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Development of an External Quality Assessment (EQA) Programme for SARS-CoV-2 Ab

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Introduction

A number of SARS-CoV-2 antibody tests are now available for use both as a Laboratory or Point of care test. These tests are used to determine the incidence of SARS-CoV-2 infection and the prevalence of immunity in the general population. More recently they have also been used to assess the durability of antibody response post vaccination and as part of the management of immunocompromised patients.

There are several types of immunoassays available, using different viral antigens for antibody detection, such as the spike, membrane, envelope and nucleocapsid proteins.

The most common antigens used are the spike protein, which contains the domain for attachment to the host cells, and the nucleocapsid protein, involved in viral replication, transcription and assembly.

These methods offer either IgG alone or total antibody and provide either qualitative or quantitative results.

Interpretation of the test

As a response to natural infection, antibodies to both N (Nucleocapsid) protein and S (Spike) protein will be produced.

A positive Nucleocapsid antibody test can be interpreted as evidence that SARS-CoV-2 infection has occurred at some time in the past few months, but cannot determine exactly when the infection happened.

A positive Spike antibody test result can be interpreted as either evidence of SARS-CoV-2 vaccination or that SARS-CoV-2 infection has occurred at some time in the past few months, but cannot determine exactly when the infection happened.

A negative test result does not exclude previous infection with SARS-CoV-2, as some patients who have had SARS-CoV-2 infection may not have detectable antibodies.

Immunosuppression and treatments such as immunoglobulin therapy, may affect antibody results.

Table 1 – Interpretation of SARS-CoV-2 Ab

	'S' Antibody	'N' Antibody
Natural infection	Positive	Positive
Vaccination	Positive	Negative
Vaccination and previous infection	Positive	Positive / Negative
No recent previous infection or vaccination history	Negative	Negative

In 2020, Weqas developed an EQA programme to assess and monitor the performance of these tests.

Method

Samples were prepared from donations collected in-house from healthy donors who had no exposure to Covid-19 i.e. pre December 2019, donations screened negative for SARS-CoV-2 Ab by at least two different spike methods, patients confirmed as positive for COVID-19, and from healthy donors following vaccination. All samples were collected into serum separation tubes, separated and frozen at -20°C. Additionally convalescent plasma samples were also used.

Three samples were distributed every month to 90 participants over a 15 month period.

Results

A wide variation of results were observed even within the same immunoassay type, (Table 2). For Anti S methods, there was a 600 fold difference in the results between the different methods, however, all the methods correctly identified the high Ab titre samples, whilst equivocal results were reported for the Beckman and Healgen methods for sample CV0721-1. For the Anti N Ab positive samples, the Roche method correctly identified all samples whilst equivocal results were reported for the Abbott method for CV11-2 and CV9-1 and a negative result for CV0621-1.

Table 2 – Summary of results for Anti-S and Anti-N Ab methods

Sample	Clinical details	Correct Interpretation	Anti- S / RBD methods U/mL						Anti-N Methods	
			Roche S (n=19)	Abbott S (n=6)	Siemens (n=7)	Beckman (n-4)	Healgen (n=3)	Lumira Dx (n=2)	Roche N (n= 21) "cut off" Index ≥ 0.8	Abbott N (n=9) "cut off" Index = 1.4
CV0921-1	Patient exposed to Covid-19 in Mar-20 – moderate symptoms, no PCR test, LFD negative 1 month after exposure. Further worsening of Covid symptoms 9 months later, positive PCR result, atypical - suggestive of a chronic rather than acute presentation, Ab results neg. Subsequently diagnosed with long Covid. This sample taken 1 month after 2nd vaccination.	Pos Anti S	All > 250, 12,667	19,432	>75, >10 pos	Pos 25.6	pos		Neg, 0.1	Neg, 0.13
CV0821-2	No exposure/ 1 months post 2nd vaccination	Pos Anti S	All> 250, 6625	8314	pos	15.7	pos	pos	Neg, 0.07	Neg, 0.03
CV0721-3	No exposure/ 3 months post 2nd vaccination (PB)	Pos Anti S	All > 250, 2500	5072	pos, >10	Pos, 8.36	Pos		Neg, 0.09	Neg, 0.02
CV0921-3	No exposure/ 4 months post 2nd vaccination	Pos Anti S	All > 250, 3411	5083	>75, >10 pos	9.2	pos		Neg, 0.1	Neg, 0.16
CV0921-2	No exposure/ 5 months post 2nd vaccination	Pos Anti S	All > 250, 918	1372	>75, >10 pos	Pos 2.1	pos		Neg, 0.1	Neg, 0.02
CV0721-2	No exposure/ 5 months post 2nd vaccination (PB)	Pos Anti S	All > 250,	1601	pos, >10	Pos, 1.82	Pos		Neg, 0.09	Neg, 0.07
CV0821-1	No exposure/ 4 months post 2nd vaccination	Pos Anti S	All > 250, 717	1130	pos	2.6	pos	pos	Neg, 0.08	Neg, 0.06
CV0821-3	No exposure/ 2 weeks post 2nd vaccination (AZ)	Pos Anti S	All> 250, 691	667	pos	1.23	pos	pos	Neg, 0.08	Neg, 0.02
CV0721-1	No exposure/ 2 months post 1st vaccination (AZ)	Pos Anti S	Pos, 52.7	274	pos, 5.3	Equ, 0.85	Equ		Neg, 0.09	Neg,0.02
CV12 - 3	No exposure/ 3 weeks post 2nd vaccination (PB)	Pos Anti S	Pos, All> 250,	Pos, 16,349	pos, >10	Pos, 24.58	Pos		Neg, 0.1	Neg, 0.01
CV0621-3	No exposure/ 3 weeks post 2nd vaccination (PB)	Pos Anti S	Pos, All> 250, 5179	Pos, 6938	pos, >10	Pos, 1.5	Pos		Neg, 0.09	Neg, 0.05
CV12 - 2	No exposure/ 3 weeks post 2nd vaccination (PB)	Pos Anti S	Pos, All> 250,	Pos, 6398	pos, >10	Pos, 12.56	Pos		Neg, 0.09	Neg, 0.05
CV12 - 1	No exposure/ 3 weeks post 2nd vaccination (PB)	Pos Anti S	Pos, All> 250,	Pos,8829	pos, >10	Pos, 15.12	Pos		Neg, 0.26	Neg, 0.02
CV10-3	No exposure / 10 weeks post 2nd vaccination	Pos Anti S	Pos >250	Pos, 4600	Pos >10	Pos, 8.0	Pos		Neg, 0.08	Neg, 0.09
CV10-2	No exposure/ 8 weeks post 2nd vaccination	Pos Anti S	Pos, 2312	Pos, 3000	Pos >10	Pos, 5.0	Pos		Neg, 0.08	Neg, 0.085
CV0621-2	No exposure/ no vaccination	Neg	Neg, All< 0.4	Neg, 5.6	neg, 0.065	Neg, 0.1	Neg		Neg, 0.1	Neg, 0.02
CV11-3	No exposure/ no vaccination	Neg	Neg, <0.4	Neg, 4.3 (1/5 pos)	Neg, 0.09	Neg, 0.035	Neg/Pos		Neg, 0.09 (7% pos)	Neg, 0.02
CV9-2	Neg Blood Transfusion donation	Neg			Neg< 0.05	Neg, 0.19	Neg		Neg, 0.07	Neg 0.03
CV11-1	Confirmed Covid infection 3 months / no vaccination	Pos Anti -S +Anti-N	Pos,240	Pos, 1310	Pos>10	Pos, 7.3	Pos		Pos, 36.9	Pos 2.7
CV11-2	Confirmed Covid infection 6 months (same patient as CV11-1) / 1 month post 2nd vaccination (PB)	Pos Anti -S +Anti-N	Pos >250	Pos, 31,887	Pos >10	Pos, 33	Pos		Pos, 21	1.14, 66.7% Neg, 33.3% Equivocal
CV0621-1	Same sample as CV11 -2	Pos Anti -S +Anti-N	Pos, All > 250, 19,904	Pos, 31,552	pos, > 10	Pos, 1.5	Pos		Pos 22.7	Neg
CV10-1	Confirmed Covid infection 1 month / no vaccination	Pos Anti -s +Anti-N	Pos, 102	Pos, 1870	Pos, 9.11	Pos, 12.0	Pos		Pos, 30.9	Pos, 4.0
CV9-3	Blood Transfusion donation screened pos for Ab	Pos Anti -S +Anti-N			Pos >10	Pos, 7.63	Pos		Pos, 48.3	Pos, 2.25
CV9-1	Blood Transfusion donation screened pos for Ab	Pos Anti -S +Anti-N			Pos, 8.6	Pos, 11.42	Pos		Pos, 2.78	Pos, 1.43 (73%), equ (27%)

Discussion

The decrease of Ab response to a natural infection over time appears to be assay specific. Patient CV11-1 was confirmed as exposed to SARS-CoV-2 virus in October 2020 and an Ab test 3 months later in January 2021 produced positive response across all platforms for both Anti-S and Anti-N. Following vaccination in January and March a further sample was taken in April, (CV11-2 and CV0621-1). The second sample produced significantly higher Anti-S Ab response of 20,000 and 31,000 U/mL for the Roche and Abbott methods with a 38% and 58% waning of the Anti-N Ab on the semi-quantitative Roche and Abbott platforms respectively. However, the majority of Abbott Anti-N users interpreted this as a negative result whilst the Roche method remained positive at a concentration 28 x "cut off" Index. The detection of antibody markers will wane over time, however this example illustrates that this appears to be assay specific and in some assays the positive signal is lost around 3 to 4 months after infection (Abbott) whilst other methods detect natural Ab for much longer periods. It is not yet clear how this will be affected by vaccination and whether the signal will be maintained for a longer period and what the variation within the available assays will be. Despite the availability of the WHO International Standard for Anti-SARS-CoV-2 immunoglobulin for harmonisation of binding antibody assays in December 2020 and the NIBSC Working Standard Anti-SARS-CoV-2 Antibody Diagnostic Calibrant, the variability of assay performance remains an area of concern with little or no harmonisation of the results between methods reported.