.45 Internal quality controls for plasma creatinine for Year 1 and Year 2 of the NDNS rolling programme

	Low	Medium	High
Mean creatinine µmol/L	62.9	170.7	607.1
SD µmol/L	4.2	3.0	13.0
CV %	6.7	1.7	2.1
N	36	36	33

Table N.46 Internal quality controls for plasma creatinine for Year 3 of the NDNS rolling programme

	Low		Medium		High	
Mean creatinineµmol/L	69.2	65.3	169.4	166.2	578.6	588.7
SD µmol/L	4.0	3.3	4.9	4.8	13.0	18.1
CV %	5.8	5.1	2.9	2.9	2.2	3.1
N	34	41	36	31	32	45

N.2.15.2 External quality controls for plasma creatinine

HNR subscribes to the UKNEQAS clinical chemistry. Figure N.13 is the UKNEQAS results for creatinine distributions 858 (19 April 2010) to 869 (4 October 2010). This covers the period when Year 1 and Year 2 creatinine retrospective reassay was performed and include an estimate of bias with respect to the All Laboratory Trimmed Mean (B score) and the consistency of that bias (C score). Results within the white area of the resulting chart indicate acceptable performance as determined by UKNEQAS. Figure N.14 is the UKNEQAS results for creatinine distributions 867

(August 2010) to 890 (July 2011). This covers the period when Year 3 creatinine analysis was performed and include an estimate of bias with respect to the All Laboratory Trimmed Mean (B score) and the consistency of that bias (C score). Results within the white area of the resulting chart indicate acceptable performance as determined by UKNEQAS.

Figure N.13 External quality controls for plasma creatinine – Year 1 and Year 2 of rolling programme (Bias and Consistency)

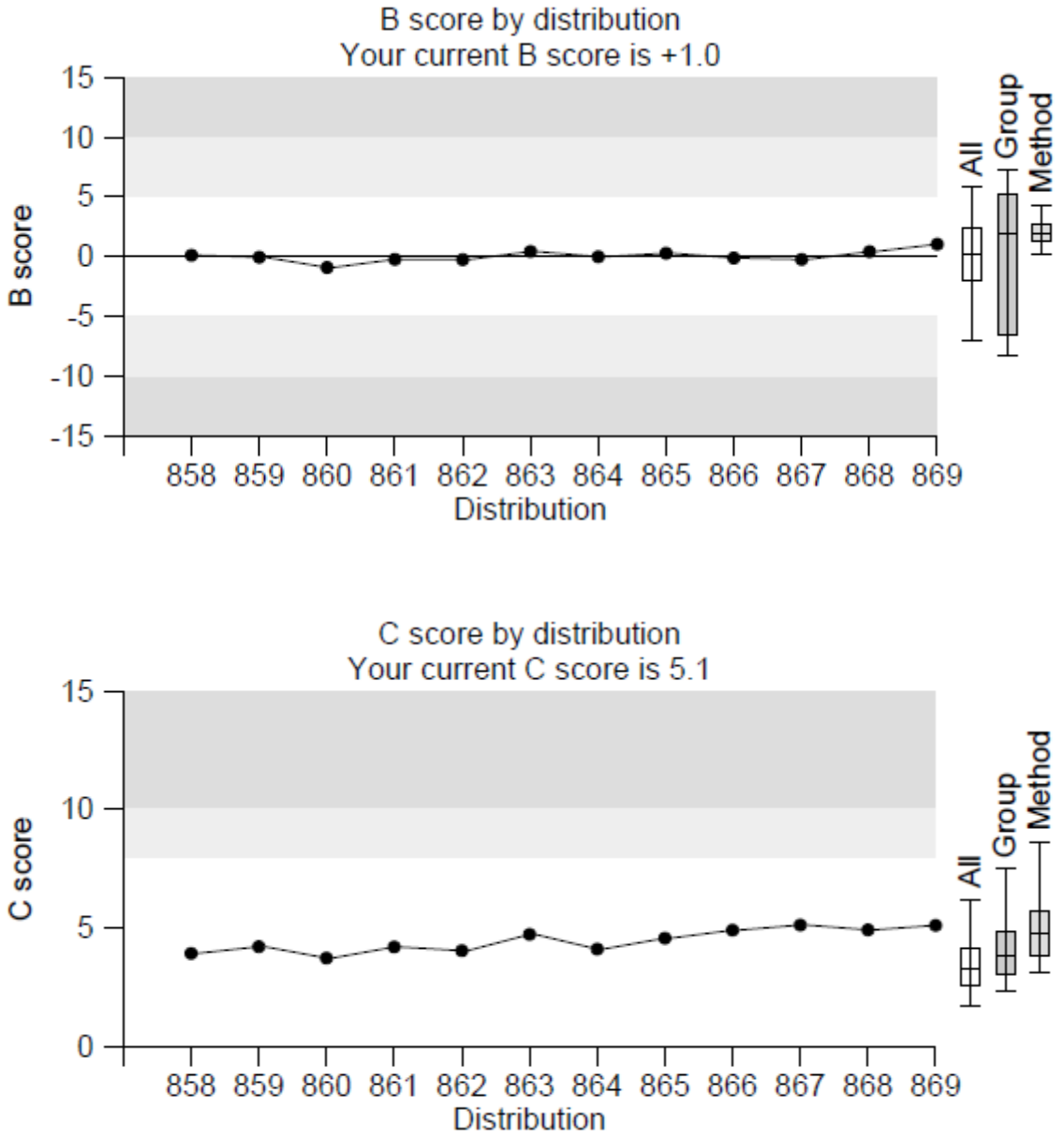
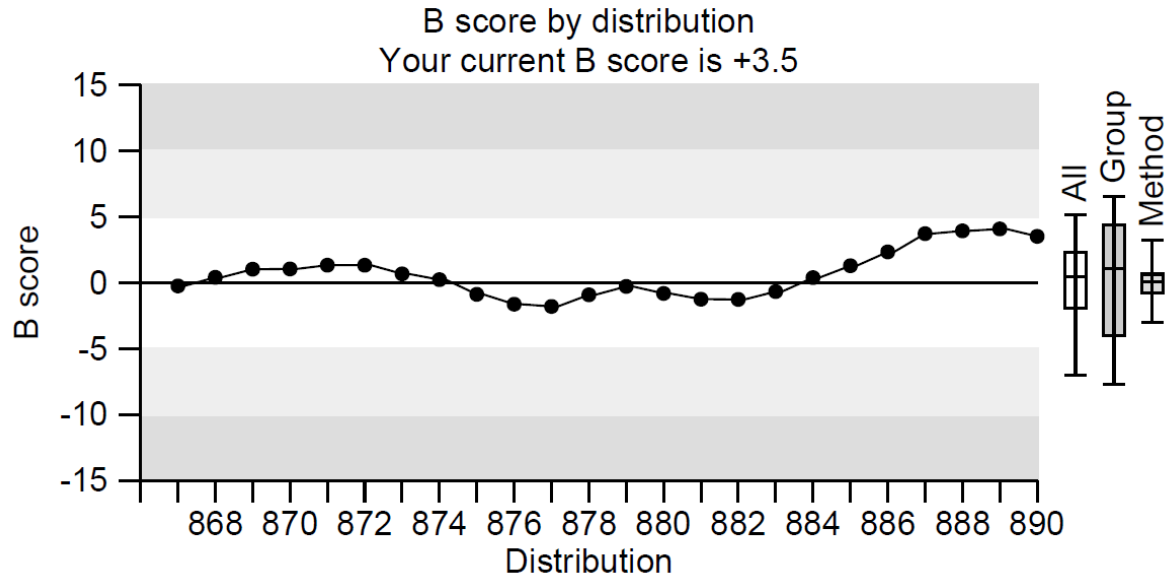
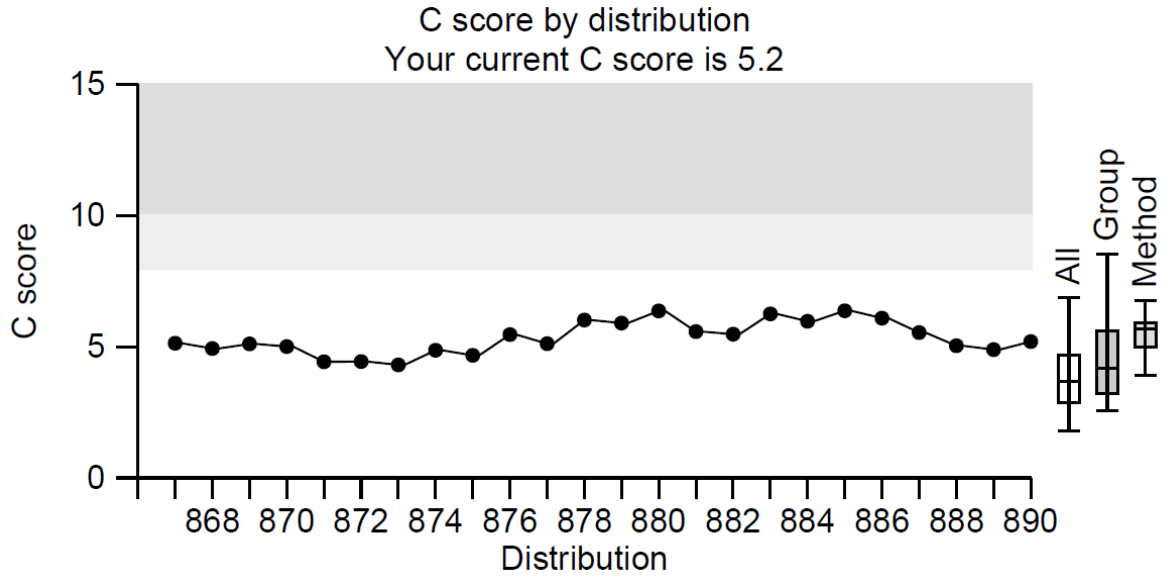


Figure N.14 External QC for creatinine – performance during Year 3 of the rolling Programme (Bias and Consistency)





N.2.16 Selenium and zinc

Total selenium (Se) and zinc (Zn) concentrations of human blood plasma were measured using a low resolution quadrupole-based inductively coupled plasma mass spectrometer (ICP-MS) equipped with a dynamic reaction cell (DRC) and two channel gas manifold.

Human blood plasma samples and QC materials were diluted (18.7 fold) in a diluent based on water with the addition of ammonia, butan-1-ol, nitric acid, Triton X-100 (surfactant) and included rhodium (Rh) as internal standard. Samples were introduced to the ICP-MS via a V-groove nebuliser and cyclonic spray chamber arrangement.

Se and Zn are easily measured by this technique however; both suffer from spectroscopic interference either from polyatomic ions formed within the instrument interface region or other isotopes with the same mass to charge ratio (m/z ratio). In addition all Se isotopes have the same m/z ratios as Argon based polyatomics (Argon is essential in the running of an ICP) causing an additional interference. To avoid these interferences and to achieve a desirable signal magnitude the ^{78}Se and ^{68}Zn isotopes were selected for measurement of Se and Zn, with methane (CH_4) used as a DRC gas to overcome Argon based interferences which affect Se measurement.

The Se and Zn isotope signals were measured alongside the ^{103}Rh (internal standard) and by comparing these signals against the internal standard, which is at a constant, any signal fluctuation due to instrument drift can be accounted for.

The table below describes the DRC and standard mode condition used, with Se measured with methane as the DRC reaction gas whilst Zn was measured under standard mode conditions.

Table N.47 Elan DRC^{Plus} DRC and standard mode conditions for Se and Zn analysis methods

	Element	Isotopic mass (amu)	Reaction gas (ml/min)	RPa	RPq
Se method	Se	77.9173	CH_4 = 0.4	0	0.5
	Rh	102.905	CH_4 = 0.4	0	0.5
Zn method	Zn	67.9249	0	0	0.7
	Rh	102.905	0	0	0.7

Prior to analysis the ICP-MS instrument was tuned for optimum signal sensitivity and minimum oxide species and doubly charged ion formation. Unknown samples, blanks, calibration standards and QCs were analysed together and the signal data generated was converted to concentration data via external calibration.

N.2.16.1 Quality controls for selenium and zinc

In order to establish quality assurance of each analytical batch and inter-batch variation across the year cohort as a whole, a series of quality control samples, of different sources, were analysed in conjunction with the blanks, calibration standards and unknown blood plasma samples that were studied. ClinChek Plasma Control Lyophilised for Trace Elements was used to establish inter-batch variation relative standard deviation (%RSD) and coefficient of variation (CV).

Tables N.48 and N.50 summarise analysis of the quality control materials used during the measurement of Se and Zn in blood plasma from Year 1 of the NDNS rolling programme. The published, or target, analyte concentrations were compared to the mean measured concentrations for each QC material. The agreement with the target concentration for analytical measurement was within 5% for all materials except those of relatively low concentration (typically materials exhibiting analyte concentrations lower than that of the bottom calibration standard) where analytical variability was within 9%. Tables N.48 and N.50 show that the analytical methodology employed, generates accurate and precise data.

Tables N.49 and N.51 show the mean measured concentration data for Se and Zn analysis of one QC material (Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1) across a series of

analytical batches in Year 1 of the NDNS rolling programme. Such data is the basis of the % RSD or CV of measurement and describes the analytical variation expected between the batches with low variation of 4.38% and 2.60% for Se and Zn respectively.

Tables N.52 and N.54 summarise analysis of the QC materials used during the measurement of Se and Zn in blood plasma from Year 2 of the NDNS rolling programme. The published, or target, analyte concentrations were compared to the mean measured concentrations for each QC material. The agreement with the target concentration for analytical measurement was within 5% for Se and 13% for Zn for all materials except those of relatively low concentration (typically materials exhibiting analyte concentrations lower than that of the bottom calibration standard) or lower number of measurements where analytical variability was within 10% for Se and 33% for Zn. Tables N.52 and N.54 show that the analytical methodology employed, generates accurate and precise data.

Tables N.53 and N.55 show the mean measured concentration data for Se and Zn analysis of one quality control material (Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1) across a series of analytical batches in Year 2 of the NDNS rolling programme. Such data is the basis of the % RSD or CV of measurement and describes the analytical variation expected between the batches with low variation of 4.77% and 6.45% for Se and Zn respectively.

Tables N.56 and N.58 summarise analysis of the QC materials used during the measurement of Se and Zn in blood plasma from Year 3 of the NDNS rolling programme. The published, or target, analyte concentrations were compared to the mean measured concentrations for each QC material. The agreement with the target concentration for analytical measurement was within 7% for Se and 11% for Zn for all materials except those of relatively low concentration (typically materials exhibiting analyte concentrations lower than that of the bottom calibration standard) or lower number of measurements where analytical variability was within 8% for Se

and 25% for Zn. Tables N.56 and N.58 show that the analytical methodology employed, generates accurate and precise data.

Tables N.57 and N.59 show the mean measured concentration data for Se and Zn analysis of one QC material (Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1) across a series of analytical batches in Year 3 of the NDNS rolling programme. Such data is the basis of the % RSD or CV of measurement and describes the analytical variation expected between the batches with low variation of 5.56% and 3.21% for Se and Zn respectively.

Table N.48 Comprehensive view of selenium quality control sample analysis for Year 1 of the NDNS rolling programme

QC Identity	QC Source	Number of Measurements (n)	Target Concentration (ug l-1, ppb)	Mean Measured Concentration (ug l-1, ppb)	Standard Deviation (SD)	%RSD (CV)	Agreement with Target (%)
QC L1	Recipe Chemicals and Instruments GmbH	55	81.00	81.70	3.98	4.87	100.87
QC L2	Recipe Chemicals and Instruments GmbH	55	118.00	117.64	5.45	4.63	99.70
Calf Serum	Sigma Aldrich	55	47.77	49.59	2.95	5.95	103.80
Calf Serum + Spike	Sigma Aldrich, Preparation at MRC - HNR	55	73.37	73.78	3.35	4.54	100.56
E-08-12	Institut National de sante Publique, Quebec	16	61.62	56.33	3.29	5.85	91.41
E-08-17	Institut National de sante Publique, Quebec	16	127.19	129.62	4.29	3.31	101.91
E-08-16	Institut National de sante Publique, Quebec	15	43.45	39.54	2.37	5.99	90.99
E-08-18	Institut National de sante Publique, Quebec	16	269.39	264.57	11.43	4.32	98.21
E-09-01	Institut National de sante Publique, Quebec	15	158.00	155.28	5.23	3.37	98.28
E-09-03	Institut National de sante Publique, Quebec	15	83.74	76.80	4.30	5.60	91.72
E-08-11	Institut National de sante Publique, Quebec	4	147.73	145.09	0.70	0.48	98.21
E-08-09	Institut National de sante Publique, Quebec	5	268.50	265.45	7.04	2.65	98.86
E-08-10	Institut National de sante Publique, Quebec	5	189.53	189.96	3.79	2.00	100.23
E-09-02	Institut National de sante Publique, Quebec	5	344.31	349.88	6.61	1.89	101.62
E-09-10	Institut National de sante Publique, Quebec	3	274.03	271.94	1.04	0.38	99.24
E-09-09	Institut National de sante Publique, Quebec	3	141.36	140.75	2.91	2.07	99.57

Table N.49 The Analysis of Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1 ([Se] = 81 µg l-1) for Year 1 of the NDNS rolling programme

Batch Number	Date of Analysis	Number of Measurements (n)	Mean Recipe QC Level 1 Concentration (ug l-1, ppb)
1	23/06/2009	8	77.49
2	10/08/2009	8	87.03
3	21/10/2009	9	80.62
4	28/01/2010	9	79.78
5	01/02/2010	9	79.43
6	02/02/2010	9	84.82
7	04/02/2010	3	85.25
Overall Mean			**82.06**
Standard Deviation (SD)			**3.60**
%RSD (%CV)			**4.38**

Table N.50 Comprehensive view of zinc quality control sample analysis for Year 1 of the NDNS rolling programme

QC Identity	QC Source	Number of Measurements (n)	Target Concentration (ug l-1, ppb)	Mean Measured Concentration (ug l-1, ppb)	Standard Deviation (SD)	%RSD (CV)	Agreement with Target (%)
QC L1	Recipe Chemicals and Instruments GmbH	48	1417.00	1438.18	62.70	4.36	101.49
QC L2	Recipe Chemicals and Instruments GmbH	48	1826.00	1835.17	55.42	3.02	100.50
Calf Serum	Sigma Aldrich	39	849.73	899.53	79.17	8.80	105.86
Calf Serum + Spike	Sigma Aldrich, Preparation at MRC - HNR	39	951.71	1040.18	94.30	9.07	109.30
E-08-12	Institut National de sante Publique, Quebec	12	693.03	724.71	44.12	6.09	104.57
E-08-17	Institut National de sante Publique, Quebec	12	1216.07	1288.30	91.38	7.09	105.94
E-08-16	Institut National de sante Publique, Quebec	15	444.58	471.66	64.47	13.67	106.09
E-08-18	Institut National de sante Publique, Quebec	12	2288.30	2332.54	44.02	1.89	101.93
E-09-01	Institut National de sante Publique, Quebec	12	1281.45	1373.26	56.50	4.11	107.17
E-09-03	Institut National de sante Publique, Quebec	12	699.57	758.46	60.32	7.95	108.42
E-08-09	Institut National de sante Publique, Quebec	5	2249.07	2277.57	142.14	6.24	101.27
E-08-10	Institut National de sante Publique, Quebec	5	1797.95	1865.68	79.05	4.24	103.77
E-09-02	Institut National de sante Publique, Quebec	5	2412.52	2327.82	58.59	2.52	96.49
E-09-10	Institut National de sante Publique, Quebec	3	2262.15	2256.28	40.15	1.78	99.74
E-09-09	Institut National de sante Publique, Quebec	3	1229.14	1219.55	59.84	4.91	99.22

Table N.51 The Analysis of Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1 ([Zn] = 1417 µg l-1) for Year 1 of the NDNS rolling programme

Batch Number	Date of Analysis	Number of Measurements (n)	Mean Recipe QC Level 1 Concentration (ug l-1, ppb)
1	21/09/2009	9	1407.55
2	22/01/2010	9	1426.28
3	28/01/2010	9	1488.97
4	01/02/2010	9	1435.13
5	02/02/2010	9	1414.63
6	04/02/2010	3	1493.28
Overall Mean			**1444.31**
Standard Deviation (SD)			**37.51**
%RSD (%CV)			**2.60**

Table N.52 Comprehensive view of selenium quality control sample analysis for Year 2 of the NDNS rolling programme

QC Identity	QC Source	Number of Measurements (n)	Target Concentration (ug l-1, ppb)	Mean Measured Concentration (ug l-1, ppb)	Standard Deviation (SD)	%RSD (CV)	Agreement with Target (%)
QC L1	Recipe Chemicals and Instruments GmbH	56	81.00	84.12	4.01	4.77	103.85
QC L2	Recipe Chemicals and Instruments GmbH	56	118.00	121.10	4.60	3.80	102.63
Calf Serum	Sigma Aldrich	56	51.50	52.41	1.95	3.71	101.77
CS + Spike	Sigma Aldrich, Preparation at MRC - HNR	56	76.23	76.58	2.80	3.66	100.45
E-08-07	Institut National de sante Publique, Quebec	12	36.00	39.78	1.46	3.67	110.49
E-08-08	Institut National de sante Publique, Quebec	12	332.00	333.20	12.65	3.80	100.36
E-08-09	Institut National de sante Publique, Quebec	12	269.00	268.85	11.76	4.37	99.95
E-08-10	Institut National de sante Publique, Quebec	12	190.00	189.95	6.96	3.66	99.98
E-08-15	Institut National de sante Publique, Quebec	12	150.00	143.33	3.79	2.65	95.56
E-09-02	Institut National de sante Publique, Quebec	12	344.31	353.27	13.23	3.75	102.60
E-09-07	Institut National de sante Publique, Quebec	12	203.59	209.17	6.70	3.20	102.74
E-09-10	Institut National de sante Publique, Quebec	12	274.00	283.88	10.10	3.56	103.61
E-09-11	Institut National de sante Publique, Quebec	12	37.88	37.71	2.38	6.30	99.56
E-09-12	Institut National de sante Publique, Quebec	12	194.00	196.11	4.48	2.29	101.09

Table N.53 The Analysis of Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1 ([Se] = 81 µg l-1) for Year 2 of the NDNS rolling programme

Batch Number	Date of Analysis	Number of Measurements (n)	Mean Recipe QC Level 1 Concentration (ug l-1, ppb)
1	19/05/2010	8	84.08
2	20/05/2010	8	86.38
3	25/05/2010	8	81.82
4	26/05/2010	8	82.92
5	05/10/2010	6	83.41
6	07/10/2010	6	79.21
7	08/10/2010	6	84.38
8	11/10/2010	6	91.18
Overall Mean			**84.12**
Standard Deviation (SD)			**4.01**
%RSD (%CV)			**4.77**

Table N.54 Comprehensive view of zinc quality control sample analysis for Year 2 of the NDNS rolling programme

QC Identity	QC Source	Number of Measurements (n)	Target Concentration (ug l-1, ppb)	Mean Measured Concentration (ug l-1, ppb)	Standard Deviation (SD)	%RSD (CV)	Agreement with Target (%)
QC L1	Recipe Chemicals and Instruments GmbH	62	1417.00	1551.87	100.08	6.45	109.52
QC L2	Recipe Chemicals and Instruments GmbH	62	1826.00	2022.89	200.81	9.93	110.78
Calf Serum (Yr 2)	Sigma Aldrich	62	1000.93	1067.74	100.06	9.37	106.68
CS + Spike (Yr2)	Sigma Aldrich, Preparation at MRC - HNR	62	1101.28	1110.63	70.32	6.33	100.85
E-08-08	Institut National de sante Publique, Quebec	15	2432.00	2616.88	88.85	3.40	107.60
E-08-09	Institut National de sante Publique, Quebec	12	2295.00	2581.87	363.74	14.09	112.50
E-08-10	Institut National de sante Publique, Quebec	12	1798.00	2384.32	484.17	20.31	132.61
E-08-15	Institut National de sante Publique, Quebec	12	1262.00	1663.95	186.08	11.18	131.85
E-09-02	Institut National de sante Publique, Quebec	12	2412.52	2699.52	295.16	10.93	111.90
E-09-07	Institut National de sante Publique, Quebec	15	1458.00	1626.80	218.78	13.45	111.58
E-09-10	Institut National de sante Publique, Quebec	15	2262	2516.321	133.186	5.293	111.243
E-09-11	Institut National de sante Publique, Quebec	15	483.81	630.646	59.320	9.406	130.350
E-09-12	Institut National de sante Publique, Quebec	12	1648	1780.461	136.410	7.661	108.038

Table N.55 The Analysis of Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1 ([Zn] = 1417 µg l-1) for Year 2 of the NDNS rolling programme

Batch Number	Date of Analysis	Number of Measurements (n)	Mean Recipe QC Level 1 Concentration (ug l-1, ppb)
1	19/05/2010	8	1597.77
2	20/05/2010	8	1561.51
3	25/05/2010	8	1547.92
4	26/05/2010	8	1524.57
5	05/10/2010	6	1484.92
6	07/10/2010	6	1410.53
7	08/10/2010	6	1544.28
8	11/10/2010	6	1654.13
9	16/12/2010	6	1633.13
Overall Mean			**1551.87**
Standard Deviation (SD)			**100.08**
%RSD (%CV)			**6.45**

Table N.56 Comprehensive view of selenium quality control sample analysis for Year 3 of the NDNS rolling programme

QC Identity	QC Source	Number of Measurements (n)	Target Concentration (ug l-1, ppb)	Mean Measured Concentration (ug l-1, ppb)	Standard Deviation (SD)	%RSD (CV)	Agreement with Target (%)
QC L1	Recipe Chemicals and Instruments GmbH	41	81	85.39	4.75	5.56	105.42
QC L2	Recipe Chemicals and Instruments GmbH	46	118	122.17	5.88	4.81	103.54
Calf Serum (Yr 3)	Sigma Aldrich	48	52	50.86	2.48	4.87	98.76
CS + Spike (Yr 3)	Sigma Aldrich, Preparation at MRC - HNR	47	73	70.54	2.57	3.65	96.07
E-08-07	Institut National de sante Publique, Quebec	6	36	39.13	2.82	7.21	108.69
E-08-08	Institut National de sante Publique, Quebec	9	332	338.13	13.06	3.86	101.85
E-08-09	Institut National de sante Publique, Quebec	9	269	282.94	12.21	4.31	105.18
E-08-10	Institut National de sante Publique, Quebec	2	190	192.94	0.64	0.33	101.55
E-08-11	Institut National de sante Publique, Quebec	14	150	150.05	6.19	4.13	100.03
E-08-14	Institut National de sante Publique, Quebec	7	205	212.07	5.24	2.47	103.45
E-08-15	Institut National de sante Publique, Quebec	2	141	148.47	7.10	4.78	105.30
E-08-16	Institut National de sante Publique, Quebec	2	43	45.51	3.29	7.23	105.83
E-09-02	Institut National de sante Publique, Quebec	4	344	365.42	2.37	0.65	106.23
E-09-07	Institut National de sante Publique, Quebec	8	204	218.14	6.25	2.87	106.93
E-09-09	Institut National de sante Publique, Quebec	12	141	145.25	4.80	3.31	103.02
E-09-10	Institut National de sante Publique, Quebec	5	274	284.12	17.23	6.06	103.69
E-09-11	Institut National de sante Publique, Quebec	2	38	42.27	1.42	3.36	111.24
E-09-12	Institut National de sante Publique, Quebec	11	194	210.68	8.68	4.12	108.60
E-09-13	Institut National de sante Publique, Quebec	4	158	165.38	3.00	1.81	104.67
E-10-05	Institut National de sante Publique, Quebec	4	117	119.18	2.69	2.26	101.86
E-10-10	Institut National de sante Publique, Quebec	3	125	130.37	2.84	2.18	104.29
E-10-12	Institut National de sante Publique, Quebec	3	35	38.87	0.72	1.85	111.06
E-10-16	Institut National de sante Publique, Quebec	5	140	144.00	8.15	5.66	102.85
E-10-17	Institut National de sante Publique, Quebec	3	98	91.51	0.66	0.72	93.38
E-10-18	Institut National de sante Publique, Quebec	5	376	387.26	24.72	6.38	102.99

Table N.57 The Analysis of Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1 ([Se] = 81 µg l-1) for Year 3 of the NDNS rolling programme

Batch Number	Date of Analysis	Number of Measurements (n)	Mean Recipe QC Level 1 Concentration (ug l-1, ppb)
1 (Q1-Q2)	09/05/2011	5	86.55
2 (Q1-Q2)	14/05/2011	5	81.97
3 (Q1-Q2)	16/05/2011	5	83.01
4 (Q1-Q2)	18/05/2011	4	79.96
5 (Q1-Q2)	01/06/2011	4	87.31
1 (Q3-Q4)	20/07/2011	5	87.06
2 (Q3-Q4)	22/07/2011	2	84.07
2a (Q3-Q4)	26/07/2011	- *	-
3 (Q3-Q4)	22/09/2011	5	89.65
4 (Q3-Q4)	23/09/2011	4	86.33
5 (Q3-Q4)	12/10/2011	2	88.66
Overall Mean			85.39
Standard Deviation (SD)			4.75
%RSD (%CV)			5.56

* No QC level 1 readings taken due to instrument problems, however level 2 measurement were within criteria

Table N.58 Comprehensive view of zinc quality control sample analysis for Year 3 of the NDNS rolling programme

QC Identity	QC Source	Number of Measurements (n)	Target Concentration (ug l-1, ppb)	Mean Measured Concentration (ug l-1, ppb)	Standard Deviation (SD)	%RSD (CV)	Agreement with Target (%)
QC L1	Recipe Chemicals and Instruments GmbH	41	1417	1355.73	137.33	10.13	95.68
QC L2	Recipe Chemicals and Instruments GmbH	45	1826	1761.89	144.10	8.18	96.49
Calf Serum (Yr3 A)	Sigma Aldrich	35	729	763.74	64.02	8.38	104.79
CS + Spike (Yr3 A)	Sigma Aldrich, Preparation at MRC - HNR	37	787	829.61	64.32	7.75	105.44
Calf Serum (Yr 3 B)	Sigma Aldrich	11	820	871.44	69.40	7.96	106.32
CS + Spike (Yr3 B)	Sigma Aldrich, Preparation at MRC - HNR	11	878	958.82	66.46	6.93	109.25
E-08-07	Institut National de sante Publique, Quebec	3	464	452.09	16.80	3.72	97.43
E-08-08	Institut National de sante Publique, Quebec	9	2432	2221.01	173.88	7.83	91.32
E-08-09	Institut National de sante Publique, Quebec	9	2295	2145.61	116.38	5.42	93.49
E-08-10	Institut National de sante Publique, Quebec	2	1798	1711.12	94.57	5.53	95.17
E-08-11	Institut National de sante Publique, Quebec	13	1262	1323.91	108.79	8.22	104.91
E-08-14	Institut National de sante Publique, Quebec	8	1327	1326.91	68.37	5.15	99.99
E-08-15	Institut National de sante Publique, Quebec	2	1229	1254.06	47.80	3.81	102.04
E-08-16	Institut National de sante Publique, Quebec	2	445	379.49	92.90	24.48	85.28
E-09-02	Institut National de sante Publique, Quebec	2	2413	2475.09	133.18	5.38	102.57
E-09-07	Institut National de sante Publique, Quebec	8	1458	1558.03	63.76	4.09	106.86
E-09-09	Institut National de sante Publique, Quebec	12	1229	1181.92	77.76	6.58	96.17
E-09-10	Institut National de sante Publique, Quebec	5	2262	2199.28	153.80	6.99	97.23
E-09-11	Institut National de sante Publique, Quebec	2	484	494.86	45.19	9.13	102.24
E-09-12	Institut National de sante Publique, Quebec	11	1648	1677.50	70.17	4.18	101.79
E-09-13	Institut National de sante Publique, Quebec	4	1281	1334.50	9.56	0.72	104.18
E-10-05	Institut National de sante Publique, Quebec	4	1059	1091.06	17.85	1.64	103.03
E-10-10	Institut National de sante Publique, Quebec	3	3269	3769.22	197.39	5.24	115.30
E-10-12	Institut National de sante Publique, Quebec	3	523	581.87	61.89	10.64	111.26
E-10-16	Institut National de sante Publique, Quebec	6	1445	1489.59	40.99	2.75	103.09
E-10-17	Institut National de sante Publique, Quebec	4	1007	1043.01	28.19	2.70	103.58
E-10-18	Institut National de sante Publique, Quebec	6	1353	1413.67	36.82	2.60	104.48

Table N.59 The Analysis of Recipe Chemicals and Instruments GmbH, ClinChek Plasma Control Lyophilised for Trace Elements QC Level 1 ([Zn] = 1417 µg l-1) for Year 3 of the NDNS rolling programme

Batch Number	Date of Analysis	Number of Measurements (n)	Mean Recipe QC Level 1 Concentration (ug l-1, ppb)
1 (Q1-Q2)	09/05/2011	5	1271.59
2 (Q1-Q2)	14/05/2011	5	1311.37
3 (Q1-Q2)	16/05/2011	5	1312.83
4 (Q1-Q2)	18/05/2011	4	1309.22
5 (Q1-Q2)	01/06/2011	4	1158.02
1 (Q3-Q4)	20/07/2011	5	1393.01
2 (Q3-Q4)	22/07/2011	2	1388.62
2a (Q3-Q4)	26/07/2011	- *	-
3 (Q3-Q4)	22/09/2011	5	1597.48
4 (Q3-Q4)	23/09/2011	4	1402.00
5 (Q3-Q4)	12/10/2011	2	1449.75
Overall Mean			1449.75
Standard Deviation (SD)			46.47
%RSD (%CV)			3.21

* No QC level 1 readings taken due to instrument problems, however level 2 measurement were within criteria

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