UK Biobank Biomarker Project

Companion Document to Accompany Serum Biomarker Data

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Contents

Document Version Control

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V1.0	*11/03/2019*	*N/A*

1.0 Introduction

In order to further enhance the value of the UK Biobank resource to researchers, UK Biobank has embarked on a project to measure a wide range of biochemical markers in biological samples collected at baseline (2006-2010) in all 500,000 participants (and also in the samples provided by 20,000 participants who returned for a Repeat Assessment in 2012/ 2013).

The project seeks to measure biomarkers in three matrices – urine, packed red blood cells (PRBC) and serum – using a phased analysis approach.

This document is provided as a companion document to the serum biomarker data available through the UK Biobank Showcase.

It is intended to provide basic information on:-

- Assay selection & project scope (refer to Section 2.0)
- Methods and equipment (refer to Section 3.0)
- Serum assay performance characteristics (refer to Section 4.0)
- Quality system and scope of accreditation (refer to Section 5.0)

2.0 Assay Selection & Project Scope

The rationale behind the project was described at the UK Biobank Frontiers meeting on the 26th June 2014. A video of the presentation may be viewed at http://www.ukbiobank.ac.uk/uk-biobank-biomarker-panel/. Overall, 34 biomarkers were selected for assay in all 500,000 participants and these are listed with a summary of the methods, instrumentation used, manufacturer, units of measurement, and assay analytical range in Appendix 1

Biomarkers were selected for analysis because they represent established risk factors for disease, are clinical diagnostic measures, or because they characterise phenotypes not otherwise well assessed.

The project was co-ordinated by the Enhancement Working Group, with input from external experts, where required (refer to Appendix 2: Expert Advisors).

A sample selection algorithm (refer to Appendix 3: Sample Selection Algorithm) was implemented to ensure that participant samples from the same geography and same collection dates & times were not picked in clusters (i.e. samples analysed evenly during the project. This minimised the risk of introducing bias or drift to analytes as the algorithm accounted for the geographical and temporal selection of samples.

This document focuses on the serum biomarkers only.

3.0 Methods and Equipment

Analysis of serum biomarkers utilised 10 immunoassay analysers (6x DiaSorin Liaison XL & 4x Beckman Coulter DXI 800 and 4 clinical chemistry analysers (2x Beckman Coulter AU5800 & 2x Siemens Advia 1800).

Each manufacturer carried out a number of quality performance tests during analyser installation to certify that each instrument was suitable for routine operation. Subsequently, in line with ISO 17025:2005 accreditation standards, the laboratory verified that analysers and individual assays achieved a level of performance that was in agreement with the manufacturer's claims and/or published total allowable error (TEA) limits based on biological variation as per the Westgard website (https://www.westgard.com/biodatabase1.htm).

Verification of assay and analyser performance was carried out using a rigorous protocol to assess the following parameters:

- **Precision**
  Including within-run and within-laboratory (total) precision. Different concentration levels of Quality Control (QC) samples and participant samples were analysed for the within-run precision experiments and the same QC material was analysed over 20 days (minimum) to estimate within-laboratory precision.
- **Accuracy (or recovery) and bias**
  A combination of External Quality Assurance (EQA) material, commercial validation material or participant samples previously analysed in an accredited laboratory were used to compare results to target values to estimate accuracy (recovery) and % bias.
- **Linearity and Reportable range including the limit of quantification (LOQ)**
  Commercial linearity standards and low concentration samples were used to verify that assays were linear over the observed reportable range.
- **Carryover**
  Low and high concentration samples were analysed consecutively in a standardised sequence to verify that there was no carryover from high concentration to low concentration samples.
- **Multi-instrument Comparison**
  The same samples were analysed over multiple platforms of the same analytical type to ensure that all instruments agreed with one other.

Biological TEA, precision and bias results were used to generate Six-Sigma[1] scores for each assay and these were then used to generate assay-specific Westgard internal quality control multi-rules[2] for sample batch acceptance during analysis.

3.1 Ongoing Verification of Assay Performance

The performance of each assay was continuously verified throughout the project with regular reports generated that summarised precision (both QC and participant results – daily and weekly means and standard deviations), accuracy and bias (EQA schemes), linearity (comparisons between reagent lots) and multiple instrument comparisons of both QC and participant results. This data was also used to continuously verify

[1] Six Sigma (6σ) is a data-based methodology to improve performance by reducing variability.

[2] A multi-rule system developed by Dr James O. Westgard based on statistical concepts using a combination of decision criteria to assess if a system is in control. Used when at least 2 levels of control are run with the examination run

that the correct internal quality control (IQC) multi-rules and standard deviation IQC acceptance limits were being applied to effectively control each assay throughout analysis.

3.2 Results Outside of the Observed Reportable Range

Each assay was verified against the manufacturer's performance information. Linearity and limit of quantification experiments determined the observed reportable range. The manufacturers' published analytical ranges for each assay are listed in Appendix 1. For most, the observed reportable range covered the manufacturer's stated analytical range. However, there were some exceptions, where the lower end of the analytical range quoted by the manufacturer referred to a limit of blank or limit of detection rather than a limit of quantification (i.e. results which fall within assay precision performance goals). Observed reportable range low and high limits are summarised in Table 1.

The analytical methods for many of the serum assays allowed samples with results exceeding the reportable range of the assay to be diluted and re-analysed (automatic dilution). (Please note that this is different to the dilution issue described in technical appendix to the document on 'Biomarker assay quality procedures: approaches used to minimise systematic and random errors'. For some assays, automatic dilution by the instrument was verified and therefore carried out by the analytical platform. If automatic dilution was not available on the instrument or where the automatic dilution was not verified, the dilution was performed manually before re-analysis to produce a result within the observed reportable range. When manual dilutions were performed, a record was kept of the result and the manual re-calculation. Manually diluted results and re-calculations were checked by a qualified individual before manual result entry onto the Modulab system and subsequent result validation by a HCPC state registered Biomedical Scientist. In some instances, due to procedural difficulties or lack of residual sample, it was not possible to carry out a dilution and in these cases the result was flagged as being above the observed reportable range.

Assay	If instrument result is below bottom of reportable range	If instrument result is above top of reportable range
ALB	<15	>60 Dilution required[3]
ALP	<3	>1500 Dilution required[3]
ALT	<3	>500 Dilution required[3]
APOA1	<0.4	>2.5 Dilution required[3]
APOB	<0.4	>2 Dilution required[3]
AST	>3	>1000 Dilution required[3]
CALC	<1	>5 Dilution required[3]
CHOL	<0.5	>18 Dilution required[3]
CREA	<4	>4420 Dilution required[3]
CRP	<0.08	>80 Dilution required[3]
CYSC	<0.1	> (7.7-8.99)[4]
DBIL	<1[5]	>171 Dilution required[3]
GGT	<5	>1200 Dilution required[3]
GLU	<0.6	>45 Dilution required[3]
HDL	<0.05	>4.65 Dilution required[3]
IGF-1	<1.30	>195 Dilution required[3]
LDL	<0.26	>10.3 Dilution required[3]
LP-a	<3.80	>189 Dilution required[3]
OEST	<175	>17993 Dilution required[3]
PHOS	<0.32	>6.4 Dilution required[3]
RF	<10	>120 Dilution required[3]
SHBG	<0.33	>(226-242)[6]
TBIL	<1[7]	>513 Dilution required[3]
TEST	<0.35	>55.48 Dilution required[3]
TP	<30	>120 Dilution required[1]
TRIG	<0.1	>11.3 Dilution required[1]
UA	<89	>1785 Dilution required[1]
UREA	<0.8	>50 Dilution required[1]
VITD	<10	>375 Dilution required[1]

Table *1*: Observed Reportable Range Flags

[3] If sufficient sample was available, a dilution was carried out and the sample reanalysed

[4] Upper end of analytical range dependent on calibrator lot.

[5] Manufacturer claimed a value of 0 for the bottom end of the reportable range, this could not be verified statistically.

[6] Upper end of analytical range dependent on calibrator lot.

[7] Manufacturer claimed a value of 0 for the bottom end of the reportable range, this could not be verified statistically.

3.3 Assay Interferences

Several diseases and pre-analytical conditions can result in increased concentrations of bilirubin, haemoglobin and lipids/turbidity in body fluids. These can interfere with the spectrophotometric measurement of some assays. Certain substances can interfere with the assay reagent ingredients and some (including drugs) may have a similar molecular structure to the analyte of interest, and can cross- react with antibodies that form part of immunoassay method reagents, thus causing falsely high or low results.

Considering the nature of the project, every effort was made to identify any potential assay interferences. This involved the utilisation of the Beckman Coulter lipaemia/turbidity, icterus and haemolysis (LIH) reagent. This is a photometric test for the semi-quantitative assessment of LIH interference and was performed for each sample. In addition, each assay instruction for use (IFU) document provided by the manufacturer contained relevant interference study data. This was used in conjunction with the LIH results to generate appropriate comments associated with the measured result. Some assay IFUs indicated that other analytes (measured as part of the biomarker project) could cause significant interference above a particular concentration. If this scenario occurred, an associated comment was generated with the result to indicate that the tested interfering substance was at a concentration that exceeded the interference limit indicated in the assay IFU.

Table 2 shows the instrument flag and numerical result generated by different concentrations of LIH interference. Appendix 4 indicates the level of LIH, or other interfering substance, required to cause significant interference with an assay, and indicates the associated comment which replaced the result. Samples with LIH Results >5, i.e. above the measurable range, had all results suppressed.

Approximate Concentration of LIH Interference				
Instrument Flag	**Result**	**LIP mg/dL (intralipid)**	**ICT (mg/dL (Bilirubin)**	**Haem (mg/dL (Haemoglobin)**
N	**0**	< 40	< 2.5	< 50
+	**1**	40 - 99	2.5 - 4.9	50 - 99
++	**2**	100 - 199	5.0 - 9.9	100 - 199
+++	**3**	200 - 299	10 - 19.9	200 - 299
++++	**4**	300 - 500	20 - 40	300 - 500
+++++	**5**	> 500	> 40	> 500

Table 2: Relationship between LIH Flag and concentration of LIH interference

4.0 Serum Assay Performance Characteristics

Throughout the project detailed quality and method performance protocols were carried out to maintain confidence that the assays were performing to the manufacturers' specifications.

One element of the quality protocol was the bracketing of participant samples with Internal Quality Control (IQC) samples of known high, medium and low concentrations. IQC samples were run prior to each batch of participant samples (opening bracket) and after each batch (closing bracket). Participant results were validated into the dataset if both the opening and closing IQC results were within the set control limits for the analytical process. The number of participant samples in a bracket averaged between 48 and 200 depending on the size of reagent pack and the time taken for analysis on a particular instrument.

Third party IQC material, (from Randox Laboratories and Technopath), were used for each assay. Third party IQC material was preferred because they are independent of a particular instrument/assay combination and therefore give a completely unbiased performance assessment of the whole analytical system. Table 3

provides information on assay performance, summarising the Coefficients of Variation (CVs[8]) derived from the IQC data for each assay over the period of the project.

Biomarker	IQC level	IQC Material in the Range	Average within-Laboratory (total) CV (%)	Average SD	Comment
IGF-1	Low	7.06-12.84	6.03	0.60	2 QC types- 4 different lots[9]
	Medium	27.23-44.97	5.29	1.54	2 QC types- 4 different lots
	High	35.85-84.62	6.18	3.44	2 QC types- 5 different lots
VIT D	Low	26.0-49.9	6.14	2.21	2 QC types- 4 different lots[9]
	Medium	54.2-85.9	5.39	3.81	2 QC types- 4 different lots
	High	78.1-116	5.04	4.73	2 QC types- 5 different lots
OEST	Low	175-417	15.26	39.05	1 QC type - 3 different lots
	Medium	446-990	8.68	61.91	1 QC type - 3 different lots
	High	1305-2503	6.47	124.24	1 QC type - 3 different lots
TEST	Low	1.04-2.20	8.34	0.13	1 QC type - 3 different lots
	Medium	13.40-22.84	3.66	0.71	1 QC type - 3 different lots
	High	29.31-49.4	4.15	1.62	1 QC type - 3 different lots
SHBG	Low	15.0-27.7	5.67	1.15	1 QC type - 3 different lots
	Medium	31.9-55.5	5.25	2.23	1 QC type - 3 different lots
	High	56.3-87.8	5.22	3.63	1 QC type - 3 different lots
APOA1	Low	0.92-1.38	2.04	0.02	1 QC type - 3 different lots
	Medium	1.42-1.92	1.70	0.03	1 QC type - 3 different lots
	High	1.78-2.49	1.85	0.04	1 QC type - 2 different lots
APOB	Low	0.77-0.94	2.68	0.02	1 QC type - 3 different lots
	Medium	1.08-1.36	2.50	0.03	1 QC type - 3 different lots
	High	1.28-1.83	2.46	0.04	1 QC type - 2 different lots
ALB	Low	27.1-33.5	2.09	0.63	1 QC type - 2 different lots
	Medium	41.8-49.7	2.20	1.00	1 QC type - 2 different lots
	High	50-61.2	2.13	1.18	1 QC type - 2 different lots
ALP	Low	57-87	3.08	2.22	1 QC type - 2 different lots
	Medium	199-252	2.84	6.42	1 QC type - 2 different lots
	High	340-514	2.87	12.23	1 QC type - 2 different lots
ALT	Low	22-30.7	2.91	0.78	1 QC type - 2 different lots
	Medium	100.4-120.4	1.54	1.39	1 QC type - 2 different lots
	High	190.4-255.3	1.16	2.58	1 QC type - 2 different lots
AST	Low	41-49	2.13	0.96	1 QC type - 2 different lots
	Medium	126-146	1.56	1.89	1 QC type - 2 different lots
	High	234-295	1.33	3.54	1 QC type - 2 different lots
CALC	Low	1.44-1.66	1.61	0.03	1 QC type - 2 different lots
	Medium	2.29-2.58	1.39	0.03	1 QC type - 2 different lots
	High	2.96-3.30	1.29	0.04	1 QC type - 2 different lots
Biomarker	IQC level	IQC Material in the Range	Average within-Laboratory	Average SD	Comment

[8] Coefficient of variation is a standardized measure of dispersion of a frequency distribution; it is defined as the ratio of the standard deviation to the mean and is widely used to express the precision and repeatability of an assay. A low CV indicates a well-controlled assay

[9] Initially 3 levels of Randox IQC material were used but due to difficulties receiving IQC material in time, there were some periods where all 3 levels could not be analysed. To continue analysis a decision was made to analyse 3 levels of Technopath IA IQC material along with the available level(s) of Randox IQC material. As soon as all 3 levels of Randox IQC were available this practice stopped.

			(total) CV (%)		
CHOL	Low	2.47-3.04	1.78	0.05	1 QC type - 2 different lots
	Medium	3.90-5.06	1.65	0.07	1 QC type - 2 different lots
	High	5.90-7.45	1.41	0.09	1 QC type - 2 different lots
CREA	Low	55-68	2.74	1.71	1 QC type - 2 different lots
	Medium	159-197	1.91	2.80	1 QC type - 2 different lots
	High	470-529	1.39	6.92	1 QC type - 2 different lots
CRP	Low	0.85-1.17	2.31	0.02	2 QC type - 2 different lots
	Medium	1.56-3.17	1.70	0.04	1 QC type - 3 different lots
	High	7.11-8.68	1.69	0.14	1 QC type - 3 different lots
DBIL	Low	5.0-6.6	2.60	0.15	1 QC type - 2 different lots
	Medium	15-17.8	2.09	0.32	1 QC type - 2 different lots
	High	27.7-53.6	1.73	0.69	1 QC type - 2 different lots
GGT	Low	22.9-29.6	2.84	0.74	1 QC type - 2 different lots
	Medium	70.8-83.8	1.94	1.21	1 QC type - 2 different lots
	High	135.2-160.9	1.44	2.14	1 QC type - 2 different lots
GLU	Low	2.51-3.35	1.82	0.05	1 QC type - 2 different lots
	Medium	6.57-8.02	1.62	0.11	1 QC type - 2 different lots
	High	14.16-17.74	1.49	0.24	1 QC type - 2 different lots
HDL	Low	0.70-0.87	1.81	0.01	1 QC type - 2 different lots
	Medium	1.05-1.38	1.76	0.02	1 QC type - 2 different lots
	High	1.39-1.81	1.72	0.03	1 QC type - 2 different lots
LDL	Low	1.46-1.94	1.71	0.03	1 QC type - 2 different lots
	Medium	2.48-3.23	1.59	0.04	1 QC type - 2 different lots
	High	3.65-4.91	1.57	0.07	1 QC type - 2 different lots
LP-a	Low	16.5-27.54	6.13	1.35	1 QC type - 3 different lots
	Medium	30.27-53.46	4.39	1.80	1 QC type - 3 different lots
	High	35.9-73.1	3.83	2.08	1 QC type - 2 different lots
PHOS	Low	0.69-0.83	2.36	0.02	1 QC type - 2 different lots
	Medium	1.22-1.48	2.05	0.03	1 QC type - 2 different lots
	High	1.96-2.54	1.74	0.04	1 QC type - 2 different lots
UREA	Low	2.70-3.30	3.04	0.09	1 QC type - 2 different lots
	Medium	12.5-16.3	2.52	0.34	1 QC type - 2 different lots
	High	18.1-23.3	2.29	0.47	1 QC type - 2 different lots
TBIL	Low	14.5-17.7	1.92	0.31	1 QC type - 2 different lots
	Medium	46.5-57.1	1.57	0.75	1 QC type - 2 different lots
	High	83.9-97.9	1.48	1.33	1 QC type - 2 different lots
TP	Low	43.4-50.3	1.22	0.57	1 QC type - 2 different lots
	Medium	63.9-70.2	1.13	0.73	1 QC type - 2 different lots
	High	81.3-89.9	1.09	0.93	1 QC type - 2 different lots
TRIG	Low	0.54-0.76	2.27	0.01	1 QC type - 2 different lots
	Medium	1.53-1.87	2.18	0.04	1 QC type - 2 different lots
	High	2.53-3.2	2.05	0.06	1 QC type - 2 different lots
UA	Low	133-153	1.54	2.21	1 QC type - 2 different lots
	Medium	338-407	1.33	4.54	1 QC type - 2 different lots
	High	446-560	1.21	6.07	1 QC type - 2 different lots
RF	low	17.0-25.6	2.55	0.54	1 QC type - 2 different lots
	High	33.6-39.5	1.58	0.58	1 QC type - 2 different lots
CYSC	Low	0.78-0.87	1.36	0.01	1 QC types- 5 different lots
	High	3.51-3.82	0.75	0.03	1 QC types- 5 different lots

Table 3: Assay performance Derived from IQC Data

5.0 External Quality Assurance (EQA)

Each assay was registered with an external quality assurance (EQA) scheme, and assay performance was externally verified via the results returned from participation in these schemes. Details of the schemes and providers used for each assay are summarised in Table 4.

Biomarker	Scheme	EQA Result Overview (No. of Good or Acceptable distributions as a % of Total no. of participated distributions as shown in brackets)
ALB	WEQAS Mainline Chemistry	97% (31)
ALP	WEQAS Mainline Chemistry	100% (31)
ALT	WEQAS Mainline Chemistry	100% (31)
APOA1	RIQAS Lipids	98% (76)
APOB	RIQAS Lipids	100% (77)
AST	WEQAS Mainline Chemistry	97% (30)
CALC	WEQAS Mainline Chemistry	100% (31)
CHOL	RIQAS Lipids	98% (76)
DBIL	WEQAS Bilirubin	100% (31)
CREA	WEQAS Mainline Chemistry	100% (31)
GGT	WEQAS Mainline Chemistry	100% (31)
GLU	WEQAS Mainline Chemistry	100% (31)
HDL	RIQAS Lipids	100% (77)
CRP	WEQAS CRP inc. hs-CRP	100% (24)
LDL	RIQAS Lipids	100% (77)
LP-a	RIQAS Lipids	100% (65)
PHOS	WEQAS Mainline Chemistry	100% (31)
RF	RIQAS Specific Proteins	100% (72)
TBIL	WEQAS Bilirubin	100% (31)
TP	WEQAS Mainline Chemistry	97% (31)
TRIG	RIQAS Lipids	100% (77)
UA	WEQAS Mainline Chemistry	100% (31)
UREA	WEQAS Mainline Chemistry	100% (13)
OEST	RIQAS Immunoassay	88% (135)
SHBG	RIQAS Immunoassay	95% (143)
TEST	RIQAS Immunoassay	99% (145)
IGF-1	RIQAS Immunoassay Speciality 1	100% (105)
VIT D	RIQAS Immunoassay Speciality 1	100% (108)
CYSC	Equalis	100% (20)

Table 4: Details of EQA Schemes and Providers

6.0 Quality System and Scope of Accreditation

The Biomarker Project was run under a strict quality regime. All assays were conducted under systems designed for and consistent with the internationally recognised standard for testing and calibration laboratories – ISO 17025:2005. During the project the UK Biobank laboratories were successfully externally audited against the ISO 17025:2005 standard. From the 17th December 2015 UK Biobank laboratories was ccredited to ISO17025 as a testing laboratory for the urine & HbA1c method. On 17th October 2016 this was extended to include all serum methods (UKAS accreditation reference: 8975).

Appendix 1: Table Summarising Assay Instrumentation & Technical Details

Serum Assay (Abbreviation)	Assay Manufacturer	Analytical Platform	Analysis Methodology	Measurement Units	Manufacturer's Analytical Range
Albumin (ALB)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Colourimetric	g/L	15 - 60
Alkaline Phosphatase (ALP)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic Rate	U/L	5 - 1500
Alanine Aminotransferase (ALT)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic Rate	U/L	3 - 500
Apolipoprotein A1 (APOA1)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Immuno-turbidimetric	g/L	0.4 - 2.5
Apolipoprotein B (APOB)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Immuno-turbidimetric	g/L	0.4 - 2
Aspartate Aminotransferase (AST)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic Rate	U/L	3 - 1000
Calcium (CALC)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Colourimetric	mmol/L	1 - 5
Cholesterol (CHOL)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic	mmol/L	0.5 - 18
Creatinine (CREA)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic	µmol/L	0 - 4420
High Sensitivity C-Reactive Protein (CRP)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Immuno-turbidimetric	mg/L	0.08 - 80
Direct Bilirubin (DBIL)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Colourimetric	µmol/L	0 - 171
Gamma-Glutamyltransferase (GGT)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic Rate	U/L	5 - 1200
Glucose (GLU)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic	mmol/L	0.6 - 45
High Density Lipoprotein (HDL)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzyme Immuno-inhibition	mmol/L	0.05 - 4.65
Low Density Lipoprotein (LDL)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic Selective Protection	mmol/L	0.26 - 10.3
Lipoprotein (a) (LP-a)	Randox Bioscience, UK	Beckman Coulter AU5800	Immuno-turbidimetric	nmol/L	5.76 - 189
Phosphate (PHOS)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Colourimetric	mmol/L	0.32 - 6.4
Rheumatoid Factor (RF)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Immuno-turbidimetric	IU/ml	10 - 120
Total Bilirubin (TBIL)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Colourimetric	µmol/L	0 - 513
Total Protein (TP)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Colourimetric	g/L	30 - 120
Triglyceride (TRIG)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic	mmol/L	0.1 11.3
Uric Acid (UA)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic	µmol/L	89 - 1785
Urea (UREA)	Beckman Coulter (UK), Ltd	Beckman Coulter AU5800	Enzymatic	mmol/L	0.8 - 50
Cystatin – C (CYSC)	Siemens plc	Siemens Advia 1800	Immuno-turbidimetric	mg/L	0.1 (7.7-8.99)
Insulin-like Growth Factor-1 (IGF-1)	Diasorin S.p.A	DiaSorin Liaison XL	Chemiluminescent Immunoassay – one step sandwich	nmol/L	1.3 – 195
Vitamin D (VITD)	Diasorin S.p.A	DiaSorin Liaison XL	Chemiluminescent Immunoassay- direct competitive	nmol/L	10 - 375
Oestradiol (OEST)	Beckman Coulter (UK), Ltd	Beckman Coulter DXI 800	Chemiluminescent Immunoassay- competitive binding	pmol/L	73 - 17621
Testosterone (TEST)	Beckman Coulter (UK), Ltd	Beckman Coulter DXI 800	Chemiluminescent Immunoassay- competitive binding	nmol/L	0.35 - 55.52
Sex Hormone Binding Globulin (SHBG)	Beckman Coulter (UK), Ltd	Beckman Coulter DXI 800	Chemiluminescent Immunoassay – 2 step sandwich	nmol/L	0.33 - (226-242)

Appendix 2: Expert Advisors

The tables below list (in alphabetical order) the members of the UK Biobank Enhancements Working Group which initiated the project, the Design Phase Expert Group who led on the selection of markers and assays, and the Biomarker Expert Working Group who guided and advised the project during its operational phase.

The UK Biobank project team would like to acknowledge the support and express thanks to all those who contributed their time and expertise to this project.

UK Biobank Enhancements Working Group

Individuals Name	Organisation
Chair: Prof Paul Elliott	Imperial College, London
Associate Prof Naomi Allen	University of Oxford/UK Biobank
Dr Rachael Almond	UK Biobank
Prof Sir Rory Collins	University of Oxford/UK Biobank
Prof Frank Kelly	Kings College London
Dr Tim Peakman	UK Biobank
Prof Naveed Sattar	University of Glasgow
Prof Augustin Scalbert	International Agency for Research on Cancer, Lyon
Dr Simon Sheard	UK Biobank
Dr Ioanna Tzoulaki	Imperial College, London
Prof Anthony Whetton	University of Manchester

Previous members: Prof Mark Caulfield (London); Prof John Gallacher (Oxford); Prof Alan Silman (Oxford); Prof Nick Wareham (Cambridge).

Biomarker Expert Working Group

Individuals Name	Organisation
Associate Prof Naomi Allen	University of Oxford/UK Biobank
Dr Rachael Almond	UK Biobank
Mrs Karen Chung	University of Oxford
Mr Richard Chung	University of Oxford
Mr Daniel Fry	UK Biobank
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Appendix 3: Sample Selection Algorithm

The standard for sample storage at UK Biobank is the SBS (Society for Biomolecular Screening) footprint tube rack, contains 96 x 1.2 ml or 96 x 0.65 ml individually identifiable microtubes.

Samples in storage at UK Biobank (both -80°C and LN_2) are grouped on storage racks by sample type and by participant in line with the sample processing and storage protocols in place at the time of sample collection (baseline sample collection).

For the Biomarker project, in order to ensure that assay drift, reagent/consumable batch effects and other systematic measurement errors did not systematically differ between cases and controls (in any case-control studies undertaken using the biomarker data), it was deemed important that the samples submitted for analysis were not grouped or submitted in a sequence which itself exhibited an underlying trend (participant phenotype, date or time of collection, geographical location, etc.)

The simplest way to achieve this would have been to request samples from storage in a randomised sequence. However, this approach (which would have led to a need for the store robotics system (on average) to access each individual rack on more than 20 occasions) would have significantly extended the project costs and timeframes.

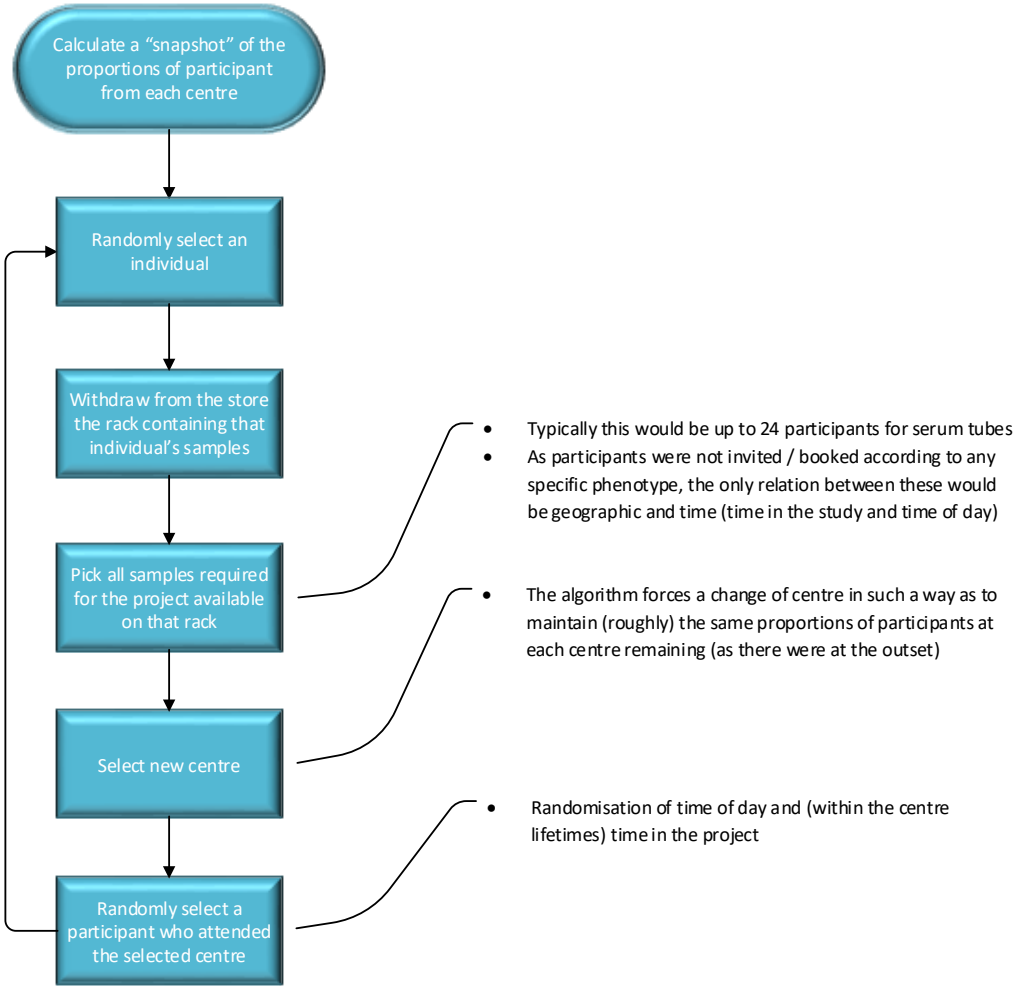
To mitigate this demand on the robotics and thus ensure that the sample retrieval process did not become the rate limiting step, a picking strategy was devised which addressed the requirement that samples should not be submitted for analysis in an order that exhibited any clustering or long term trends in collection timing/ location nor in any participant phenotypes.

To achieve this algorithm was developed. The approach adopted is summarised below (Appendix 3; Figure 1):

1. Snapshot the proportion of the total samples of a particular type that contributed to the UK Biobank archive collection by each assessment centre
2. Pick one tube (and thus rack) at random from a randomly selected assessment centre (the seed) and pick all samples required for the project from that rack (typically up to 24 / 32 / 48 per plate depending on sample type)
3. Assess the new proportions and select the assessment centre whose recalculated proportion has the greatest positive deviation from its respective baseline proportion (assessed in step 1), and select a tube (and thus rack) at random from this new assessment centre
4. Repeat from step 2 until all samples were picked.

This approach typically mixes 3 or 4 source plates (in some cases more) to create one destination (output) plate. Adopting this method has the following advantages:

- Ensures randomness of participant ages, ethnicity, sex etc. via the randomness of the attendance at the baseline centres
- Randomises time of day via the pseudo-random selection of the 3 (or more) source plates bundled to create one destination plate (though there will be a limited spread of time of day within the each single source plate)
- Generally forces a mixture of collection centres to be bundled in each destination plate
- Generally leads to a regional variation on the output plates (as a consequence of the distribution and sizes of the collection centres)
- Keeps the relative proportions of each assessment centre in the store in line with the original proportions and thus avoids long term drift and bias associated with prematurely exhausting the samples from specific (smaller) centres.



Appendix 3; Figure 1: Overview of the Sample Selection Algorithm

The algorithm was simulated against a model which accurately reflected the location of samples within the store (before use) to confirm its performance in ensuring a mix of centres, times, dates etc. and was then checked (during the project) against 3 sets of 14 sequentially created destination plates (picked at different times through the project). The approach was confirmed to prevent clustering against the following 8 characteristics:

- Assessment centre
- Date of sample collection
- Time of day of sample collection
- Age
- Sex
- Smoking status
- Townsend score
- Ethnicity

Appendix 4: Table Summarising Assay Interference

Level of Interference	Comment appended	Assays Effected
LIP = 3	Sample lipaemic level 3 (200-299mg/dl) indicating significant interference with this assay.	APOB
LIP = 4	Sample lipaemic level 4 (300-500mg/dl) indicating significant interference with this assay.	APOB, ALT, AST, CRP, DBIL
LIP ≥ 5	Sample lipaemic level 5 (>500mg/dl) indicating significant interference with this assay.	ALB, ALP, ALT, APOA1, APOB, AST, CALC, CHOL, CREA, CRP, CYSC, DBIL, GGT, GLU, HDL, LDL, LP-a, PHOS, RF, SHBG, TBIL, TEST, TP, TRIG, UA, UREA, VITD
LIP ≥ 5	Lipaemia measurement exceeds maximum measurement (>500mg/dL), interference for assay significant at 1800mg/dL	OEST
LIP ≥ 5	Lipaemia measurement exceeds maximum measurement (>500mg/dL), interference for assay significant at 3000mg/dL.	IGF-1
ICT = 2	Sample Icterus level 2 (86 - 170umol/L) indicating significant interference with this assay.	CHOL, TRIG
ICT = 3	Sample Icterus level 3 (171 - 340umol/L) indicating significant interference with this assay.	CHOL, GLU, OEST, TEST, TRIG, UREA
ICT = 4	Sample Icterus level 4 (342 - 684umol/L) indicating significant interference with this assay.	ALP, CHOL, GLU, IGF-1, LDL, OEST, TEST, TP, TRIG, UREA
ICT ≥ 5	Sample Icterus level 5 (>684umol/L) indicating significant interference with this assay.	ALB, ALP, ALT, APOA1, APOB, AST, CALC, CHOL, CREA, CRP, CYSC, DBIL, IGF-1, GGT, GLU, HDL, LDL, LP-a, OEST, PHOS, RF, SHBG, TBIL, TEST, TP, TRIG, UA, UREA, VITD
HAEM = 1	Sample haemolysed level 1 (50-99mg/dl) indicating significant interference with this assay.	AST, DBIL, TBIL
HAEM = 2	Sample haemolysed level 2 (100-199mg/dl) indicating significant interference with this assay.	AST, DBIL, TBIL
HAEM = 3	Sample haemolysed level 3 (200-299mg/dl) indicating significant interference with this assay.	AST, DBIL, GGT, TBIL, TP, UREA, VITD
HAEM = 4	Sample haemolysed level 4 (300-500mg/dl) indicating significant interference with this assay.	ALB, ALP, AST, DBIL, GGT, LDL, PHOS, TBIL, TP, UREA, VITD
HAEM ≥ 5	Sample haemolysed level 5 (>500mg/dl) indicating significant interference with this assay.	ALB, ALP, ALT, APOA1, APOB, AST, CALC, CHOL, CREA, CRP, CYSC, DBIL, IGF-1, GGT, GLU, HDL, LDL, LP-a, OEST, PHOS, RF, SHBG, TBIL, TEST, TP, TRIG, UA, UREA, VITD
Cholesterol ≥7.78	Cholesterol result >7.78mmol/L, this exceeds the cut off for <10% interference in assay result.	VITD
Total Protein ≥120	Total protein result is >120g/L, this exceeds the cut off for <10% interference in vitamin D assay result.	VITD
TP = <55 or >120	Significant interference is present when the sample concentration of Total Protein is outside of the range 55 - 85g/L.	TEST
TRIG ≥ 11.3	Triglyceride level indicates significant interference with assay.	HDL, LDL
TRIG ≥ 20	Triglyceride concentration indicates significant interference with assay.	OEST, TEST
Uric Acid ≥1190	Uric acid result is >1190umol/L, this exceeds the cut off for <10% interference in vitamin D assay result.	VITD