Pathology Laboratory, Department of Blood Sciences			
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Validation and verification of new or existing equipment			

Initiator:	Subash Gurung	**Change control number:**	
Department:	Blood Sciences	**Date of Procedure:**	24/03/2020
Validation (Y/N)	N	**Verification (Y/N)**	Y

1. User Requirements (URS)

Provide a description of the equipment that requires validation and verification:

Validation and verification for ELISA assay for the Detection of IgG antibodies to SARS-CoV-2 N Protein on DS2, automated benchtop ELISA instrument.

Kit: EDI Novel Coronavirus COVID-19 IgG ELISA KIT

Instrument: DS2

Purpose of the examination and clinical significance:

Measuring serum antibodies to the SARS-CoV-2 (COVID-19) virus is useful to assess if patient have been infected with this virus. It is of use to confirm infection in suspected PCR/RNA+ individuals, but in particular in symptomatic individuals who have been found to be negative on PCR/RNA testing. It can be used to screen for evidence of exposure in asymptomatic individuals as in most cases the virus will not be detectable any more. It is therefore a valuable tool in contact tracing.

SARS-COV-2 virus infections have emerged in the end of 2019 and have since then rapidly grown to a pandemic reaching in May 2020 more than 3,6 Million cases and 250 thousand deaths worldwide and more than 200 thousand cases with 30 thousand deaths in the UK (see: John Hopkins Coronavirus resource center; coronavirus.jhu.edu). SARS-COV-2 is a positive sense RNA virus. One major SARS-COV-2 proteins have been used in immune assay to detect specific IgG levels, the Nucleocapsid protein (N). The N protein is implicated in viral replication. It is therefore a main target of neutralizing antibodies (Walls et al, Cell 180, 2020).

Principle and method of the procedure:

This ELISA kit is designed, developed, and produced for the qualitative measurement of the human anti-COVID-19 IgG antibody in serum. This assay utilizes the microplate based enzyme immunoassay technique. Assay controls and 1:100 diluted human serum samples are added to the microtiter wells of a microplate that was coated with COVID recombinant full length nucleocapsid protein. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step.

A horseradish peroxidase (HRP) labelled polyclonal goat anti-human IgG tracer antibody is added to each well. After an incubation period, an immunocomplex of "COVID-19 recombinant antigen – human anti-COVID-19 IgG antibody - HRP labelled anti human IgG tracer antibody" is formed if there is specific coronavirus IgG antibody present in the tested specimen. The unbound tracer antibody is removed by the subsequent washing step. HRP tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the anti-COVID-19 IgG on the wall of the microtiter well is proportional to the amount of the anti-COVID-19 IgG antibody level in the tested specimen.

Performance characteristics and regulatory standards required for equipment, reagent, service, and environment

Equipment and reagents required:

Item	Stock storage	In-use storage	Supplier	Part number
Dynex DS2 EIA Processor	Blood Sciences Lab	Blood Sciences Lab, Room temp	Werfen	1dsa1585
NOVEL CORONAVIRUS COVID-19 IgG ELISA	Cold Room 1	Serology Fridge 3	Pathway Diagnostics	KT1032
DS2 reagent tips	Microbiology store room	Blood Sciences Lab, Room temp	Werfen	65920
DS2 sample tips	Microbiology store room	Blood Sciences Lab, Room temp	Werfen	65910
Sarstedt Universal containers	Microbiology store room	Blood Sciences Lab, Room temp	Werfen	63.9922.252

The Kit and reagents are handled per manufacturers recommendations. The test kit is stored at 2 – 8oC upon receipt. Prior to use, all reagents are allowed to come to room temperature. Reagents from different kit lot numbers are not combined or interchanged. ELISA WASH CONCENTRATE is diluted as instructed prior to use. All other reagents are ready for use. Below lists the steps involved if the assay were to be performed manually:

Sample Preparation

1. Dilute sample by a 1:100 dilution ratio with the COVID-19 IgG Sample Diluent (31218). For each 10 µL of sample, 1000 µL of COVID-19 IgG Sample Diluent (31218) is needed.
2. Mix well prior to performing the assay

Assay Procedure

1. Place a sufficient number of microwell strips (31217) in a holder to run controls (31221, 31222) and samples in duplicate.
2. Add 100 µL of controls (31221, 31222) and 1:100 diluted samples into the designated microwells.
3. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 30 minutes.
4. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
5. Add 100 µL of the HRP labeled Anti-hIgG Tracer Antibody (31220) into the microwells.
6. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 30 minutes.
7. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add 100 µL of the substrate (10020) into the microwells.
9. Mix gently and cover the plate with aluminum foil. Incubate at room temperature (20-25 ºC) for 20 minutes.
10. Remove the aluminum foil and add 100 µL of stop solution (10030) into each of the microwells. Mix by gently by tapping the plate.
11. Read the absorbance at 450 nm within 10 minutes with a microplate reader

INTERPRETION OF RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).

2. Calculate the cuttoffs using the following formulas:

Positive cutoff = 1.1 X (xNC + 0.18)

Negative cutoff = 0.9 (xNC + 0.18)

Calculate the Background Adjustment Factor (BAF) using the following formulas: • BAF = xNC - 0.10 3.

Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value ≤ Negative cuttoff	The sample does not contain the new coronavirus (COVID-19) IgG related antibody
Positive	Measured value ≥ Positive cuttoff	The sample contains novel coronavirus (COVID-19) IgG associated antibodies.
Borderline	Negative cuttoff < Measured value > Positive cuttoff	Retest the sample in conjunction with other clinical tests.

Epitope Diagnostics claims the diagnostic sensitivity is 100% and the diagnostic specificity is 100%.

CLINICAL TESTING

Serum samples from two cohorts of patients were tested using the IgG ELISA kit at the Jiaxing City Center for Disease Control and Prevention and Zhejiang University Hospital. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak [December 3, 2019] (n = 54) and RT-PCR confirmed positive patients in after the second week of the onset of the disease (n = 30). The results are as follows:

	Test Positive	Test Negative
Confirmed Positive	30	0
Confirmed Negative	0	54

The diagnostic sensitivity is 100%.
The diagnostic specificity is 100%.

Cross reactivity

Epitope Diagnostics claims there were no Cross-reactivity from the following diseases or infectious agents:

Anti-infulenza A
Anti-Influenza B
Hepatitis C
Anti-nuclear antibodies
Respiratory syncytial

Evaluation of potentially interfering drugs and interfering substances:

LIMITATIONS OF THE PROCEDURE

1. This test is only for qualitative detection. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction to other tests.
2. In the first week of the onset of the infection with the novel coronavirus (COVID-19) patients results may be negative for IgG. In addition, patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgG. Previous infection of SARS or other coronavirus strain may cause a light IgG positive in view of similarity of different strains.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

Training requirements (could be manufacturer training or in-house training):

Training was not offered by the manufacturer. In-house training and competency is sufficient. Only competency tested individuals should be attempting this test unsupervised. Once a member of staff is fully trained by a competent assessor and the relevant paperwork is complete, they will be able to run this test and report patient results.

2. Scope of validation/verification:

Reason for validation/verification:

Validation and verification of new assay kit for Measuring serum antibodies to the SARS-CoV-2 (COVID-19).

Performance qualification (PQ)
Plan:

To test the manufacturers claims of 100% sensitivity and 100% specificity

To assess the assays parameters: Linearity, intra-assay variation, inter-assay variation and assay carryover**.**

3. Validation/verification plan:

Timescale for validation/verification study to be complete: 01/06/2020

1) **Validation process**

- To test the manufacturers claims of 100% sensitivity and 100% specificity
- To assess the assays parameters: Linearity, intra-assay variation, inter-assay variation and assay carryover.

2) **Verification process**

Results:

Sensitivity –

	A	B	C	D	E	F	G	H	I	J
76	20V027101	05/05/2020	20249833	20M036149	POS	21/04/2020	Serum	POS		
77	20W042434	05/05/2020	31174516	20M036396	POS	24/04/2020	Serum	POS		
78	20W042435	05/05/2020	2021 2093	20M032294	POS	05/04/2020	Serum	POS		
79	20S167270S				POS		Serum	POS		
80	20S169372S				POS		Serum	POS		
81	20S165402				POS		Serum	POS		
82	20W042436				POS		Serum	POS		
83	20S170093S				POS		Serum	POS		
84	20S014672S				POS		Serum	POS		
85	20S170329S				POS		Serum	POS		
86	20S169136S				POS		Serum	POS		
87	20W42438S				POS		Serum	POS		
88	20S170351S				POS		Serum	POS		
89	20S170364S				POS		Serum	POS		
90	20S169509S				POS		Serum	POS		
91	20S013994				POS		Serum	POS		
92	20S163846				POS			POS		
93	20W042149S				POS			POS		
94	20W043466S				POS			POS		
95	20S010124	24-Mar	20269599	20M029179	POS	20-Mar	Serum	Negative	POS	Fresh sample for re-test
96	20V024975	25-Mar	10802849	20M029224	POS	21-Mar	Serum	Negative	POS	Fresh sample for re-test
97	20S146460	24-Mar	10966804	20M029278	POS	22-Mar	Serum	BORDERLINE	POS	Fresh sample for re-test
98	20V024888	24-Mar	31060048	20M029332	POS	23-Mar	Serum	Negative	POS	Fresh sample for re-test
99	20S163189				POS		Serum	Negative	POS	Fresh sample for re-test
100	20S167066	05/05/2020	3107 5064	20m032795	POS	15/04/2020	Serum	Negative	T 9 PARAPLEGIC ON VANCOMYCIN FOR OSTEOMYELITI	NO FRESH SAMPLES
101	20S014029	04/05/2020	2018 2869	20M036017	POS	19/04/2020	Serum	Negative	25% FLAME BURNS - ON ANTIBIOTIC	NO FRESH SAMPLES
102										
103	**N**	100								
104	**Positive**	98								
105	**Negative**	2								
106	**Sensitivity %**	**98%**								

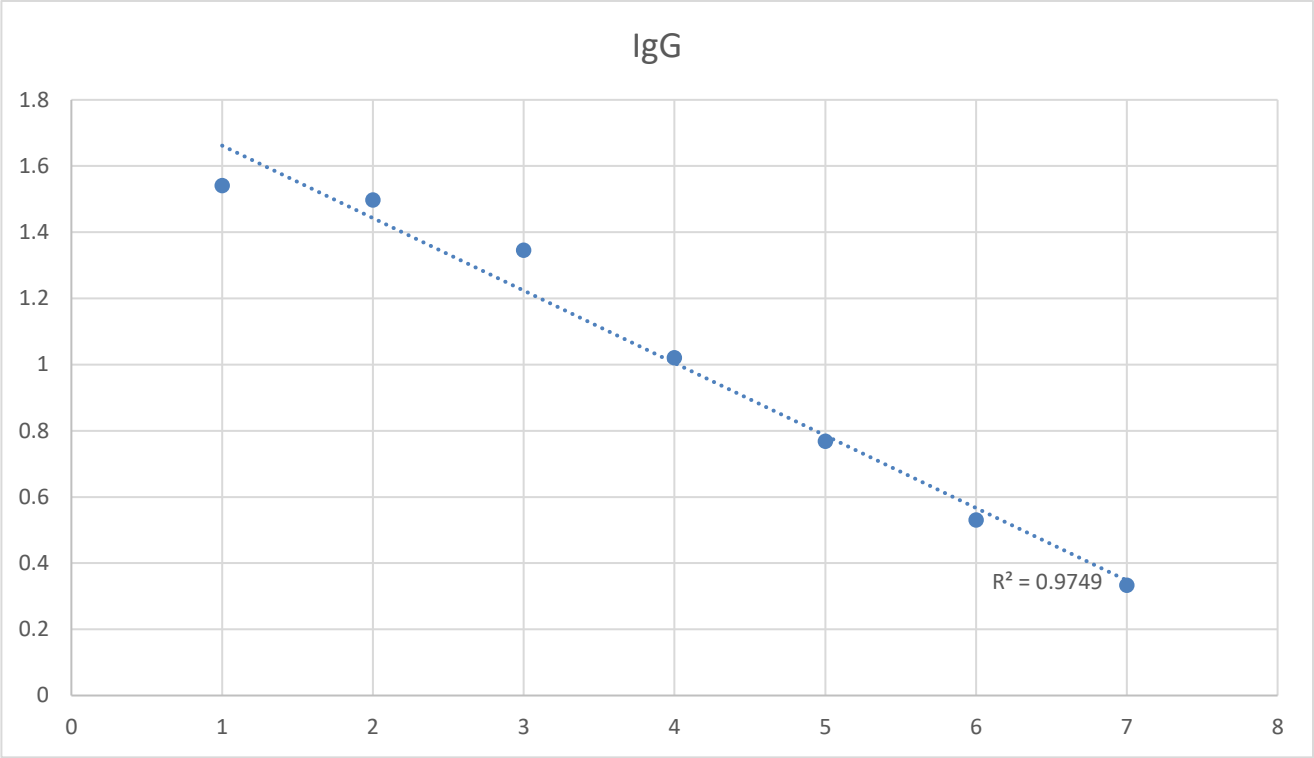
To assess the sensitivity of the assay 100 PCR positive samples were processed. Out of 100 PCR positive samples, 7 samples were classified as negative. Further investigation revealed the serum samples were taken just 2 – 4 days post PCR positive. Rerun of these patients' samples post >10 PCR positive cleared the discrepancy for 5 samples. We were unable to obtain fresh samples for 2 of the negative classified patients. Sensitivity of 98% was achieved. **ACCEPTED**

Specificity –

	A	B	C	D	E	F	G	H	I
75	17V058156	04/10/2017	10768643	N/A	N/A	N/A	N/A	Serum	Negative
76	17V058155	03/01/2017	4.262E+09	N/A	N/A	N/A	N/A	Serum	Negative
77	17V058146	04/10/2017	20221719	N/A	N/A	N/A	N/A	Serum	Negative
78	17V058142	04/10/2017	20153209	N/A	N/A	N/A	N/A	Serum	Negative
79	18V036318	29/05/2018	31071164	N/A	N/A	N/A	N/A	Serum	Negative
80	18V036307	29/05/2018	20317335	N/A	N/A	N/A	N/A	Serum	Negative
81	18V036317	29/05/2018	20343877	N/A	N/A	N/A	N/A	Serum	Negative
82	18V036358	29/05/2018	31106195	N/A	N/A	N/A	N/A	Serum	Negative
83	18V036110	24/05/2018	7.108E+09	N/A	N/A	N/A	N/A	Serum	Negative
84	18V036037	24/05/2018	20272307	N/A	N/A	N/A	N/A	Serum	Negative
85	18V036121	25/05/2018	11369245	N/A	N/A	N/A	N/A	Serum	Negative
86	18V036156	24/05/2018	31104427	N/A	N/A	N/A	N/A	Serum	Negative
87	18V036201	24/05/2018	20184572	N/A	N/A	N/A	N/A	Serum	Negative
88	18V036331	29/05/2018	31065561	N/A	N/A	N/A	N/A	Serum	Negative
89	18V036083	24/05/2018	31054800	N/A	N/A	N/A	N/A	Serum	Negative
90	18V033723	12/02/2018	10631291	N/A	N/A	N/A	N/A	Serum	Negative
91	18V036285	10/05/2018	N/A	N/A	N/A	N/A	N/A	Serum	Negative
92	18V036023	24/05/2018	11305478	N/A	N/A	N/A	N/A	Serum	Negative
93	18V036208	24/05/2018	4.323E+09	N/A	N/A	N/A	N/A	Serum	Negative
94	18V036012	25/05/2018	4.744E+09	N/A	N/A	N/A	N/A	Serum	Negative
95	18V036118	25/05/2018	31066696	N/A	N/A	N/A	N/A	Serum	Negative
96	18V036178	25/05/2018	20385876	N/A	N/A	N/A	N/A	Serum	Negative
97	18V036177	25/05/2018	31104169	N/A	N/A	N/A	N/A	Serum	Negative
98	18V035967	22/05/2018	20373249	N/A	N/A	N/A	N/A	Serum	Negative
99	18V036119	25/05/2018	10616505	N/A	N/A	N/A	N/A	Serum	Negative
100	18V036198	25/05/2018	20192437	N/A	N/A	N/A	N/A	Serum	Negative
101	18V035979	24/05/2018	20194445	N/A	N/A	N/A	N/A	Serum	Negative
102									
103	**N**	100							
104	**Negative**	100							
105	**Positive**	0							
106	**Specificity**	**100%**							

To assess the specificity of the assay 100 frozen sample from 2017/2018 pre COVID-19 infection. All of the 100 samples were classified as negative. Specificity of 100% was achieved. **ACCEPTED**

Linearity –



For linearity assessment a Positive serum was serially diluted at fixed ratios (i.e., 1:2; 1:4; 1:8; 1:16; 1:32 and 1:64) to cover the most clinically significant range of concentrations for this parameter. Serial dilutions were analysed and Linearity was assessed with calculation of linear regression analysis with R^2 value of = 0.9749. **ACCEPTED**

Intra-Assay Precision – (Acceptable < 10% CV)

For intra-assay evaluation 2 samples (Positive and negative) were repeated 10 times in a single batch in different location of the ELISA plate. CV of 8.8% was achieved for positive sample and 3.4% for negative sample. **ACCEPTED**

Inter-Assay Precision – (Acceptable < 15% CV)

For intra-assay evaluation a positive sample was analysed on separate batch 8 times. CV of 3.66% was achieved. **ACCEPTED**

Assay carryover –

To assess possible assay carryover one strong positive (ID-20S165992) and one negative (ID-218V036023) were run in pattern shown below:

	A	B	C	D	E	F	G	H	I
1	**Pos Sample ID**	20S165992							
2	**Neg Sample ID**	18V036023							
3	LAB NUMBER (ORIGINAL)	SAMPLE DATE	MRN/NHS	PCR LAB NUMBER	CT VALUE	PCR TEST	PCR RESULT DATE	SAMPLE TYPE	IgG Results
4	20S165992					POS		Serum	0.632
5	20S165992					POS		Serum	0.622
6	20S165992					POS		Serum	0.572
7	20S165992					POS		Serum	0.522
8	20S165992					POS		Serum	0.636
9	18V036023					N/A		Serum	0.117
10	18V036023					N/A		Serum	0.129
11	18V036023					N/A		Serum	0.117
12	18V036023					N/A		Serum	0.122
13	18V036023					N/A		Serum	0.123
14	20S165992					POS		Serum	0.546
15	20S165992					POS		Serum	0.53
16	18V036023					N/A		Serum	0.129
17	18V036023					N/A		Serum	0.125
18	20S165992					POS		Serum	0.528
19	20S165992					POS		Serum	0.509
20	18V036023					N/A		Serum	0.122
21	18V036023					N/A		Serum	0.121
22	20S165992					POS		Serum	0.501
23	18V036023					N/A		Serum	0.118
24									
25	**Pos Sample ID**	20S165992							
26	Mean	0.559							
27	SD	0.049							
28	CV %	8.858							
29									
30	**Neg Sample ID**	18V036023							
31	Mean	0.122							
32	SD	0.004							
33	CV %	3.411							

No carryover was observed - **ACCEPTED**

Clinical Cut-Offs and Comments:

Positive cutoff = 1.1 X (xNC + 0.18)

Negative cutoff = 0.9 (xNC + 0.18)

Acceptance criteria (analytical):

4. Validation/verification report:

Have the performance criteria been met?

YES

If performance criteria have not been met, what are the actions and associated timeframes?

(further paperwork as required, eg risk assessments)

Prepared by (name / signature / date):	**Subash Gurung** **12/05/2020/** 
Authorised by (name / signature / date):	

5. Summary and Comments

Additional Documentation:

COVID-19 masterData.xlsx

KT1032 EDI Novel Coronavirus COVID-1

Coronavirus IgG testing on the Dynex

KT1032+33 Change Notification CN 2020-