UK Biobank

Serological measurement of infectious agents in UK Biobank: a pilot study in 10,000 samples

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Introduction

In order to assess the feasibility of large-scale multiplex measurement of antibodies against 20 infectious agents and to determine their estimated sero-prevalence in the UK Biobank cohort, a pilot study was performed in approximately 10,000 samples. Development and validation of the multiplex panel was performed in collaboration with Drs. Tim Waterboer and Nicole Brenner at the German Cancer Research Centre (DKFZ), Heidelberg, Germany.

The purpose of this document is to provide explanatory information on the antibody response and sero-positivity status of 20 pathogens for ~10,000 UK Biobank samples measured in the pilot study.

Pathogens and antigens included in the panel

The 20 pathogens selected for the panel were chosen because they are either established risk factors for outcomes such as cancer, and cardiovascular or neurodegenerative diseases, or are of novel scientific interest (see Table 1). The final selection of antigens was based on their known biological functions and/or existing assays. Furthermore, the performance characteristics of each pathogen-specific assay consisting of 1-6 antigens was validated before inclusion in the final panel run on a Luminex platfom. During validation, each pathogen-specific assay was compared with a gold-standard reference assay using independently sourced reference sera. The sensitivity and specificity of each of the pathogen-specific assays was above the pre-specified validation criteria of 85%, with a median sensitivity and specificity of 97.0% and 93.7%, respectively. Further details of assay validation, conducted by Drs Tim Waterboer and Nicole Brenner are available (see Table 1).

Multiplex Serology measurements

9,724 UK Biobank participant samples selected at random were assayed at DKFZ in July 2016 using Multiplex Serology. Of these, 9,695 (99.7%) samples passed validity checks and are available for data analysis.

Multiplex Serology was performed over a 2-week period at serum dilution 1:1000. The methodology was previously developed (Waterboer *et al.* 2005, 2006) and applied in various sero-epidemiological studies on a broad range of pathogens (e.g. Sankaranarayanan *et al.* 2016, Kreimer *et al.* 2017, Butt *et al.* 2019, Hammer *et al.* 2015).

The output from the Luminex reader produced quantitative data expressed in median fluorescence intensity (MFI) values per pathogen-specific antigen and serum. Sero-positivity for each antigen was determined by examining percentile plots and cut-offs were chosen to optimise sensitivity and specificity, based on the validation work.

Based on the quantitative properties of the data, MFI values can also be analysed in categories (e.g. tertiles) among the sero-positives to evaluate dose-response relationships.

Table 1. List of pathogens and pathogen-specific antigens included in the multiplex panel including suggested cut-offs to determine sero-positivity

Pathogen name	Pathogen abbrev.	Antigen	suggested cut-off [MFI] to assign sero-positivity for each antigen (>)	Definition of pathogen sero-positivity (for pathogens with more than 1 antigen)	References
Herpes Simplex virus-1	HSV-1	1gG	150	n/a	Brenner *et al.* 2018
Herpes Simplex virus-2	HSV-2	2mgG unique	150	n/a	Brenner *et al.* 2018
Varicella Zoster Virus	VZV	gE / gI[1]	100	n/a	Brenner *et al.* 2018
Epstein-Barr Virus	EBV	VCA p18	250	Positive for 2 or more antigens	Brenner *et al.* 2018
		EBNA-1	250		
		ZEBRA	100		
		EA-D	100		
Human Cytomegalovirus	CMV	pp150 Nter	100	Positive for 2 or more antigens	Brenner *et al.* 2018
		pp 52	150		
		pp 28	200		
Human Herpesvirus-6	HHV-6	IE1A	100	HHV-6 overall[2]: Positive for any antigen HHV-6A: Positive for IE1A HHV-6B: Positive for IE1B	Engdahl *et al.* (in preparation)
		IE1B	100		
		p101 k	100		
Human Herpesvirus-7	HHV-7	U14[1]	100	n/a[2]	
Kaposi's Sarcoma-Associated Herpesvirus	KSHV	LANA	100	Positive for any antigen[2]	
		K8.1	175		
Hepatitis B Virus	HBV	HBc	100	Positive for both antigens[3]	Brenner *et al.* 2019
		HBe	150		
Hepatitis C Virus	HCV	Core	150	Positive for both antigens[4]	Brenner *et al.* 2019; Dondog *et al.* 2015
		NS3	150		
Toxoplasma gondii	T. gondii	p22	100	Positive for any antigen	Brenner *et al.* 2019
		sag1	160		
Human T-Lymphotropic Virus-1	HTLV-1	HTLV-1 gag	1500	Positive for any antigen	Brenner *et al.* 2019
		HTLV-1 env	150		

Human Immunodeficiency Virus	HIV-1	HIV-1 gag	600	Positive for both antigens	Kranz *et al.* submitted
		HIV-1 env	150		
Human Polyomavirus BKV	BKV	BK VP1	250	n/a	Gossai *et al.* 2016
Human Polyomavirus JCV	JCV	JC VP1	250	n/a	Gossai *et al.* 2016
Merkel Cell Polyomavirus	MCV	MC VP1	250	n/a	Gossai *et al.* 2016
Human Papillomavirus type-16	HPV 16	L1	175	Two definitions are available. Definition I: positive for L1 (cumulative exposure marker) Definition II: positive for E6 and/or E7 (cancer marker)[5]	L1: Combes *et al.* 2014 E6, E7: Kreimer *et al.* 2017
		E6	120		
		E7	150		
Human Papillomavirus type-18	HPV 18	L1	175	n/a	Combes *et al.* 2014
Chlamydia trachomatis	C. trachomatis	momp D	100	Two definitions are available.[6] Definition I: positive for pGP3 Definition II: (for pGP3 sero-negatives): positive for 2 out of 4 antigens (highest value for momp A or momp D, and tarpD F1, tarpD F2 and porB)	Definition I: Trabert *et al.* 2018 Definition II: Hulstein *et al.* 2018
		momp A	100		
		tarp-D F1	100		
		tarp-D F2	100		
		PorB	80		
		pGP3	200		
Helicobacter pylori	H. pylori	CagA	400	Two definitions are available[7]. Definition I: positive for 2 or more antigens Definition II: positive for 2 or more antigens (excluding CagA)	Michel *et al.* 2009 Butt *et al.* 2019
		VacA	100		
		OMP	170		
		GroEL	80		
		Catalase	180		
		UreA	130		

1 VZV antigens gE and gI were co-loaded onto the same Luminex bead set.
2 Because there are no universally applicable serological gold standard assays available for HHV-6, HHV-7 and KSHV, formal validation of these assays was not performed yet. As such, these classifications should be treated with caution.
3 The definition of HBV sero-positivity depends on the research question. Please see Brenner *et al* b for details.
4 This definition for HCV sero-positivity increases specificity, while an AND/OR algorithm optimizes sensitivity.
5 HPV16 early proteins E6 and E7 are oncoproteins. Sero-positivity for HPV16 oncoproteins is associated with (potentially) HPV16-driven cancers (e.g. Combes *et al.* 2014, Kreimer *et al.* 2015). HPV16 E6 sero-positivity is a highly sensitive and specific prospective marker for HPV16-driven oropharyngeal cancer (Kreimer *et al.* 2017, Holzinger *et al.* 2017).
6 Definition 1 should be used as the main definition for sero-positivity for *C. trachomatis.*
7 The CagA antigen was excluded from the data generated in the 2nd week because of a lab-based handling mistake. Hence, two definitions are available, one with and one without the CagA antigen.

Sero-prevalence estimates

The sero-prevalence of each pathogen was calculated using the cut-offs and algorithms described in Table 1 among the 9,695 participants in the pilot study (shown in Table 2).

Table 2: Sero-prevalence estimates for each infectious agent included in the pilot study

Pathogen	Sero-prevalence in 9,695 UK Biobank participants [%]
HSV-1	69.8
HSV-2	16.2
VZV	92.5
EBV	94.7
CMV	58.2
HHV-6	90.8
HHV7	94.7
KSHV	8.1
HBV	2.5
HCV	0.3
HIV	0.2
HTLV-1	1.6
HPV-16	4.4
HPV-18	2.7
BKV	95.4
JCV	57.5
MCV	66.7
C. trachomatis	21.4
H. pylori	31.5
T. gondii	28.0

Data uploaded into the Data Showcase

- MFI values for each antigen
- Sero-positivity statuses for each pathogen (true/false)
- Error flag
- Date of assay

Contact for queries about the data:

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