UK Biobank Biomarker Enhancement Project

Companion Document to Accompany HbA1c Biomarker Data

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Document Version Control

Version	*Issued*	*Changes*
V1.0		*N/A*

1.0 Introduction

In order to enhance further 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 HbA1c (glycated haemoglobin) biomarker data available through the UK Biobank Showcase.

It is intended to provide basic information on:-

- Assay selection & project scope
- Methods and equipment
- HbA1c assay performance characteristics
- Quality system and scope of accreditation

2.0 Assay Selection & Project Scope

Overall, 34 biomarkers were selected for assay in all 500,000 participants, full details of which can be found at http://www.ukbiobank.ac.uk/uk-biobank-biomarker-panel/

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

The project was co-ordinated by the Enhancement Working Group, with input from external experts, where required (listed in Appendix 1 – Expert Advisors).

This document focuses on the HbA1c biomarker only.

3.0 Assay Equipment and Information

The HbA1c assay was performed using five Bio-Rad Variant II Turbo analysers. These analysers are manufactured by Bio-Rad Laboratories, Inc. and employ a High Performance Liquid Chromatography (HPLC) method. The units of measurement are in mmol/mol (International Federation of Clinical Chemistry [IFCC] units), with a manufacturers' analytical range of 15-184 mmol/mol. All reagents and calibrators used were sourced from Bio-Rad, and quality control material was sourced from both Bio-Rad and a 3rd party provider, Randox Bioscience.

The five analysers underwent a rigorous validation protocol including matrix validation for the use of PRBC, an atypical matrix for this CE marked assay, to ensure they were all compliant with ISO 17025:2005 standards before analysis commenced. The validation study also included a multi-instrument comparison to ensure all five instruments were in agreement.

The Bio-Rad Variant II Turbo Haemoglobin Testing System (Figure 1) uses HPLC to determine the relative concentration of HbA1c in PRBCs.

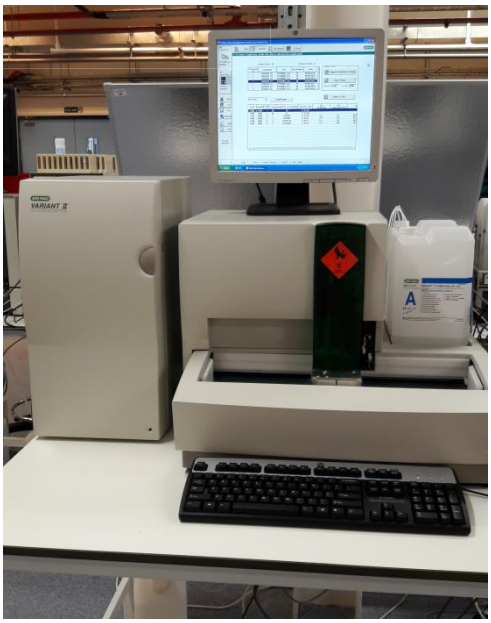

Figure 1: Bio-Rad Variant II Turbo Haemoglobin Testing System

3.1 Results Outside of the Reportable Range

The HbA1c assay was validated against the manufacturer's performance information. Linearity experiments determined the reportable range. The observed reportable range covered the manufacturer's analytical range of 15 to 184 mmol/mol.

Showcase data only includes HbA1c results returned by the instrument that are within the reportable range; where a valid result was not obtained, the result field is populated with either:

'< 15' if the result returned is lower than the bottom of the reportable range.

'> 184' if the result returned is greater than the top of the reportable range.

3.2 Pre-Diluted Samples

Due to the nature of the sample type used for this assay (PRBCs) a suitable acceptable range for total area was determined in excess of that determined by the manufacturer for whole blood samples. Samples outside of the range of 1.0 to 4.0 million µvolt/second (to 1 d.p) were reanalysed after being manually diluted 'off-board'. Any sample subjected to an 'off-board' manual dilution will have the comment field populated with '1:300 off-board dilution performed. Dilution volumes were 5 µL PRBC sample in 1.5 mL sample diluent'.

3.3 Total Area Results Outside of the Allowable Total Area Range

Showcase data only includes results returned by the instrument where the Total Area is within the acceptable range. When it was not possible to obtain a valid Total Area result, the result field is populated with the comment 'HbA1c result Invalidated'. The comment field contains the reason for the invalidation, either 'Total Area greater than analytical range - HbA1c result invalidated' or 'Total Area less than analytical range - HbA1c result invalidated'.

3.4 Invalidation of Results

Showcase data only includes numerical values for HbA1c when valid results are returned from the analysers. If the result set becomes invalidated for any of the reasons listed below in Table 1, the result field is populated with the comment 'HbA1c result invalidated' and the comment field details the reason (see Table 1).

Invalidation Reason	Associated comment
P3 peak area exceeds 10%	P3 peak >10% interferes with HbA1c measurement, HbA1c result invalidated.
P4 peak area exceeds 10%	P4 peak >10% interferes with HbA1c measurement, HbA1c result invalidated.
A1a peak area exceeds 5%	A1a peak >5% HbA1c result invalidated.
A1b peak area exceeds 5%	A1a peak >5% HbA1c result invalidated.
Unable to detect an HbA1c peak	Abnormal chromatogram, no HbA1c detected.
Labile HbA1c peak exceeds 5%	Labile HbA1c peak is >5%, HbA1c result invalidated.
Peak detected within the Variant Window	Unidentified peak detected in the Variant Window, HbA1c result invalidated.
Unknown peak area exceeds 3%	Unknown peak with area >3% detected, HbA1c result invalidated.
HbF peak area exceeds 25%	HbF>25% interferes with HbA1c measurement. HbA1c result invalidated.

Table 1 – HbA1c Invalidation reasons and associated comments

4.0 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' specification.

One element of the quality protocol was the bracketing of participant samples with Internal Quality Control (IQC) samples of known high and low concentration. Randox IQC samples were run prior to each batch of participant samples (opening bracket) and after each batch (closing bracket)[1]. Bio-Rad IQC samples were run once each day prior to analysis to ensure optimum performance of the analysers. Participant results were validated into the dataset if the start of day Bio-Rad IQC and opening and closing bracket Randox IQC results were within the set control limits for the analytical process.

Table 2 provides information on IQC performance, summarising the Coefficients of Variation[2] (CV) derived from the IQC data for Randox IQC material used for bracketing purposes over the period of the project.

Material	IQC level	IQC material in the range[3]	CV (%)
Randox IQC	Low	36.35 – 40.70	2.13
	High	92.41 – 105.17	1.46

Table 2 – Performance of HbA1c IQC

[1] The batch size maximum was set at 40 samples; typically IQC brackets were run every 32 samples

[2] 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

[3] For each assay, a number of IQC lots were used; the table presents the range of Lot means

Additional information:

The HbA1c assay was registered with UK NEQAS for Glycated Haemoglobins External Quality Assurance (EQA) scheme and assay performance was externally verified via the results returned from participation in this scheme.

A sample selection algorithm was implemented to ensure no bias or drift was introduced to the assay as a consequence of the order of sample analysis.

5.0 ISO 17025:2005 Quality 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 - ISO17025:2005.

During the project the UK Biobank laboratories were successfully externally audited against the ISO17025:2005 standard. From the 17th December 2015, the UK Biobank laboratories have accreditation to ISO17025:2005 as a testing laboratory. The HPLC method for HbA1c appears on the scope of accreditation (UKAS accreditation reference: 8975).

Appendix 1 – Expert Advisors

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

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
Mr Mark Gordon	UK Biobank
Dr Michael Hill	University of Oxford
Dr Gareth McClean	University of Oxford
Mr Stewart Moffat	UK Biobank
Dr Simon Sheard	UK Biobank
Mrs Jane Wintour	University of Oxford

Design Phase Expert Working Group

Individuals Name	Organisation
Prof Jane Armitage	University of Oxford
Prof Peter Burney	Imperial College, London
Dr Karen Canfell	University of Sydney, Australia
Prof Robert Clarke	University of Oxford
Prof John Danesh	University of Cambridge
Prof Paul Foster	Moorfields Eye Hospital, London
Dr Silvia Franceschi	International Agency for Research on Cancer (IARC), Lyon, France
Dr Marc Gunter	Imperial College, London (now IARC)
Prof Ian Hall	University of Nottingham
Dr Anna Hansell	Imperial College, London
Prof Nick Harvey	MRC Life-course Epidemiology Unit, University of Southampton
Dr Michael Hill	University of Oxford
Prof Debbie Jarvis	Imperial College, London
Prof Tim Key	University of Oxford
Prof Michael Kidd	Flinders University, Adelaide, Australia
Prof Dave Leon	London School of Hygiene & Tropical Medicine, London
Prof Gordon Lowe	University of Glasgow
Dr Teri Manolio	National Institute of Health, Bethesda, USA
Prof Stephen MacMahon	George Institute, University of Sydney/University of Oxford
Prof Naveed Sattar	University of Glasgow
Prof Liam Smeeth	London School of Hygiene & Tropical Medicine, London
Prof David Strachan	St George's Hospital, London
Prof Martin Tobin	University of Leicester
Prof Paolo Vineis	Imperial College, London
Prof Cyrus Cooper	MRC Life-course Epidemiology Unit, University of Southampton