

## Blood biomarkers



### BRHS 30 year follow-up (Q30)

#### 2010 – 2012

##### Fasting blood samples

At the 30 year follow-up physical examination in 2010-2012 (Q30), the BRHS study participants were asked to fast for a minimum of 6 hours, during which they were instructed to drink only water and to attend for measurement at a prespecified time between 0800 and 1800 h.

All men were asked to provide a blood sample, collected using the BD Vacutainer system. The filled tubes were gently agitated and placed in a rack. Serum gel tubes stood for a minimum of 30 minutes before being centrifuged on site to preserve the sample during transit. The blood collection tubes were delivered overnight from the examination centres to the Department of Chemical Pathology at the Royal Free Hospital in London. Plasma samples were centrifuged and both plasma and serum gel samples were aliquoted and then frozen for longer term storage at  $-70^{\circ}\text{C}$ .

The blood sampling procedure is described in [section 4.6](#) of the Physical examination protocol ([BRHS 2010-12 \(Q30\) 30yr follow-up Physical examination protocol.pdf](#)).

The handling of the blood specimens is described in [Appendix C](#) of the physical examination protocol ([BRHS 2010-12 \(Q30\) 30yr follow-up Physical examination protocol.pdf](#)).

##### Blood marker adjustments

\* Adjusted blood markers: A number of blood markers (Glucose, insulin, triglycerides, LDL, HDL, cholesterol, HbA1c) were adjusted for fasting duration (using the time the participants reported as having something to eat or drink other than water) which was recorded on the [physical examination data collection form](#). These blood markers are available both in unadjusted and adjusted form.

**Methods for blood marker adjustments – work in progress (GW).**

A list of all available BRHS bloods markers for 2010-2012(Q30) can be found in the table below followed by the laboratory methods used for each blood marker where available.

A double star (\*\*) against a blood marker indicates that the blood marker has been adjusted for fasting duration the corresponding unadjusted(raw data) variable is indicated with a single \*.

## Blood biomarkers - BRHS 30 year follow-up (Q30) 2010-12

| Blood marker (Q30 2010-12)   | Units              | Methods section | BRHS variable name | Mean   | SD     | Min   | Max     | N    | N Miss | Data access |
|--|--------------------|-----------------|--------------------|--------|--------|-------|---------|------|--------|-------------|
| Adiponectin  | ug/ml              | B.38            | q30adiponectin     | 9.05   | 6.23   | 0.42  | 30.00   | 1603 | 119    | yes         |
| Albumin  | g/l                | B.8             | q30alb             | 44.81  | 2.47   | 35.00 | 54.00   | 1638 | 84     | yes         |
| Alanine Aminotransferase (ALT)                                     | u/l                | B.12            | q30alt             | 20.36  | 9.81   | 4.00  | 178.00  | 1635 | 87     | yes         |
| Alkaline Phosphatase (ALP)?  | mmol/l             | B.10            | q30aphos           | 73.59  | 28.57  | 12.00 | 564.00  | 1638 | 84     | yes         |
| Aspartame Transaminase (AST)                                       | u/l                | B.11            | q30ast             | 23.65  | 7.77   | 5.00  | 110.00  | 1630 | 92     | yes         |
| Basophils Absolute Value   |                    | B.23            | q30baso_abs        | 0.03   | 0.02   | 0.00  | 0.30    | 1590 | 132    | yes         |
| Calcium  | mmol/L             | B.1             | q30calc            | 2.29   | 0.09   | 2.00  | 2.78    | 1638 | 84     | yes         |
| Corrected Calcium  |                    |                 | q30ccalc           | 2.19   | 0.09   | 1.92  | 2.72    | 1638 | 84     | yes         |
| CHDR (Cholesterol/HDL ratio)                                       | ratio              |                 | q30chdr            | 3.38   | 1.04   | 1.10  | 8.40    | 1638 | 84     | yes         |
| *Cholesterol (raw/unadjusted)                                      | mmol/l             | B.13            | q30chol            | 4.63   | 1.03   | 2.20  | 8.50    | 1638 | 84     | yes         |
| ** Cholesterol (adjusted)  | mmol/l             |                 | q30chol_adj        | 4.63   | 1.03   | 2.20  | 8.50    | 1638 | 84     | yes         |
| Carboxymethyl lysine (CML)   | uM                 | B.40            | q30cml             | 2.72   | 1.38   | 0.23  | 16.76   | 1553 | 169    | yes         |
| Lysine Percentage of lysine that is glycated (CML to Lysine ratio) | ratio              | B.40            | q30cml_per_lys     | 0.07   | 0.03   | 0.02  | 0.43    | 1553 | 169    | yes         |
| Creatinine   | μ mol/l            | B.7/B.6         | q30creat           | 98.61  | 28.79  | 50.00 | 365.00  | 1636 | 86     | yes         |
| C-Reactive protein (CRP)   | mg/L               | B.26            | q30crp             | 3.19   | 9.51   | 0.05  | 239.84  | 1601 | 121    | yes         |
| Cystatin C   | mg/L               | B.32            | q30cystatinc       | 1.06   | 0.37   | 0.20  | 3.59    | 1604 | 118    | yes         |
| D-dimer  | ng/ml              | B.27            | q30ddimer          | 303.59 | 302.27 | 25.00 | 2000.00 | 1610 | 112    | yes         |
| eGFR   |                    |                 | q30egfr            | 72.71  | 18.10  | 14.91 | 146.01  | 1636 | 86     | yes         |
| Eosinophils Absolute Value   | 10 <sup>9</sup> /l | B.23            | q30eosin_abs       | 0.18   | 0.14   | 0.00  | 1.35    | 1590 | 132    | yes         |
| Gamma-Glutamyl transferase (gamma-GT, GGT)                         | u/l                | B.20            | q30gggt            | 32.51  | 35.93  | 6.00  | 420.00  | 1636 | 86     | yes         |
| *Glucose (raw/unadjusted)  | mmol/l             | B.21            | q30gluc            | 5.79   | 1.74   | 3.20  | 24.50   | 1536 | 186    | yes         |
| **Glucose (adjusted)   | mmol/l             | B.21            | q30gluc_adj        | 5.77   | 1.58   | 2.75  | 22.15   | 1536 | 186    | yes         |
| Haemoglobin(hb)  | g/l                | B.23            | q30hb              | 14.13  | 1.36   | 8.10  | 18.20   | 1610 | 112    | yes         |
| *Glycated Haemoglobin (HbA1c) (raw/unadjusted)                     | %                  | B.22            | q30hba1c           | 5.85   | 0.68   | 3.70  | 11.50   | 1597 | 125    | yes         |
| ** Glycated Haemoglobin (HbA1c) (adjusted)                         | %                  | B.22            | q30hba1c_adj       | 5.85   | 0.67   | 3.71  | 12.35   | 1597 | 125    | yes         |
| Haematocrit  | l/l                | B.23            | q30hct             | 0.43   | 0.04   | 0.27  | 0.56    | 1610 | 112    | yes         |
| *HDL High Density Lipoprotein cholesterol (raw/unadjusted)         | mmol/l             | B.15            | q30hdlc            | 1.45   | 0.42   | 0.50  | 4.50    | 1638 | 84     | yes         |
| ** HDL High Density Lipoprotein cholesterol (adjusted)             | mmol/l             | B.15            | q30hdlc_adj        | 1.45   | 0.42   | 0.47  | 4.48    | 1638 | 84     | yes         |

\* Raw data. Not adjusted

\*\* Adjusted for fasting duration

| Blood marker (Q30 2010-12) (cont. 1)                               | Units               | Methods section | BRHS variable name | Mean    | SD     | Min    | Max     | N    | N Miss | Data access |
|--|---------------------|-----------------|--------------------|---------|--------|--------|---------|------|--------|-------------|
| High-Sensitivity Troponin T (hs cTnT) - pg/ml or ng/L equivalent   | pg/ml               | B.35            | q30hstnt           | 14.51   | 16.08  | 1.50   | 407.10  | 1603 | 119    | yes         |
| Insulin-like growth factor 1 (IGF-1)                               | ng/ml               | B.31            | q30igf_1           | 75.81   | 24.00  | 16.43  | 200.25  | 1604 | 118    | yes         |
| Interleukin-6 (IL-6)   | pg/ml               | B.28            | q30il6             | 4.32    | 4.61   | 0.15   | 30.00   | 1618 | 104    | yes         |
| *Insulin (raw/unadjusted)  | mU/L                | B.24            | q30rawinsulin      | 10.30   | 10.58  | 0.33   | 136.88  | 1619 | 103    | yes         |
| ** Insulin (adjusted)  | mU/L                | B.24            | q30insulin_adj     | 10.35   | 9.37   | 0.36   | 110.99  | 1612 | 110    | yes         |
| *LDL Low Density Lipoprotein Cholesterol (direct) (raw/unadjusted) | mmol/l              | B.16            | q30ldlc            | 2.60    | 0.94   | 0.30   | 6.40    | 1632 | 90     | yes         |
| ** LDL Low Density Lipoprotein Cholesterol (direct) (adjusted)     | mmol/l              | B.16            | q30ldlc_adj        | 2.60    | 0.93   | 0.31   | 6.53    | 1632 | 90     | yes         |
| Leptin   | ng/ml               | B.39            | q30leptin          | 9.93    | 10.08  | 0.41   | 130.58  | 1603 | 119    | yes         |
| Lymphocytes Absolute Value   | 10 <sup>9</sup> /l  | B.23            | q30lymph_abs       | 1.79    | 2.46   | 0.31   | 71.74   | 1590 | 132    | yes         |
| Lysine concentration   | uM                  | B.40            | q30lys             | 39186.1 | 6731.5 | 8343.7 | 58231.3 | 1565 | 157    | yes         |
| Magnesium (Mg)   | mmol/L              | B.18            | q30magn            | 0.82    | 0.07   | 0.44   | 1.14    | 1637 | 85     | yes         |
| Mean Cell Haemoglobin (MCH)  | pg                  | B.23            | q30mch             | 30.67   | 1.72   | 21.70  | 38.50   | 1610 | 112    | yes         |
| Mean Cell Haemoglobin Concentration (MCHC)                         | g/dl                | B.23            | q30mchc            | 32.50   | 1.43   | 26.40  | 36.20   | 1610 | 112    | yes         |
| Mean Cell volume (MCV)   | fl                  | B.23            | q30mcv             | 94.48   | 5.83   | 77.10  | 130.80  | 1610 | 112    | yes         |
| Monocytes Absolute Value   | 10 <sup>9</sup> /l  | B.23            | q30monocy_abs      | 0.59    | 0.21   | 0.00   | 2.19    | 1590 | 132    | yes         |
| Mean Platelet volume (MPV)   | fl                  | B.23            | q30mpv             | 11.28   | 0.97   | 8.70   | 14.90   | 1605 | 117    | yes         |
| Neutrophils Absolute Value   | 10 <sup>9</sup> /l  | B.23            | q30neutro_abs      | 4.30    | 1.46   | 0.44   | 13.18   | 1590 | 132    | yes         |
| NT-proBNP (units: pg/ml or ng/L equivalent)                        | pg/ml               | B.34            | q30ntprobnp        | 393.9   | 946.0  | 2.5    | 15899.0 | 1603 | 119    | yes         |
| Phosphate  | mmol/l              | B.2             | q30phosph          | 1.09    | 0.16   | 0.47   | 3.30    | 1637 | 85     | yes         |
| Platelets  | 10 <sup>9</sup> /l  | B.23            | q30plat            | 225.2   | 59.2   | 64.0   | 646.0   | 1608 | 114    | yes         |
| Potassium  | mmol/l              | B.5             | q30potass          | 4.39    | 0.40   | 2.70   | 6.50    | 1628 | 94     | yes         |
| Proinsulin   | pmol/L              | B.36            | q30proinsulin      | 18.2    | 20.3   | 1.8    | 132.0   | 1604 | 118    | yes         |
| Rage (sRage)   | pg/ml               | B.33            | q30rage            | 1213.9  | 758.0  | 157.0  | 7411.0  | 1604 | 118    | yes         |
| Red blood cell count (RBC)   | 10 <sup>12</sup> /l | B.23            | q30rbc             | 4.61    | 0.44   | 2.48   | 6.09    | 1610 | 112    | yes         |
| Red Blood Cell Distribution Width                                  | %                   | B.33            | q30rdw             | 14.12   | 1.21   | 11.60  | 28.90   | 1610 | 112    | yes         |
| Sodium   | mmol/l              | B.4             | q30sodium          | 141.24  | 3.06   | 126.00 | 162.00  | 1637 | 85     | yes         |
| Total Bilirubin  | μ mol/l             | B.9             | q30tbili           | 12.24   | 5.34   | 3.00   | 42.00   | 1634 | 88     | yes         |
| Tissue plasminogen activator(tPA)                                  | ng/ml               | B.29            | q30tpa             | 10.05   | 5.43   | 1.50   | 50.00   | 1610 | 112    | yes         |
| Protein (Total Protein)  | g/l                 | B.19            | q30tprot           | 69.99   | 4.05   | 56.00  | 91.00   | 1638 | 84     | yes         |

\* Adjusted for fasting duration

\*\* Raw data. Not adjusted

| Blood marker (Q30 2010-12) (cont. 2) | Units              | Methods section | BRHS variable name | Mean   | SD    | Min   | Max    | N    | N Miss | Data access |
|--------------------------------------|--------------------|-----------------|--------------------|--------|-------|-------|--------|------|--------|-------------|
| **Triglycerides (raw/unadjusted)     | mmol/l             | B.14            | q30trig            | 1.30   | 0.67  | 0.40  | 8.90   | 1638 | 84     | yes         |
| * Triglycerides (adjusted)           | mmol/l             | B.14            | q30trig_adj        | 1.30   | 0.66  | 0.36  | 8.73   | 1638 | 84     | yes         |
| Urate                                | mmol/l             | B.17            | q30urat            | 0.36   | 0.08  | 0.12  | 0.77   | 1570 | 152    | yes         |
| Urea                                 | mmol/l             | B.3             | q30urea            | 6.84   | 2.45  | 2.60  | 30.40  | 1638 | 84     | yes         |
| Vitamin D                            | ng/ml              | B.37            | q30vitd            | 22.36  | 13.18 | 1.50  | 70.00  | 1582 | 140    | yes         |
| Von Willebrand factor (VWF)          | IU/dl              | B.30            | q30vwf             | 134.91 | 73.74 | 19.45 | 433.17 | 1610 | 112    | yes         |
| White blood cell count (WBC)         | 10 <sup>9</sup> /l | B.23            | q30wbc             | 6.91   | 3.09  | 1.99  | 87.49  | 1610 | 112    | yes         |

\* Adjusted for fasting duration

\*\* Raw data. Not adjusted

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# Methodological Summaries

## Routine Biochemistry

Measured at the Department of Clinical Biochemistry, Royal Free Hospital, London. (Prof Mike Thomas).

### **B.1 Calcium** mmol/L

Principal: The method is based on a blanked colorimetric endpoint assay using o-cresolphthalein complexone which produces a purple coloured complex whose intensity is directly proportional to the calcium concentration.

Between batch Imprecision: <2%

Reference: Gindler EM & King JD. 1972 "Rapid colorimetric determination of calcium in biologic fluids with methythymol blue." Am J Clin Pathol 58: 376 - 382

### **B.2 Phosphate** mmol/l

Principal: A colorimetric assay with endpoint determination and sample blanking. Inorganic phosphate forms an ammonium phosphomolybdate complex with ammonium molybdate in the presence of sulphuric acid. The complex is determined photometrically in the ultraviolet region (340 nm).

Between batch Imprecision: <2%

Reference: Weissman N, Pileggi VJ. 1974 "Inorganic anions." In: Henry RJ, Cannon DC, Winkelman JW, eds. "Clinical chemistry: principles and techniques" 2nd ed. New York: Harper and Row:723 - 727

### **B.3 Urea** mmol/l

Principal: A kinetic UV assay. Urea is enzymatically converted via urease to ammonium ions which are utilised by the enzyme glutamate dehydrogenase. This enzyme consumes NADH, the decrease of which is measured kinetically.

Between batch Imprecision: 3.4%

Reference: Kaplan, L.A., 1984. Urea. In: Clinical Chemistry; Theory, Analysis and Correlation, Kaplan, L.A. and A.J. Pesce (Eds.). CV Mosby Co., St. Louis, pp: 1257-1261.

### **B.4 Sodium** mmol/l

Principal: Sodium is measured by an ion selective electrode. A membrane composed of crown ether with a neutral PVC carrier forms a selective membrane for sodium ions, creating an electrical potential as sodium ions traverse the membrane. The electrical potential can be compared to a reference electrode to determine the sodium ion concentration.

Between batch Imprecision: <2%

Reference: GB Levy,. 1981. "Determination of Sodium with Ion-Selective Electrodes." Clin Chem. 27: 1435-1438.

### **B.5 Potassium mmol/l**

Principal: Potassium is measured by an ion selective electrode. A selective membrane composed of valinomycin creates an electrical potential as only potassium ions traverse the membrane. The electrical potential can be compared to a reference electrode to determine the potassium ion concentration.

Between batch Imprecision: <2%

Reference: Fiedler U, Růžička J. 1973. "Selectrode—the universal ion-selective electrode: Part VII. A valinomycin-based potassium electrode with nonporous polymer membrane and solid-state inner reference system". *Analytica Chimica Acta*. 67: 179-193.

### **B.6 Creatinine - Jaffe $\mu$ mol/l**

Principal: Rate blanked kinetic assay. Creatinine reacts with picric acid in alkaline conditions to produce an orange-red product which can be measured photometrically. The rate blank step is introduced to minimise interferences from bilirubin. A negative factor of -26  $\mu$ mol/L is applied to reduce non specific interferences.

Between batch Imprecision: 2.3%

Reference: Peake M, Whiting M. 2006. "Measurement of Serum Creatinine – Current Status and Future Goals" *Clin Biochem Rev*. 27: 173-184.

### **B.7 Creatinine - Enzymatic $\mu$ mol/l**

Principal: Enzymatic colorimetric assay. Creatinine is converted via a series of enzymes to hydrogen peroxide, which can be measured spectrophotometrically at 546nm following a modified Trinder reaction with absorbance blanking at 700nm.

Between batch Imprecision: 2.1%

Reference: Peake M, Whiting M. 2006. "Measurement of Serum Creatinine – Current Status and Future Goals" *Clin Biochem Rev*. 27: 173-184.

### **B.8 Albumin g/l**

Principal: Endpoint colorimetric assay. In acidic conditions, albumin can bind bromocrescol green to produce a coloured complex, the intensity of which is directly proportionate to the concentration of albumin.

Between batch Imprecision: <2%

Reference: Hill PG, 1985. "The measurement of albumin in serum and plasma." *Ann Clin Biochem* 22:565–78

### **B.9 Total Bilirubin $\mu$ mol/l**

Principal: Colorimetric assay. Indirect/Unconjugated bilirubin is liberated following interaction with detergents. After this step, the bilirubin reacts with diazonium ions under strongly acidic conditions to produce azobilirubin, which can be measured spectrophotometrically.

Between batch Imprecision: <2%

Reference: Watson D, Rogers JA. 1961 "A study of six representative methods of plasma bilirubin analysis" *J Clin Pathol*. 14: 271-278.

### **B.10 Alkaline Phosphatase** mmol/l

Principal: Enzymatic colorimetric assay. In the presence of zinc and magnesium, ALP is able to convert p-nitrophenyl phosphate into p-nitrophenol, the concentration of which is measured spectrophotometrically and is proportionate to the concentration of ALP.

Between batch Imprecision: <2%

Reference: Bowers GN Jr, McComb RB. 1975 "Measurement of total alkaline phosphatase activity in human serum." Clin Chem 21: 1988-1995.

### **B.11 Aspartate Transaminase (AST)** u/l

Principal: Kinetic enzymatic assay. AST is able to produce oxaloacetate when L-aspartate and alpha-ketoglutarate are present. Oxaloacetate can then be utilised by malate dehydrogenase along with NADH and H<sup>+</sup> to produce L-Malate and NAD<sup>+</sup>. The rate of consumption of NADH is measured spectrophotometrically and the decrease in absorbance is directly proportionate to the rate of production of oxaloacetate and therefore the AST concentration.

Between batch Imprecision: 3.2%

Reference: Bergmeyer HU, Horder M, Rej R. 1986 "Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes" J. Clin. Chem. Clin. Biochem. 24: 497-510.

### **B.12 Alanine Aminotransferase (ALT)** u/l

Principal: Kinetic enzymatic assay. ALT is able to form pyruvate and L-Glutamate from alpha-ketoglutarate and L-aspartate. Pyruvate can then be utilised with NADH and H<sup>+</sup> by lactate dehydrogenase to produce L-Malate and NAD<sup>+</sup>. The rate of consumption of NADH can be measured spectrophotometrically and is proportionate to the concentration of ALT.

Between batch Imprecision: 3.2%

Reference: Bergmeyer HU, Horder M, Rej R. 1986 "Approved Recommendation (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes" J. Clin. Chem. Clin. Biochem. 24: 481-495.

### **B.13 Cholesterol** mmol/l

Principal: Enzymatic colorimetric assay. Cholesterol and cholesterol esters are converted via a series of enzymatic reactions to hydrogen peroxide which is then utilised to produce a red dye via a peroxidase enzyme. The colour intensity of the dye is directly proportionate to the concentration of cholesterol.

Between batch Imprecision: <2%

Reference: Deeg R, Ziegenhorn J. 1983 "Kinetic enzymic method for automated determination of total cholesterol in serum." Clin Chem 29: 1798-1802.

### **B.14 Triglycerides** mmol/l

Principal: Enzymatic colorimetric assay. Triglycerides are converted via a series of enzymatic reactions to produce hydrogen peroxide, which is then utilised in a Trinder endpoint reaction to form a red dye. The concentration of the dye is used to determine the concentration of triglyceride.

Between batch Imprecision: <2%

Reference: Wahlefeld, A. W. 1974. Triglycerides. Determination after enzymatic hydrolysis. In: "Methods of Enzymatic Analysis." H. U. Bergmeyer, editor. Academic Press, New York, NY.

### **B.15 High Density Lipoprotein Cholesterol (HDL) mmol/l**

Principal: Homogenous enzymatic colorimetric assay. A detergent containing a sugar compound in conjunction with magnesium is used to favour the conversion of HDL cholesterol esters to cholesterol over other sources of cholesterol such as LDL-cholesterol. This liberated HDL-cholesterol is further converted to hydrogen peroxide which can react with a dye via peroxidase to form a purple-blue pigment which can be detected spectrophotometrically. The concentration of the purple-blue pigment is directly proportionate to the concentration of HDL-Cholesterol.

Between batch Imprecision: <2%

Reference: Nauck M, März W, Jarausch J, Cobbaert C, Sägers A, Bernard D, Delanghe J, Honauer G, Lehmann P, Oestrich E, von Eckardstein A, Walch S, Wieland H, Assmann G. 1997 "Multicenter evaluation of a homogeneous assay for HDL-cholesterol without sample pretreatment." Clin Chem. 43: 1622-1629.

### **B.16 Low Density Lipoprotein Cholesterol (direct) (LDL) mmol/l**

Principal: Homogenous enzymatic colorimetric assay. A detergent containing a sugar compound in conjunction with magnesium is used to favour the conversion of LDL cholesterol esters to cholesterol over other sources of cholesterol such as HDL-cholesterol. This liberated LDL-cholesterol is further converted to hydrogen peroxide which can react with a dye via peroxidase to form a purple-blue pigment which can be detected spectrophotometrically. The concentration of the purple-blue pigment is directly proportionate to the concentration of LDL-Cholesterol.

Between batch Imprecision: <2%

Reference: Esteban-Salán M, Guimón-Bardesi A, de La Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Río E. 2000 "Analytical and clinical evaluation of two homogeneous assays for LDL-cholesterol in hyperlipidemic patients." Clin Chem 46:1121-1131.

### **B.17 Urate mmol/l**

Principal: Enzymatic colorimetric assay. Uric acid is converted via uricase to hydrogen peroxide which can react via a peroxidase to a quinone-diimine dye which can be measured spectrophotometrically. The concentration of the dye is proportionate to the urate concentration.

Between batch Imprecision: <2%

Reference: Zhao Y, Yang X, Lu W, Liao H, Liao F. 2009 "Uricase based methods for determination of uric acid in serum" Microchimica Acta 164: 1-6.

### **B.18 Magnesium mmol/L**

Principal: Colorimetric endpoint assay. The sample is incubated with EGTA to minimise calcium interference and an alkaline buffer, after which xylydyl blue is added which will react with magnesium to produce a purple product. The decrease in absorbance at 600nm by xylydyl blue is measured and used to determine the magnesium concentration.

Between batch Imprecision: <2%

Reference: D. Stankov, T. Jovanović, M. Jelikić-Stankov. 1997 "Spectrophotometric Determination of Magnesium in Serum Using Xylydyl Blue Reagent in Micellar Medium" In: "Magnesium: Current Status and New Developments" p51-52. Kluwer Academic Publishers.



### **B.19 Total Protein g/l**

Principal: Colorimetric assay. In alkaline conditions protein can complex with copper ions to produce a copper protein complex, the concentration of which is determined spectrophotometrically and is directly proportionate to the concentration of protein in the sample.

Between batch Imprecision: <2%

Reference: Savory J, Heintges MG, Sonowane M, Cross RE. 1976 "Measurement of total protein and albumin in serum with a centrifugal analyzer" Clin Chem. 22: 1102-1104.

### **B.20 Gamma-Glutamyltransferase (GGT) u/l**

Principal: Enzymatic colorimetric assay. Following addition of substrate, GGT catalyses the conversion of reagent into 5-amino-2-nitrobenzoate which can be measured via spectrophotometry at 410nm.

Between batch Imprecision: <2%

Reference: Tietz, N.W., 1986. "Fundamentals of Clinical Chemistry", 3rd Edition, W.B. Saunders,

### **B.21 Glucose mmol/l**

Principal: Enzymatic colorimetric method. Glucose is enzymatically converted into hydrogen peroxidase which can be utilised by a peroxidase enzyme to produce a red dye which is read via spectrophotometry at 505nm. The concentration of the dye is directly proportionate to the concentration of glucose in the sample.

Between batch Imprecision: 3.8%

Reference: Trinder P. 1969 "Determination of blood glucose using 4-amino phenazone as oxygen acceptor."

### **B.22 Glycated Haemoglobin (HbA1c) %**

Method not yet available.

## Routine Haematology markers

### B.23 Routine Haematology markers

Routine haematology analyses were carried out at the Department of Haematology, Royal Free Hospital, London (Dr Chris McNamara)

Available haematology markers include:

|   | units        |
|---|--------------|
| Haemoglobin                               | g/l          |
| White Blood Cell Count                    | $10^9$ /l    |
| Platelets Red Blood Cell Count            | $10^9$ /l    |
| Red blood cell count (RBC)                | $10^{12}$ /l |
| Red Blood Cell Distribution Width         | %            |
| Haematocrit                               | l/l          |
| Mean Cell Volume (MCV)                    | fl           |
| Mean Cell Hemoglobin (MCH)                | pg           |
| Mean Cell Hemoglobin Concentration (MCHC) | g/dl         |
| Neutrophils Absolute Value                | $10^9$ /l    |
| Lymphocytes Absolute Value                | $10^9$ /l    |
| Monocytes Absolute Value                  | $10^9$ /l    |
| Eosinophils Absolute Value                | $10^9$ /l    |
| Basophils Absolute Value                  |              |
| Mean Platelet Volume (MPV)                | fl           |

## OTHER BLOOD MARKERS

### B.24 Insulin mU/L

Measured at the Institute of Cellular Medicine, Newcastle University. (Prof Sally Marshall)

Method not yet available.

### B.26 C-Reactive protein (CRP) mg/L

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using ultrasensitive assay on an automated analyser (Cobas c311, Roche Diagnostics, Burgess Hill, UK) with the manufacturers calibrators and quality controls.

|                         | Limits of detection and assigned values |                             |                          |                             |
|-------------------------|---|-----------------------------|--------------------------|-----------------------------|
|                         | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| C-Reactive protein(CRP) | <0.1 mg/L                               | 0.05                        | n/a                      | n/a                         |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

Laboratory notes: C reactive protein (CRP): There are a few outliers >200mg/L at upper end. These outliers are to be expected for CRP, and have been checked. *Paul Welsh*

### B.27 D-dimer ng/ml

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Asserachrom , Stago, Theale, UK).

|         | Limits of detection and assigned values |                             |                          |                             |
|---------|---|-----------------------------|--------------------------|-----------------------------|
|         | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| D-dimer | <50 ng/ml                               | 25                          | >200                     | 200                         |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

### B.28 Interleukin-6 (IL-6) pg/mL

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using a high-sensitivity enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Abingdon, UK)

|                      | Limits of detection and assigned values |                             |                          |                             |
|----------------------|---|-----------------------------|--------------------------|-----------------------------|
|                      | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| Interleukin-6 (IL-6) | n/a                                     | n/a                         | >30                      | 30                          |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

### B.29 Tissue plasminogen activator(tPA) ng/mL

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Asserachrom , Stago, Theale, UK).

|                                   | Limits of detection and assigned values |                             |                          |                             |
|-----------------------------------|---|-----------------------------|--------------------------|-----------------------------|
|                                   | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| Tissue plasminogen activator(tPA) | <3 ng/ml                                | 1.5                         | >50                      | 50                          |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

### B.30 Von Willebrand factor (VWF) IU/dL

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using high-sensitivity enzyme-linked immunosorbent assays (ELISA) (Technozym , Pathway Diagnostics, Dorking, UK)

### B.31 Insulin-like growth factor 1 (IGF-1) ng/ml

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using an enzyme-linked immunosorbent assays (ELISA) R&D Systems, Abingdon, UK)

### B.32 Cystatin C mg/L

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using an automated analyser (Cobas c311, Roche Diagnostics, Burgess Hill, UK) with the manufacturers calibrators and quality controls.

### B.33 Rage pg/ml

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using an enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Abingdon, UK)

Reference: Wannamethee SG, Welsh P, Papacosta O, Ellins EA, Halcox JPJ, Whincup PH, et al. Circulating soluble receptor for advanced glycation end product: Cross-sectional associations with cardiac markers and subclinical vascular disease in older men with and without diabetes. *Atherosclerosis*. 2017;264:36-43 Epub 2017/08/02

### B.34 NT-proBNP ng/L or pg/ml (equivalent)

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using an automated immunoassay analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers' calibrators and quality controls. The lower limit of sensitivity was 5 pg/ml

|             | Limits of detection and assigned values |                             |                          |                             |
|-------------|---|-----------------------------|--------------------------|-----------------------------|
|             | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| NT - proBNP | < 5 pg/ml                               | 2.5                         | n/a                      | n/a                         |

<sup>1</sup> A laboratory reported result of “< than the lower limit of detection” was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of “> than the upper limit of detection” was assigned the value the upper limit of detection.

#### Laboratory note:

NT-proBNP outlier checks

- NTproBNP values of 15899 and 12627 were confirmed to be correct (Dr Paul Welsh)

### B.35 High-Sensitivity Troponin T (hsTnT) ng/L or pg/ml (equivalent)

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using an automated immunoassay analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers' calibrators and quality controls. The lower limit of sensitivity was 3 pg/ml

|                                     | Limits of detection and assigned values |                             |                          |                             |
|-------------------------------------|---|-----------------------------|--------------------------|-----------------------------|
|                                     | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| High sensitivity troponin T (hsTnT) | <3 pg/ml                                | 1.5                         | n/a                      | n/a                         |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

#### Laboratory note:

hsTnT outlier checks

- hsTnT value of 407.1 was confirmed to be correct (Dr Paul Welsh)

### B.36 Proinsulin pmol/L

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using an enzyme-linked immunosorbent assays (ELISA) (Merckodia, Diagenics Milton Keynes, UK)

|            | Limits of detection and assigned values |                             |                          |                             |
|------------|---|-----------------------------|--------------------------|-----------------------------|
|            | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| Proinsulin | <3.5 pmol/L                             | 1.75                        | >132                     | 132                         |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

### B.37 Vitamin D ng/mL

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)

Measured using an automated analyser (Cobas e411, Roche Diagnostics, Burgess Hill, UK) with the manufacturers calibrators and quality controls.

|           | Limits of detection and assigned values |                             |                          |                             |
|-----------|---|-----------------------------|--------------------------|-----------------------------|
|           | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| Vitamin D | < 3 ng/ml                               | 1.5                         | >70                      | 70                          |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

Laboratory note: Values not measurable.

\* 22 with insufficient blood have been set to MISSING value =.

\* 38 were recorded as <3 ng/mL. These were assigned the value of 1/2 of 3 --> 1.5 ng/mL

\* 9 were recorded as >70 ng/mL. These were assigned to the value of 70 ng/mL

### B.38 Adiponectin ug/ml

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using an enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Abingdon, UK)

|             | Limits of detection and assigned values |                             |                          |                             |
|-------------|---|-----------------------------|--------------------------|-----------------------------|
|             | Lower limit <sup>1</sup>                | Assigned value <sup>1</sup> | Upper limit <sup>2</sup> | Assigned value <sup>2</sup> |
| Adiponectin | n/a                                     | n/a                         | >30 ug/ml                | 30                          |

<sup>1</sup> A laboratory reported result of "< than the lower limit of detection" was assigned the value of ½ of the lowest limit of detection.

<sup>2</sup> A laboratory reported result of "> than the upper limit of detection" was assigned the value the upper limit of detection.

Laboratory note: Values Not measurable.

Adiponectin values recorded as ">30" were assigned the value of 30. There are 19 such values

### B.39 Leptin ng/ml

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Measured using an enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Abingdon, UK)

### B.40 Carboxymethyl lysine (CML) uM, Lysine uM, CML to Lysine ratio

Measured at the University of Glasgow, Institute of Cardiovascular & Medical Sciences (Dr Paul Welsh)  
Isotope dilution Mass spectrometry

Chromatography was carried out on a Dionex UltiMate 3000 RSLC system (Thermo Fisher Scientific, Hemel Hempstead, UK)

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