Performance Evaluation	thermoscientific
CC-0530-7020	Performance Evaluation Report for 384 Max SARS-CoV-2 N-Protein IgG ELISA
	Project Prince

Title	Performance Evaluation Report for 384 Max SARS-CoV-2 N-Protein IgG ELISA
Project Number	CC-0530-7020
Document Number	CC-0530-7020_PER
Document Version	1
Authors	Matt Donaldson, Jeffrey McBride

1 Change history

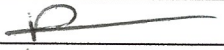
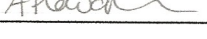
Issue	Changes	Changed by	Date
A	Original version	N/A	Mar 2021
1	Reviewed Version	Jeffrey McBride	19th March 2021

2 Document Approval

Author:

Name	Position	Sign	Date
Matt Donaldson	Manufacturing Sciences Manager	Approved by Email	24th Mar 2021
Jeffrey McBride	Biomolecular Lead	Approved by Email	22nd Mar 2021

Approved By

Name	Position	Sign	Date
Phil Brame	Manager, Regulatory Affairs	Approved by Email	23rd Mar 2021
Fay Curd	Manager, Quality Assurance	Approved by Email	23rd Mar 2021
Diane Ruscoe	Senior R&D Project Manager		24th Mar 2021
Alison Howarth	Project Manager Oxford University		22/03/2021
James Stringer	R&D Manager	Approved by Email	22nd Mar 2021

3 Table of Contents

5 Background and Scope

Remel Europe Limited (part of Thermo Fisher Scientific) have been asked to develop a family of IVD medical device kits in the serology field to assist with the national effort to create reliable tools to deal with the current SARS CoVid Pandemic.

The project will cover the development of two CE marked products for the qualitative detection of the SARS-CoV-2 Nucleocapsid (N) protein. The kits will be developed in the 96 and 384 well format and are intended to work in conjunction with the OmniPATH Combi Max SARS-CoV-2 IgG ELISA. Where possible the kits will be designed to work with the reagents developed as part of the S-trimer ELISA program (CC-0511-7019).

There is a requirement in the UK from the Department of Health and Social Care (DHSC) to rapidly expand serology surveillance testing using an N protein assay. To make use of the existing testing platform at the university of Oxford we have been asked to develop and release an RUO product to be used alongside the existing S trimer product. Based on this request the project will include the interim release of an RUO kit to be used exclusively by Oxford university in the UK.

The N-protein assay will use the SARS-CoV-2 ELISA Nucleocapsid protein as the target antigen. When used in combination with the OmniPATH Combi SARS-CoV-2 IgG Elisa it will be possible for researchers to identify whether a sample has been taken from a vaccinated individual or someone who has been previously infected with the virus. The N-protein assay will be developed as a single sample qualitative assay for both RUO use and CE certification.

The development will include close collaboration with Oxford University, UK and other Thermo Fisher entities globally and will transfer technology developed at Freiburg on the ELIA platform to an ELISA platform manufactured at the Dartford site.

Although the primary intended market is the UK the UKCA / CE marked device will be developed to be suitable for global distribution.

The performance evaluation activities detailed in this report form the basis for release of the RUO product. They will also be used along with additional V&V testing to support the release of a CE marked product in due course.

6 Kit Description

- 384 Max SARS-CoV-2 N-Protein IgG ELISA RUO
  - A 10 plate 384-well microplate assay kit comprising of a 384 well microplate pre-coated with SARS-CoV-2 nucleocapsid protein. The kit will contain all associated reagents required to perform an enzyme-linked immunoassay (ELISA). Kit is to be initially sold as a Research Use Only (RUO) kit. The device will be developed to be suitable for ongoing development and testing required for CE registration. This kit is intended to be used as a high-throughput assay for large serological studies.

7 Intended Use (RUO)

The 384 Max SARS-CoV-2 N-Protein IgG ELISA is a solid phase immunoassay intended for the detection of IgG class antibodies against the nucleocapsid protein of SARS-CoV-2 virus in serum or plasma. Used as part of a high-throughput serological survey workflow to identify individuals with an immune response to SARS-CoV-2, indicating a recent or prior infection. This device is intended for Research Use Only (RUO) and is suitable for automated use.

The target population are members the UK population selected by the ONS to take part in serology screening for SARS-CoV-2 infection.

8 Document References

DOC Code	Name	Location
CC-0530-7020_UN	User Needs (Draft)	Design History File (CC-0530-7020_DHF)
CC-0530-7020_PRS	Product Requirement Specification (Draft)	Design History File (CC-0530-7020_DHF)
CC-0530-7020_PEP	Performance Evaluation Plan	Design History File (CC-0530-7020_DHF)

9 Test Protocol

This document outlines the performance evaluation assessment that has been performed for the OmniPATH 384 Max SARS-CoV-2 N-Protein IgG ELISA as per the performance evaluation plan to support the following requirements. These requirements have been derived from the Draft Design Input documents: User Needs; Product Requirements Specifications written for the CE marked product. This document is only intended to provide evidence for the use of this product for Research Use Only (RUO). A select number of the User Needs/Product Requirement Specifications (or parts of these requirements), listed below, have been identified to satisfy the requirements of a RUO product.

All technical requirements have been covered in the below tables apart for UN-3061 and PRS-3061. Testing of the RUO device for these PRSs is not required because any interference affecting the result on the assay will be identified through the combination of the S-trimer and N-protein result. It has been demonstrated that the disease states/substances listed in UN/PRS-3061 do not have an impact on the performance of the S-trimer assay. In the event of these substances impacting the performance of the assay and affording false positive results this would be identified by a negative S-trimer result and a positive N-protein assay result for a sample. This combination of results will be treated as an invalid result and the sample will either be re-run or reported as an invalid result. If a significant number of invalid results are seen during the testing this could be indicative of an issue with interference and further testing may be needed. In addition, the S-trimer and N-protein assays are based on the same core reagents (Conjugate, wash and sample diluents). It has been shown with the S-trimer testing that the performance of the reagents is not impacted by the disease state/substances listed in the UN/PRS. As such, the risk on the N-protein assay being affected by these agents is low. It is for these reasons that the testing will not be conducted for the RUO device.

ID	Requirement
RUO Intended use.	Intended Use: The 384 Max SARS-CoV-2 N-Protein IgG ELISA is a solid phase immunoassay intended for the detection of IgG class antibodies against the nucleocapsid protein of SARS-CoV-2 virus in serum or plasma. Used as part of a high-throughput serological survey workflow to identify individuals with an immune response to SARS-CoV-2, indicating a recent or prior infection. This device is intended for Research Use Only (RUO) and is suitable for automated use.
UN-0030	Assay Procedure: User requires a specimen volume that is high enough to ensure accuracy and low enough to allow all required reagents to fit within the wells.
UN-0040	Assay Procedure: User requires a reagent volume that is low enough to allow all required reagents to fit within the wells.
UN-3010	Sample Type: The user needs a test that works with sample types (plasma/serum) that contain antibodies to the SARS-CoV-2 virus.
PRS-3010	Sample Type: The kit will work with serum and plasma samples.
PRS-3011	Sample Type: The kit will work with venous and capillary blood.
UN-3020	Desired output: The user needs a kit that can provide a qualitative result.
PRS-3021	The kit will give a clinical sensitivity of greater than 95% Desired Output: The kit will give a clinical sensitivity of greater than 98% confidence interval of 96-100%.
PRS-3022	The kit will give a clinical sensitivity of greater than 95% Desired Output: The kit will give a clinical sensitivity of greater than 98% confidence interval of 96-100%.
PRS-3023	The kit will give repeatability with an OD CV of less than 15% when tested with normal, strong, medium and light positive serum and plasma as samples.
PRS-3024	The kit will give reproducibility with an OD CV of less than 15% when tested with normal, strong, medium and light positive serum and plasma as samples.
PRS-3025	The kit will give a test signal response that is directly proportional to the concentration of anti-SARS-CoV-2 IgG. The correlation coefficient of anti-SARS-CoV-2 IgG concentration versus mean OD will be ≥ 0.95.
PRS-3026	The kit will return the correct result (positive or negative) during robustness testing.
PRS-3027	The kit will be provided with instructions on how to create a standard Cut-off control and use this to interpret the test result.

ID	Requirement
UN-3060	Analytical Specificity: User requires minimal cross-reactivity with other known coronavirus, or common respiratory pathogens. If such activity does exist user needs to be informed.
PRS-3060	Analytical Specificity: The kit should be tested for analytical specificity with the list of infective agents detailed in annex 3 of the MHRA standard: 1. Pre-pandemic samples (Desired: specimens collected at least 66 months before the known appearance of the SARSCoV-2 virus, minimum of 3 months is required) 2. Other coronavirus, MERS 3. hCOV 229E, OC43, HKU1, NL63 epitopes 4. Adenovirus (e.g. C1 Ad. 71) 5. Human metapneumovirus (hMPV) 6. Parainfluenza virus 1 – 4 7. Influenza A & B 8. Enterovirus (e.g. EV68) 9. Respiratory syncytial virus 10. Rhinovirus 11. Chlamydia pneumoniae 12. Haemophilus infuenzae 13. Legionella pneumophila 14. Mycobacterium tuberculosis 15. Streptococcus pneumoniae 16. Streptococcus pyogenes 17. Bordetella pertussis 18. Mycoplasma pneumoniae Pneumocystis jirovecii (PJP)
UN-3070	Results: User requires a simple way to identify the result of the test.
PRS-3070	Results: The kit will be supplied with an easy manual calculation to determine the outcome of the test.
UN-3090	Hook effect: It will be confirmed that high dose hook effect will not change the positive/negative result of the assay.
PRS-3090	Hook effect: It will be confirmed that high dose hook effect will not change the positive/negative result of the assay.

ID	Validation Approach
RUO Intended Use	Successful completion of the performance evaluation plan will demonstrate that the assay works in accordance with the Intended Use.
UN-0030	Successful completion of the performance evaluation plan will demonstrate that the devices work in accordance with claims made for sample volume, type and make-up.
UN-0040	
UN-3010	
PRS-3010	
PRS-3011	
UN-3020	The qualitative aspect of this device will be assessed in all stages of the performance evaluation
PRS-3021	Clinical Sensitivity will be derived from the clinical performance studies.
PRS-3022	Clinical Specificity will be derived from the clinical performance studies.
PRS-3023	Repeatability will be assessed as part of study 1.
PRS-3024	Reproducibility will be assessed as part of study 1.
PRS-3025	The signal response will be tested as part of study 1
PRS-3026	Robustness will be assessed as part of study 1.
PRS-3027	The use of the assay methodology will be tested in all stages of the performance evaluation.
UN-3060	A small number of common coronaviruses in current circulation (as identified by the MHRA) will be assessed for interference with the N-protein assay as part of study 2. The remaining disease states/interfering substances will be assessed as part of the wider verification and validation assessment. Successful completion of the performance evaluation plan will demonstrate that the device is not impacted by the identified interfering substances/disease states. The successful running and interpretation of these samples by the assay will provide evidence that there is no interference with substances commonly found in plasma/serum samples.
PRS-3060	
UN-3070	The completion of the performance validation by the team at Oxford University will confirm the usability and interpretation of the assay. The assay format and workflow are sufficiently similar to that of the OmniPATH™ Max Combi SARS-CoV-2 IgG ELISA that the usability testing completed for that product can be used to prove usability for this family of devices (See report CC-0511-7019_UVR).
PRS-3070	
UN-3090	The hook effect will be assessed as part of study 1.
PRS-3090	

All experimental assays have followed the assay protocol as described for the assay in the performance evaluation plan and the appendices of this document.

9.1 Study Design

The performance evaluation of the 384 Max SARS-CoV-2 N-Protein Elisa includes studies completed by the team at the University of Oxford, product support at the Thermo Fisher Dartford site and the IDD team based in Freiburg in Germany. The studies are designed to demonstrate the performance of the assay as a Research Use Only kit that will be used to support the UK governments expansion of population serology testing. Additional studies will be required prior to any CE marking submission. The study outlines are detailed in the table below. All testing has been carried out using the final presentation of the kits and following the assay protocols described in the performance evaluation plan and Appendices in this document.

Study Number	Description	Testing Outcomes
1	Analytical Performance of the Assay: **Linearity:** To demonstrate test signal response is directly proportional to the concentration of analyte (anti-SARS-CoV-2 N protein) in samples. **Trueness: Use of Anti-SARS-CoV-2 Verification Panel for Serology Assays** (**NIBSC 20/B770**): Verify positives and negatives are identified in accordance with a standard panel **Precision: Repeatability and Reproducibility:** • Assess repeatability (intra-assay) - the precision estimate obtained when measurement tests and results are produced in one facility with identical samples according to standard protocol/conditions during a short interval of time by one operator. • Assess reproducibility (inter-assay) - the precision estimate when a series of measurements are made under more variable conditions, i.e. standard test method, same samples but different operators, different equipment, different times **Robustness:** The capacity to remain unaffected by small, but deliberate variations in the method parameters. Herein: • End point stability – consistency of results when the microplate is read > 30 minutes later • Temperature **- consistency of results when the assay is performed at 15 – 25ºC** **Hook Effect:** Demonstration of the impact of the high-dose hook effect on the assay	• Repeatability (PRS 3023) • Reproducibility (PRS 3024) • Linearity (PRS-3025) • Robustness (PRS-3026) • Controls (PRS-3027) • Hook Effect (UN-3090; PRS-3090)
2	Analytical Specificity: Assessment for interference of the assay with other common Coronaviruses: hCOV 229E; OC43; HKU1 and NL63.	• Analytical Specificity (UN-3060; PRS-3060)
3	Assessment of 200 true positive samples (Venous and Capillary draw) and 200 pre-pandemic negatives.	• Sample type & Volume: Venous Serum and Capillary Plasma (UN-0030; UN-0040; UN-3010; PRS-3010; PRS-3011) • Qualitative Output (UN-3100) • Determination of the Cut-off control (PRS-3027) • Qualitative Result (UN-3020); Qualitative Sensitivity (PRS-3021) and Qualitative Specificity (PRS-3022) • Results of test (UN-3070; PRS-3070) • Assay Procedure (UN-0030) • Analytical Specificity and Interferants cross-reactivity (UN-3060; PRS-3060 and UN-3061; PRS-3061)

9.1.1 Study 1

Objective:	To demonstrate the analytical performance of the assay, the following parameters will be studied: 1. **Linearity**: To demonstrate that the test signal response is directly proportional to the concentration (amount) of anti-SARS-CoV-2 N Protein IgG as analyte. 2. **Trueness**: Use of Anti-SARS-CoV-2 Verification Panel for Serology Assays (NIBSC 20/B770): Verify that positive and negative samples from individuals with infection are identified in accordance with the reference panel (NIBSC 20/B770). Demonstrate that a preliminary cut-off anti-SARS-CoV-2 N Protein IgG concentration is able to distinguish individuals as positive or negative using the OmniPATH ELISA method. 3. **Precision: Repeatability and Reproducibility:** To demonstrate the closeness of agreement between a series of measurements obtained from multiple sampling of the same defined anti-SARS-CoV-2 N protein IgG containing serum and plasma samples using the OmniPATH ELISA method and will be assessed as follows: **Intra-assay precision (Repeatability):** Intra-assay validation shows the reproducibility between wells within the assay plate under the same ELISA operating conditions over a short interval of time. **Inter-assay precision (Reproducibility):** shows the reproducibility between assays done on different days within the laboratory, to ensure that the ELISA method will provide the same results when the same human-anti-SARS-CoV-2 IgG sample is analysed by the ELISA method with a different user and on different days. 4. **Robustness:** Demonstrate the capacity to remain unaffected by small, but deliberate variations in the method parameters. Herein: • End point stability – consistency of results when the microplate is read > 30 minutes later • Temperature – Consistency of results when the assay is performed at 15-25^{o}C. 5. **Hook Effect:** Demonstration of the impact of the high-does hook effect on the assay
Top-Level Procedure	1. **Linearity** Prepare Standard solutions of Human monoclonal anti-SARS-CoV-2 N Protein IgG of at least five concentrations and to cover the range to 200ng mL in sample diluent (see 10.1.2). Perform ELISA method as per section 10.1.3. Prepare a standard curve by plotting the mean OD for each standard concentration (y-axis) against anti-spike mAb (x-axis). The best fit line can be generated using any curve-fitting capable software by regression analysis using a 4-parameter curve fit:

$Signal = \mathrm{D} + \frac{\mathrm{A-D}}{(1+(\frac{\mathrm{concentration}}{\mathrm{C}})^{\mathrm{B}})}$ ↔ Concentration = $C\,(\frac{A-D}{Signal-D} - 1)^{1/B}$

A = theoretical response at zero concentration; B = Slope factor; C = mid-range concentration (inflection point); D = theoretical response at infinite concentration

- Obtain the *r*2 value from the calibration curve(s).
- Record and attach results to the Appendix.

2. **Trueness: Anti-SARS-CoV-2 Verification Panel for Serology Assays** (**NIBSC 20/B770**)

Perform Assay as per section 10.1.3 using the NIBSC 20/B770 panel (23 positive, 14 negative plasma samples). Perform assay with preliminary Human monoclonal anti-SARS-CoV-2 N Protein IgG cut-off control of appropriate concentration (10 – 30ng mL).

- Record the OD of samples and cut-off control's (minus sample diluent as blank).
- Determine the sample index ratio as *Sample Index Ratio = (Blanked Test Sample OD) / (Blanked Cut-off OD)*. Record results as Negative (≤ cut-off) or positive (>cut-off) with each cut-off control
- Record and attach results to the Appendix.

3. **Precision: Repeatability and Reproducibility:**

Repeatability (Intra-assay Precision)

Perform Assay as per section 10.1.3 with normal, strong, medium and light positive serum and plasma as sample (or normal human serum and plasma as sample matrix spiked with monoclonal human anti-SARS-CoV-2 N Protein IgG representative of high, medium, and low levels.).

- Use a preliminary cut-off control using monoclonal human anti-SARS-2 N Protein IgG at appropriate concentration.
- Record the OD of samples (minus sample diluent as blank).
- Determine the sample index ratio as *Sample Index Ratio = (Blanked Test Sample OD) / (Blanked Cut-off control OD)*. Record results as Negative (≤ cut-off) or positive (>cut-off)
- Calculate the mean, standard deviation, and CV of the OD signal of samples.

Reproducibility (Inter assay precision)

Inter-assay precision (within-laboratory variation) will be demonstrated by two analysts using the ELISA procedure on 3 separate days as per Repeatability (intra-assay precision).

Operator 1 (Day 1 and/or Day 3): Using the ELISA method (section 10.1.3) perform assay of spiked samples of at least 16 replicates .

Operator 2 (Day 2 and/or day 3): Using the ELISA method (section 10.1.3) repeat the assay with the spiked samples, at least 16 replicates.

- Use a cut-off control Hu mAb IgG to SARS-CoV-2 N protein at appropriate concentration.
- Record the OD of samples (minus sample diluent as blank).

	• Determine the sample index ratio as *Sample Index Ratio = (Blanked Test Sample OD) / (Blanked Cut-off control OD)* Record results as Negative (≤ cut-off) or positive (>cut-off) • Calculate the mean, standard deviation, and % CV of the OD signal of samples. 4. **Robustness** **End point stability** Perform Assay as per section 10.1.3 with normal, strong, medium and light positive serum and plasma samples (or normal human serum and plasma as sample matrix spiked with monoclonal human anti-SARS-CoV-2 N Protein IgG representative of high, medium, and low levels). Read microplate after colour development stopped. Re-read the microplate >30 minutes later. • Use a preliminary cut-off control Hu mAb IgG to SARS-CoV-2 N protein at appropriate concentration. • Record the OD of samples (minus sample diluent as blank). • Determine the sample index ratio as *Sample Index Ratio = (Blanked Test Sample OD) / (Blanked Cutoff OD)* Record results as Negative (≤ cut-off) or positive (>cut-off). **Temperature** Perform Assay as per section 10.1.3 at 15°C and 25°C with normal, a strong medium and a light positive serum and plasma samples (or normal human serum and plasma as sample matrix spiked with monoclonal human anti-SARS-CoV-2 N Protein IgG representative of high, medium, and low levels). • Use a preliminary cut-off control monoclonal human anti-SARS-CoV-2 N Protein IgG at appropriate concentration. • Record the OD of samples (minus sample diluent as blank). • Determine the sample index ratio as *Sample Index Ratio = (Blanked Test Sample OD) / (Blanked Cutoff OD)* Record results as Negative (≤ cut-off) or positive (>cut-off). 5. **Hook Effect** Perform ELISA as per section 10.1.3. Double dilute a high-level positive sample or normal human serum and plasma as sample matrix spiked with monoclonal human anti-SARS-CoV-2 N Protein IgG until at least 3 doubling dilutions to fall into the signal range of the assay. • Use a preliminary cut-off control monoclonal human anti-SARS-CoV-2 N Protein IgG ~~at~~ appropriate concentration. • Record the OD of samples (minus sample diluent as blank). • Determine the sample index ratio as *Sample Index Ratio = (Blanked Test Sample OD) / (Blanked Cutoff OD)* Record results as Negative (≤ cut-off) or positive (>cut-off) • Record if hook effect is observed
Acceptance Criteria	**1. Linearity:** Signal (OD) shall be proportional to monoclonal human anti-SARS-CoV-2 N Protein IgG concentration. The correlation coefficient (*r*2) for Hu mAb IgG concentration to mean OD will be ≥ 0.95. **2. Trueness: Anti-SARS-CoV-2 Verification Panel for Serology Assays (NIBSC 20/B770)**

	Verify at a given preliminary cut-off monoclonal human anti-SARS-CoV-2 N Protein IgG concentration positive (sample index ratio > 1) and negative samples (sample index ratio ≤ 1) are identified in accord with the NIBSC 20/B770 sample panel. **3. Precision** • Strong, medium and light positive samples should give a positive result (sample index ratio > 1). Normal samples should give a negative result (sample index ratio ≤ 1). • For intra-assay, CV of the OD signal for positive samples should be ≤ 15%. • For inter assay, the OD signal for positive samples obtained by two operators over three days should have a statistical CV ≤ 15%. **4. Robustness** • Positive samples should remain positive (sample index ratio > 1), negative sample remain negative (sample index ratio ≤ 1). **5. Hook Effect** • Observe for hook effect and review impact on assay working parameters.

9.1.2 Study 2

Objective:	To demonstrate that there is no false positive/interference associated with the presence of antibodies to other common coronaviruses in circulation. This assessment will focus on the 4 specific coronavirus strains which have been identified within the Target Product Profile document from the MHRA (229E; OC43; HKU1 and NL63).
Top-Level Procedure	Serum samples from individuals with known presence of the identified coronaviruses will be run on the assay by the team at the University of Oxford.
Acceptance Criteria	All samples for the similar coronaviruses must give a negative result on the assay, when compared to negative serum samples. A negative result for the similar coronavirus will indicate that there is no demonstrable interference associated with the presence of antibodies to that disease in this assay. Any positive interference will be documented within the limitations section of the IFU.

9.1.3 Study 3

Objective:	To demonstrate the clinical performance of 384 Max SARS-CoV-2 N-Protein IgG ELISA assay. This study has been designed to assess the following sections of the User Needs (UN) and Product Requirement Specifications (PRS).
Top-Level Procedure	Assessment of 200 true positive serum/plasma samples (venous/capillary draw) and 200 pre-pandemic negative serum/plasma samples (venous/capillary draw) using the qualitative protocol. Each sample will be run in triplicate, with two runs being conducted on the 384 well format and one run being conducted on the 96 well format. The samples will be pre-diluted at 1/50 using at least 5 µl of sample. A qualitative result for each sample will be obtained and compared to the known result of the sample. Sensitivity and Specificity for the qualitative assay will be derived and reported. This study will be run on samples collected through the University of Oxford and all processing shall be conducted by members of the university faculty and on university premises.
Acceptance Criteria	**Minimum:** Sensitivity > 95% Specificity > 95% **Desirable:** Sensitivity - ≥98% with a 95% confidence interval of 96-100% Specificity - ≥98% with a 95% confidence interval of 96-100% The qualitative results for each sample run on both assay formats (96 Well and 384 Well) must match. Successful reporting of results for each sample run, with good correlation to the known results.

10 Results

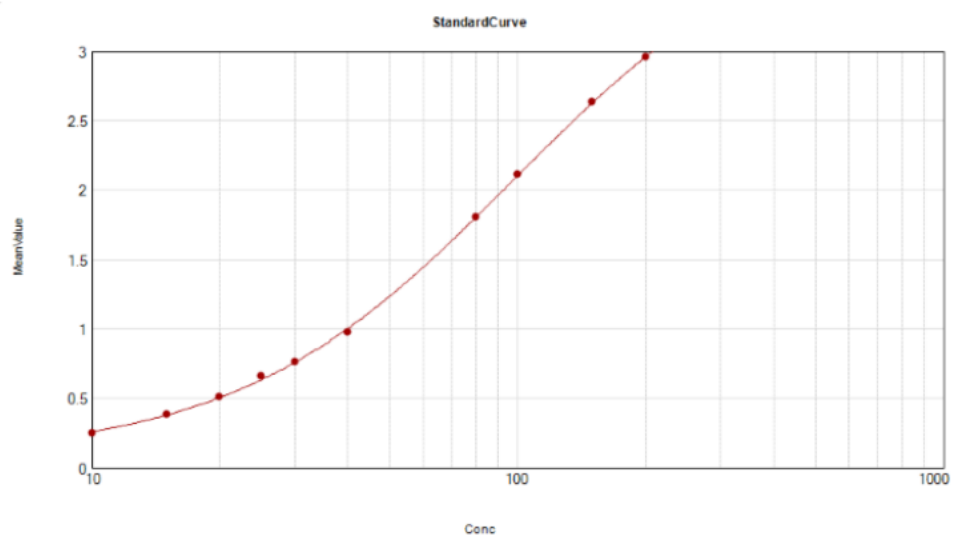
The performance evaluation testing has been completed according to the performance evaluation plan and the results documented. The comments in the table below result from a review of the findings of CC-0530-7020 Performance Evaluation Plan for 384 Max SARS-CoV-2 N-Protein IgG ELISA.

Table: Summary of the Test results

Study Plan	Test Description	Section(s)	Results (Pass / Fail)	Deviation Raised (Yes / No)
1	Linearity	9.1.1, 1 10.1.1 11.2.1.1	Pass	No
	Trueness	9.1.1, 2 10.1.2 11.2.1.2	Pass	No
	Precision	9.1.1, 3 10.1.3 11.2.1.3	Pass	No
	Robustness	9.1.1, 4 10.1.4 11.2.1.4	Pass	No
	Hook Effect	9.1.1, 5 10.1.5 11.2.1.5	Pass	No
2	Seasonal coronavirus	9.1.2 10.2 11.2.2	Pass	No
3	Positive and Negative samples	9.1.3 10.3 11.2.3	Fail	Yes

10.1 Study 1 Results

10.1.1 Linearity

Objective:	To demonstrate linearity of the OmniPATH ELISA procedure. To show that the test signal response is directly proportional to the concentration (amount) of anti-SARS-CoV-2 N Protein IgG as analyte.
Required Materials	• As per section 11.1.2 • monoclonal human anti-SARS-CoV-2 N Protein IgG of varying concentrations.
Procedure	• As per section 9.1.1, 1. and 11.1.3
Results	 Curve Fit Results ▲ Curve Fit : 4-Parameter Logistic $y = D + \frac{A-D}{1+\left(\frac{x}{C}\right)^B}$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Std R^2 = 1.000 EC50 = 97.63	A	0.080	0.038	[-0.013, 0.173]
	B	1.340	0.070	[1.169, 1.511]
	C	97.63	5.535	[84.09, 111.2]
	D	4.063	0.159	[3.673, 4.452]

Results Summary			
Verification Step	**Expected Results**	**Actual**	**Meet Acceptance Criteria (Yes / No)**
Linearity (Calibration Model Validation)	Correlation coefficient $\geq$ 0.95	Correlation coefficient = 1	**Yes**

COMMENTS/REFERENCES/DEVIATIONS:

Linearity of calibration was demonstrated by preparing anti-SARS-CoV-2 N-Protein IgG at 10 different calibrant (section 9.1.1,1; 11.1.3) concentrations from 10 to 200ng mL in sample diluent. Each calibrant was assessed in duplicate by the OmniPATH SARS-CoV-2 N-Protein IgG ELISA procedure (section 11.1.3). The test signal response (OD) is directly proportional to the concentration (amount) of anti-SARS-CoV-2 N-Protein IgG as analyte.

All acceptance criteria were met.

Data:	Section 11.2.1.1
Testing met acceptance criteria (Pass/Fail)	**Pass**

10.1.2 Trueness

Objective:	Verify that positive and negative samples from individuals with infection are identified in accordance with the reference panel (NIBSC 20/B770). Demonstrate that a preliminary cut-off anti-SARS-CoV-2 N Protein IgG concentration is able to distinguish individuals as positive or negative using the OmniPATH ELISA method.
Required materials	• As per section 11.1.2 • Anti-SARS-CoV-2 Verification Panel for Serology Assays (NIBSC 20/B770)
Procedure	As per section 9.1.1, 2. and 11.1.3
Results	

NIBSC Anti-SARS-CoV-2 Verification Panel for Serology NIBSC 20/B770 (n = 37)						
Preliminary Cut-off level (ng mL Anti-SARS-CoV-2 N Protein IgG)	Positive samples (n = 23)		Negative Samples (n = 14)		Sensitivity %	Specificity %
	Sample index ratio > 1 (True Positive)	Sample index ratio ≤1 (False Negative)	Sample index ratio ≤1 (True Negative)	Sample index ratio > 1 (False Positive)		
10	23	0	14	0	100	100
15	23	0	14	0	100	100
20	23	0	14	0	100	100
25	23	0	14	0	100	100
30	22	1	14	0	95.65	100

Table: Summary of results

Results Summary			
Verification Step	**Expected Results**	**Actual**	**Meet Acceptance Criteria**
Trueness	Positive and negative samples distinguished with a cut-off control at an appropriate anti-SARS-CoV-2 IgG concentration	All positive and negative samples are identified and distinguished at cut-off control levels from 10 to 25ng mL giving equivalent sensitivity and specificity to 100%.	**Yes**

COMMENTS/REFERENCES/DEVIATIONS:

Correction: The objective in this section in the Performance evaluation plan document was stated incorrectly due to a copy and paste error. The objective has been corrected in this report and is as per section 9.1.1, 2: Verify that positive and negative samples from individuals with infection are identified in accordance with the reference panel (NIBSC 20/B770). Demonstrate that a preliminary cut-off anti-SARS-CoV-2 N Protein IgG concentration is able to distinguish individuals as positive or negative using the OmniPATH ELISA method.

The NIBSC Anti-SARS-CoV-2 Verification Panel for Serology Assays comprises 37 samples; 23 positive and 14 negative samples. All samples were assessed in duplicate with preliminary cut-off control levels equivalent to 10, 15, 20, 25, 30 ng mL anti-SARS-CoV-2 N Protein IgG evaluated.

At levels of 10, 15, 20, 25 ng mL anti-SARS-CoV-2 N Protein all samples are distinguished by their signal index ratio (> or ≤ 1.0 respectively) i.e. 23/23 positives correctly identified, and 14/14 negatives correctly identified. This gives a sensitivity and specificity equivalent to 100%.

At 30ng mL, one positive sample gave a sample index ratio close to 1 (average sample 0.9) i.e. 22/23 positives correctly identified, and 14/14 negatives correctly identified giving an equivalent sensitivity and specificity of 95.65 and 100% respectively.

At all cut-off levels, from 10 to 30ng mL, > 95% sensitivity and specificity are met according to PRS-3022 and PRS-3021.

Data:	Section 11.2.1.2
Testing met acceptance criteria (Pass/Fail)	**Pass**

10.1.3 Precision – Repeatability (intra-assay precision) & Reproducibility (inter-assay precision)

Objective:	To demonstrate the closeness of agreement between a series of measurements obtained from multiple sampling of the same defined anti-SARS-CoV-2 N Protein IgG serum and plasma samples using the OmniPATH ELISA method.		
Required materials	• As per section 11.1.2. • Spiked serum and plasma samples representative of high, medium, low levels of anti-SARS-CoV-2 N Protein IgG		
Procedure	As per section 9.1.1, 3. and 11.1.3.		
Results - Serum			
Verification Step	**Expected Results**	**Actual**	**Meet Acceptance Criteria (Yes / No)**
Intra assay precision	Spiked positive serum samples CV should be ≤ 15%; normal sample CV ≤ 25%	**High Spike Serum Concentration:** 150 ng mL n: 16 Mean OD: 2.63 SD: 0.05 **CV: 1.97%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	Yes
		Med. Spike Serum Concentration: 80 ng mL n: 16 Mean OD: 1.83 SD: 0.07 **CV: 3.64%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Low Spike Serum Concentration: 40 ng mL n: 16 Mean OD: 1.07 SD: 0.03 **CV: 3.19%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	

		Normal Serum n: 16 Mean OD: 0.11 SD: 0.003 **CV: 3.06 %** **No. Positive (Sample Index ratio > 1):** 0 **No. Negative (Sample Index ratio ≤ 1):** 16	
Inter assay precision	Spiked positive serum samples CV should be ≤ 15%; normal sample CV ≤ 25%	**High Spike Serum Concentration:** 150 ng mL n: 48 Mean OD: 2.72 SD: 0.07 **CV: 2.89%** **No. Positive (Sample Index ratio > 1):** 48 **No. Negative (Sample Index ratio ≤ 1):** 0	Yes
		Med. Spike Serum Concentration: 80ng mL n: 48 Mean OD: 1.92 SD: 0.08 **CV: 4.35%** **No. Positive (Sample Index ratio > 1):** 48 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Low Spike Serum Concentration: 40ng mL n: 48 Mean OD: 1.12 SD: 0.05 **CV: 4.29%** **No. Positive (Sample Index ratio > 1):** 48 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Normal Serum n: 48 Mean OD: 0.1 SD: 0.01 **CV: 10.36%** **No. Positive (Sample Index ratio > 1):** 0 **No. Negative (Sample Index ratio ≤ 1):** 48	

Results - Spiked Plasma			
Verification Step	**Expected Results**	**Actual**	**Meet Acceptance Criteria (Yes / No)**
Intra assay precision	Spike positive plasma sample CV should be ≤ 15%; normal sample CV ≤ 25%	**High Spike Plasma Concentration:** 150ng mL n: 16 Mean OD: 2.65 SD: 0.05 **CV: 2.05%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	Yes
		Med, Spike Plasma Concentration: 80ng mL n: 16 Mean OD: 1.81 SD: 0.04 **CV: 2.2%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Low Spike Plasma Concentration: 40ng mL n: 16 Mean OD: 1.07 SD: 0.03 **CV: 2.55%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Normal Plasma n: 16 Mean OD: 0.07 SD: 0.003 **CV: 4%** **No. Positive (Sample Index ratio > 1):** 0 **No. Negative (Sample Index ratio ≤ 1):** 16	
Inter assay precision	Spike positive plasma sample CV should be ≤ 15%; normal sample CV ≤ 25%	**High Spike Plasma Concentration:** 150ng mL n: 48 Mean OD: 2.73 SD: 0.07 **CV: 2.65%** **No. Positive (Sample Index ratio > 1):** 16 **No. Negative (Sample Index ratio ≤ 1):** 0	Yes

		Med. Spike Plasma Concentration: 80ng mL n: 48 Mean OD: 1.87 SD: 0.06 **CV: 3.25%** **No. Positive (Sample Index ratio > 1):** 48 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Low Spike Plasma Concentration: 40ng mL n: 48 Mean OD: 1.08 SD: 0.03 **CV: 2.92%** **No. Positive (Sample Index ratio > 1):** 48 **No. Negative (Sample Index ratio ≤ 1):** 0	
		Normal Plasma n: 48 Mean OD: 0.05 SD: 0.01 **CV: 23.27%** **No. Positive (Sample Index ratio > 1):** 0 **No. Negative (Sample Index ratio ≤ 1):** 48	

COMMENTS/REFERENCES/DEVIATIONS:

Intra-assay (repeatability) precision of the method for assay was demonstrated by spiking normal human serum and plasma solutions with recombinant human anti-SARS-CoV-2 N-Protein IgG at high, medium, low concentration or (150, 80, 40 ng mL respectively, 1 in 50 serum or plasma respectively as per procedure). 16 replicates of each sample were analyzed on each microplate according to the OmniPATH ELISA method (section 10.1.2; 10.1.3). The intra-assay validation shows the reproducibility between wells within an OmniPATH assay plate. Intra-assay signal (OD) CV for spiked serum/plasma samples was $\leq$ 3.65%. All spiked samples were identified as positive (16/16 each sample; sample index ratio > 1) at preliminary cut-off concentrations equivalent to 10, 15, 20, 25, 30ng mL anti-SARS-CoV-2 N-Protein IgG. All un-spiked, normal samples were identified as negative (16/16 each sample; sample index ratio $\leq$ 1) at the same preliminary anti-SARS-CoV-2 N-Protein IgG cut-off concentrations. The results demonstrate that samples run in different wells of the plate will give comparable results.

Inter-assay precision (reproducibility) of the method was demonstrated by repeating the intra-assay repeatability experiment firstly with the same operator day two, then with a second operator on day three. A total of 48 replicates of each sample was thus used, 3 microplates, 1 per day, 2 operators. Each microplate had 16 replicates each of the serum and plasma samples described for intra-assay precision above. Inter-assay signal (OD) CV was $\leq$4% for the spiked serum and plasma samples. Inter-assay signal (OD) CV was 10.36% for un-spiked normal serum and 23.27% for un-spiked plasma. The higher CV values of normal samples merely reflects the expected much lower signal (OD) for these and both normal serum and plasma were identified as negative (48/48 each; signal index ratio $\leq$ 1) at preliminary cut-off concentrations equivalent to 10, 15, 20, 25, 30ng mL anti-SARS-CoV-2 N-Protein IgG. All spiked-serum and plasma samples were identified as positive (48/48 each; signal index ratio > 1) at the same at the same preliminary anti-SARS-CoV-2 N-Protein IgG cut-off concentrations. The results demonstrate plate-to-plate consistency and reproducibility between assays done on different days and by different operators.

All criteria were met.

Data:	Section 11.2.1.3
Testing met acceptance criteria (Pass/Fail)	**Pass**

10.1.4 Robustness

Objectives:	Verify robustness of the OmniPATH ELISA by evaluating small but deliberate variations in the key method parameters: • **End-point Stability:** Assess the end point stability (time window during which signal remains valid; ≥ 30 minutes) following colour development the signal is stable with regard to OD signal and sample index ratio (positive or negative) • **Temperature:** Verify different ambient temperatures, 15 and 25°C result in negligible change to determined sample [anti-SARS-CoV-2] concentration.
Required materials	• As per section 11.1.2.
Procedure:	**End-point Stability** • Perform Assay as per section 9.1.1, 4. and 11.1.3. **Temperature variation** • Perform Assay as per section 9.1.1, 4. and 11.1.3.

Results: End Point Stability, Serum samples

Verification Step	Expected Criteria	Serum	After addition of stop	>30minutes after 1st Microplate reading	Meet Acceptance Criteria (Yes / No)
Robustness: End point stability	OD change ≤ 20 % when re-read > 30minutes after first read. Sample index ratio > 1 (pos serum or plasma) or ≤ 1 (neg serum or plasma)	High Serum	Mean OD: 2.63 Sample Index ratio: 3.97	Mean OD: 2.33 % Change: 18.37 Sample Index ratio: 3.92	Yes
		Med. Serum	Mean OD: 1.83 Sample Index ratio: 2.76	Mean OD: 1.68 % Change: 7.82 Sample Index ratio: 2.84	Yes
		Low Serum	Mean OD: 1.07 Sample Index ratio: 1.62	Mean OD: 0.98 % Change: 8.17 Sample Index ratio: 1.66	Yes
		Normal serum	Mean OD: 0.11 Sample Index ratio: 0.16	Mean OD: 0.08 % Change: 21.43 Sample Index ratio: 0.14	Yes, see comments

Results: End Point Stability, Plasma samples					
Verification Step	**Expected Criteria**	**Plasma**			**Meet Acceptance Criteria (Yes / No)**
			After addition of stop	**>30minutes after 1st Microplate reading**	
Robustness: End point stability	OD change ≤ 20 % when re-read > 30minutes after first read. Sample index ratio > 1 (pos serum or plasma) or ≤ 1 (neg serum or plasma)	High Plasma	Mean OD: 2.65 Sample Index ratio: 4	Mean OD: 2.33 % Change: 12.13 Sample Index ratio: 3.92	Yes
		Med. Plasma	Mean OD: 1.81 Sample Index ratio: 2.74	Mean OD: 1.68 % Change: 7.01 Sample Index ratio: 2.84	Yes
		Low Plasma	Mean OD: 1.07 Sample Index ratio: 1.61	Mean OD: 0.98 % Change: 8.29 Sample Index ratio: 1.65	Yes
		Normal Plasma	Mean OD: 0.07 Sample Index ratio: 0.1	Mean OD: 0.05 % Change: 22.76 Sample Index ratio: 0.14	Yes, see comments

Results: Temperature					
Verification Step	**Expected Criteria**	**Serum/ Plasma**	**Anti-IgG-SARS-CoV-2 IgG Sample Concentration ng mL**		**Meet Acceptance Criteria (Yes / No)**
			Assay at 15ºC	**Assay at 25ºC**	
Robustness: Temperature	Sample index ratio > 1 (pos serum or plasma) or ≤ 1 (neg serum or plasma)	High Serum	Sample Index: 5.29	Sample Index: 4.36	Yes
		Med. Serum	Sample Index: 3.35	Sample Index: 3.16	Yes
		Low Serum	Sample Index: 1.88	Sample Index: 1.88	Yes
		Normal Serum	Sample Index: 0.25	Sample Index: 0.16	Yes
		High Plasma	Sample Index: 5.14	Sample Index: 4.38	Yes
		Med. Plasma	Sample Index: 3.14	Sample Index: 3.02	Yes
		Low Plasma	Sample Index: 1.79	Sample Index: 1.74	Yes
		Normal Plasma	Sample Index: 0.16	Sample Index: 0.082	Yes

COMMENTS/REFERENCES/DEVIATIONS:

Robustness was determined by observing how the method stands up to slight variations in normal operating parameters. Method robustness was demonstrated by both end-point stability (signal when samples re-read at > 30minutes following addition of stop reagent and initial readout) and operating at the extremes of the working temperature range (15 and 25ºC). Operating at the extremes of the working temperature range (15ºC c.f. 25ºC) and readout of the completed assay up to 30 minutes after stopping the assay did not have impact on results.

In all studies, normal serum and plasma samples were spiked with high, medium and low levels of Human monoclonal anti-SARS-CoV-2 N-Protein IgG (150, 80, 40 ng mL or un-spiked respectively; 1 in 50 serum or plasma dilution in sample buffer) and assessed in duplicate. Change in sample interpretation using the sample index ratio from positive (above cut-off level; > 1) or negative (below cut-off level; ≤1) was monitored as was change in signal (OD) for end-point stability. Preliminary cut-off control levels equivalent to 10, 15, 20, 25, 30 ng mL anti-SARS-CoV-2 N Protein IgG were evaluated (as per section 10.1.3). Signal ratio's given in the results tables above are at the preliminary cut-off 25ng mL. In all cases, both at 25ng mL or the other cut-off levels, the signal ratio of the spiked samples remained > 1 (positive) and that of the un-spiked serum and plasma ≤ 1 (negative) - see Appendix 11.3.1.4.

For end-point stability, monitoring change in the OD signal of spiked samples demonstrated ≤ 18.5% change for spiked samples. For normal un-spiked samples this was > 20% but < 23% and reflects the much lower signal (OD) for these samples - both remained negative with respect to their sample index ratio.
All criteria were met.

Data:	Section 11.2.1.4
Testing met acceptance criteria (Pass/Fail)	**Pass**

10.1.5 Hook Effect

Aim	To observe if there is a hook effect with the OmniPATH ELISA
Required materials include:	• Materials and reagents as specified in section 8.2. • Hu mAb anti-SARS-CoV-2 IgG
Procedure:	As per section 9.1.1, 5. and 11.1.3.

Hook Effect			
Verification Step	**Expected Results**	**Actual Results**	**Meet Acceptance criteria (Yes/No)**
Hook Effect	No hook effect	**Evaluated anti-SARS-CoV-2 N Protein IgG concentration range:** **Plasma:** double dilution's down from 1.28µg mL anti-SARS-CoV-2 N Protein IgG, 1 in 50 plasma. **Serum:** double dilution's down from 1.28µg mL anti-SARS-CoV-2 N Protein IgG, 1 in 50 serum. **Hook Effect Observed?** No	Yes

COMMENTS/REFERENCES/DEVIATIONS:

Normal Human serum and plasma were spiked at 64ug mL human monoclonal anti-SARS-CoV-2 N-protein IgG to represent a high sample. These samples were diluted at 1 in 50 in sample diluent as per the standard procedure given in section 10.1.3 to give a high level anti-SARS-CoV-2 IgG concentration, 1.28µg mL, in excess of that demonstrating linearity (10-200ng mL; see 10.2.1.1). The samples were further diluted as a series of doubling dilutions and assayed in parallel with preliminary cut-off control anti-SARS-CoV-2 N-protein IgG at10, 15, 20, 25, 30ng mL (as per procedure 10.1.3). At all dilutions with concentrations above those preliminary cut-off control levels, all samples gave a positive result (sample index ratio > 1) demonstrating no hook effect.

All criteria were met.

Data:	Section 11.2.1.5
Testing met acceptance criteria (Pass/Fail)	**Pass**

10.2 Study 2 Results

<table>
<tr><td>Objective:</td><td>To demonstrate that there is no false positive/interference associated with the presence of antibodies to other common coronaviruses in circulation. This assessment will focus on the 4 specific coronavirus strains which have been identified within the Target Product Profile document from the MHRA (229E; OC43; HKU1 and NL63).</td></tr>
<tr><td>Top-Level Procedure</td><td>Serum samples from individuals with known presence of the identified coronaviruses will be run on the assay by the team at the University of Oxford.</td></tr>
<tr><td>Acceptance Criteria</td><td>All samples for the similar coronaviruses must give a negative result on the assay, when compared to negative serum samples. A negative result for the similar coronavirus will indicate that there is no demonstrable interference associated with the presence of antibodies to that disease in this assay. Any positive interference will be documented within the limitations section of the IFU.</td></tr>
<tr><td>Results and Conclusion:</td><td>17 samples positive for seasonal coronavirus including 229E; OC43; HKU1 and NL63 were assessed by the standard procedure.

At 10ng mL anti-SARS-CoV-2 N protein IgG cut-off level, 2 samples had a sample index ratio > 1 (Positive).

At 15, 20, 25, 30ng mL anti-SARS-CoV-2 N protein IgG cut-off level, all samples had a sample index ratio ≤ 1 (Negative).

<table>
<tr><th rowspan="2">Preliminary Cut-off level (ng mL Anti-SARS-CoV-2 N Protein IgG)</th><th colspan="2">Samples (n = 17)</th></tr>
<tr><th>Sample index ratio > 1 (Positive)</th><th>Sample index ratio ≤ 1 (Negative)</th></tr>
<tr><td>10</td><td>2</td><td>15</td></tr>
<tr><td>15</td><td>0</td><td>17</td></tr>
<tr><td>20</td><td>0</td><td>17</td></tr>
<tr><td>25</td><td>0</td><td>17</td></tr>
<tr><td>30</td><td>0</td><td>17</td></tr>
</table>
</td></tr>
<tr><td>Comments:</td><td>At a cut-off level ≥ 15ng mL, all criteria passed. A cut-off level of > 10ng mL is suitable.</td></tr>
<tr><td>Data:</td><td>Section 11.2.2</td></tr>
<tr><td>Acceptance Criteria (Pass/Fail)</td><td>Pass</td></tr>
</table>

10.3 Study 3 Results

Objective:	To demonstrate the clinical performance of 384 Max SARS-CoV-2 N-Protein IgG ELISA assay. This study has been designed to assess the following sections of the User Needs (UN) and Product Requirement Specifications (PRS).
Top-Level Procedure	Assessment of 200 true positive serum/plasma samples (venous/capillary draw) and 200 pre-pandemic negative serum/plasma samples (venous/capillary draw) using the qualitative protocol. Each sample will be run in triplicate, with two runs being conducted on the 384 well format and one run being conducted on the 96 well format. The samples will be pre-diluted at 1/50 using at least 5 µl of sample. A qualitative result for each sample will be obtained and compared to the known result of the sample. Sensitivity and Specificity for the qualitative assay will be derived and reported. This study will be run on samples collected through the University of Oxford and all processing shall be conducted by members of the university faculty and on university premises.
Acceptance Criteria	**Minimum:** Sensitivity > 95% Specificity > 95% **Desirable:** Sensitivity - ≥98% with a 95% confidence interval of 96-100% Specificity - ≥98% with a 95% confidence interval of 96-100% The qualitative results for each sample run on both assay formats (96 Well and 384 Well) must match. Successful reporting of results for each sample run, with good correlation to the known results.
Results and Conclusion:	**Correction:** It was not intended to run samples on the 96 well format for this study and thereby not also a criteria that Sensitivity % = TP / (TP + FN) Specificity % = TN / (TN + FP) Study 3 was performed as singlet samples on the 384 well format.

- 12 False negatives were re-tested independently for anti-SARS-C0V-2-spike protein IgG and anti-SARS-C0V-2-nucleoprotein protein IgG on established assay systems. 8 of these proved negative for both. One sample was positive for both, the remaining 3 were positive for spike protein but only weakly reactive for nucleoprotein. The 8 samples that proved negative for both spike and nucleoprotein were removed from the results (The results with the 8 samples included are available in the appendix).

Cut-off level ng mL									
10		**15**		**20**		**25**		**30**	
Total (n = 399)									
Positive (n = 192)									
True positive	189	True positive	188	True positive	187	True positive	185	True positive	181
False negative	3	False negative	4	False negative	5	False negative	7	False negative	11
Negative (n = 207)									
False positive	80	False positive	44	False positive	31	False positive	22	False positive	15
True negative	127	True negative	163	True negative	176	True negative	185	True negative	192
Results									
Sensitivity	98.44%	Sensitivity	97.92%	Sensitivity	97.4%	Sensitivity	96.35%	Sensitivity	**94.3%**
Specificity	61%	Specificity	78.74%	Specificity	85%	Specificity	89%	Specificity	**92.8%**

In parallel to study 3, the NIBSC verification panel (see also section 9.1.1, 2, 10.1.2) was also run at Oxford. Summary results are given below.

NIBSC Anti-SARS-CoV-2 Verification Panel for Serology NIBSC 20/B770 (n = 37)						
Preliminary Cut-off level (ng mL Anti-SARS-CoV-2 N Protein IgG)	Positive samples (n = 23)		Negative Samples (n = 14)		Sensitivity %	Specificity %
	Sample index ratio > 1 (True Positive)	Sample index ratio ≤ 1 (False Negative)	Sample index ratio ≤ 1 (True Negative)	Sample index ratio > 1 (False Positive)		
10	23	0	13	1	100	93
15	22	1	14	0	95.65	100
20	22	1	14	0	95.65	100
25	22	1	14	0	95.65	100
30	22	1	14	0	95.65	100

- Sensitivity & specificity results for the NIBSC panel when performed at Oxford University are as per results found at Thermo Fisher Scientific, Dartford at 30ng mL cut -off.

Comments:	The sensitivity of and specificity of the assay for samples did not meet the requirements > 95% (PRS-3021; PRS-3022). 12 of the false negative results recorded were also tested on other pre-existing ELISA assay/systems by an independent party. 8 samples recorded a negative result for both anti-SARS-CoV-2 Spike protein and anti-SARS C0V-2 Nucleoprotein IgG indicating these patients had not seroconverted and as such no IgG to either antigen was present. The remaining 4 samples returned a positive response for anti-SARS-CoV-2 Spike protein, 1 of these was also positive for anti-SARS-CoV-2 N protein, the other 3 were weak positive for anti-SARS-CoV-2 nucleoprotein IgG. The latter may reflect either lower sensitivity in general for nucleoprotein IgG assay or differences in seroconversion for spike and nucleoprotein. 8 false negative samples were thus discounted from this study. At 30ng mL cut-off level, 94.3 & 92.8 % sensitivity and specificity respectively. In parallel to the positive and negative samples, the NIBSC verification panel was also screened. This gave sensitivity and specificity > 95% (95.65 and 100% respectively) and was also in agreement with 'trueness' evaluated in section 10.2.2. At these levels, > 95% sensitivity and specificity are met according to PRS-3022 and PRS-3021.
Data:	Section 11.2.3.1 and 11.2.3.2
Acceptance Criteria (Pass/Fail)	**Fail**

11 Conclusion

Study 3 failed to meet the minimum acceptance criteria of 95%. Results were sensitivity 94.3%, and specificity 92.8%. This was discussed with representatives from the university of Oxford who agreed to accept this performance on an interim basis while work is conducted to optimise plate performance for a further RUO version. The performance of the subsequent RUO plate version once completed will be assessed in a further performance evaluation plan and report.

On this basis it is acceptable to release the RUO plate for sale to the University of Oxford.

12 Deviations

Deviation #	Description
1	Plan 1 (Section 9.1.1, 2; 10.1.2). The objective for Plan 1; 9.1.1, 1 was included in error in CC-0530-7020_Performance Evaluation Plan results section (section 10.1.2 herein) but is as that already given in section 9.1.1, 2.
2	Plan 3 (Section 9.1.3; 10.3): Singlet rather than triplet samples were used due to sample volume limitations.
3	Plan 3 (Section 9.1.3; 10.3): 96 well microplate testing was included in error in CC-0530-7020_Performance Evaluation Plan – the current intention is for a 384 well RUO format so no 96 well microplate test was intended nor performed.
4	Plan 3 (Section 9.1.3; 10.3): Sensitivity and specificity $\leq$ 95% criteria (PRS-3021; PRS-3022). The NIBSC verification panel was performed in parallel to study 3 samples and demonstrated $\geq$ 95% sensitivity and specificity. For clarity, the actual sensitivity (94.3%) and specificity (92.8%) derived from the testing will be provided to the customer.

13 Appendix

13.1 Assay Protocols for OmniPATH SARS-CoV-2 N-Protein IgG ELISA

11.1.1 Equipment

Test instruments used in the execution of this protocol must be calibrated at the time of use. Equipment used for the execution of this validation should also be appropriately validated/verified. In the event this is not feasible due to outstanding or concurrent validation, the equipment/procedure used should reflect what would routinely be used during actual process. Following the completion of outstanding or concurrent validation activities, the impact on any associated process validations will be assessed and further validation completed if deemed necessary.

11.1.2 Materials and reagents

Materials and reagents used in OmniPATH ELISA are given in Table 1

Table : Materials and reagents required for testing OmniPATH™ SARS-CoV-2 kits.

Reagent	Description
OmniPATH 384 Well SARS-CoV-2 N-Protein IgG microplate	Prepared using Maxisorp™ microplates coated with recombinant SARS-CoV-2 Nucleocapsid Protein (NP) and surface blocking/glaze.
Detector Conjugate	Mouse Anti Human IgG antibody 504, Identity F16c50, Isotype Mouse IgG1 conjugated to Horseradish Peroxide (HRP) at working strength in calibrant/conjugate buffer.
Conjugate Buffer and Positive / Negative control buffer	Phosphate-Citrate buffer containing 2% v/v Glycerol, 5% w/v Sucrose, 0.3% Sodium Casein. 30mL foetal calf serum, 0.005% v/v Triton X100, 0.1% Proclin300 pH 7.4, 0.8um filtered.
20x Wash Buffer	20x concentrated (to give when diluted 9mM diSodium Phosphate, 1.1% w/v Sodium Chloride, 0.05% tween-20, 0.0025% v/v Proclin300).
Cut-off control: human-anti-SARS-CoV-2 IgG	SARS2_Ab_human_mAb207_(SARS2_NP)_(293T), recombinant Anti-SARS-CoV-2 NP antibody human sequence prepared in OmniPATH Conjugate and Control Buffer.
Sample Diluent	9mM diSodium Phosphate, 1% w/v Sodium Chloride, 0.05% tween-20, 0.05% v/v Proclin300.pH 7.4 ± 0.1 0.8um filtered.
Negative Control	As sample diluent.
Substrate	TMB ready to use, commercially supplied.
Stop solution	Dilute Sulphuric acid ready to use, commercially supplied.
Normal Human Serum	Normal Human Serum.
Normal Human Plasma	Normal Human Plasma.
Positive sera	Positive human serum or normal human serum as sample matrix spiked with monoclonal human anti-SARS-CoV-2 IgG.
Positive plasma	Positive human serum or normal human plasma as sample matrix spiked with monoclonal human anti-SARS-CoV-2 NP IgG.

Sample collection and storage instructions

Infectious items should be handled in accordance with relevant national standards and guidelines. Serum (citrate, heparin, EDTA) and plasma from venous and capillary blood were tested with this assay. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly haemolysed or lipemic samples or sodium azide preserved samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive SARS-CoV-2 IgG antibody. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Reagent preparation instructions

Wash Buffer

1. If crystals have formed in the Wash Buffer Concentrate, warm them gently until they have completely dissolved.
2. The volume of Wash Buffer concentrate required for the assay should be brought to room temperature and should be diluted as per the instructions prior to use.
3. Pour entire contents of Wash Buffer Concentrate into a clean 1000 mL graduated cylinder.
4. Bring to final volume (1000mL) with deionized water and mix gently to avoid foaming
5. Transfer to a clean wash bottle and store 2°C – 25°C. Please note the Wash Buffer (1x) is stable for 30 days.

Qualitative Control(s)s

The following samples may be run as preliminary cut-off control samples:

1. Prepare monoclonal human anti-SARS-CoV-2 N Protein IgG at 2µg mL in sample diluent as cut-off control stock solution.
2. Dilute the cut-off control stock as follows to make cut-off controls 10 – 30ng mL:

Tube	anti-SARS-CoV-2 N Protein IgG concentration ng mL	Calibrant Stock (µl)	Sample Diluent (µl)
Cut off Control 1	10	10	1990
Cut off Control 2	15	15	1985
Cut off Control 3	20	20	1980
Cut off Control 4	25	25	1975
Cut off Control 5	30	30	1970

Table 2: Cut-off control(s)

3. Swirl or mix gently to obtain a homogeneous solution.

Calibration Standards

For information purposes e.g. study 1, Linearity, in addition to the cut-off control (s), the following calibrant concentrations can also be prepared using stock 2µg mL monoclonal human anti-SARS-CoV-2 N Protein IgG

Tube	anti-SARS-CoV-2 N Protein IgG concentration ng mL	Cut-off control stock (2µg mL)	Sample Diluent (µl)
CAL1	200	200	1800
CAL2	150	150	1850
CAL3	100	100	1900
CAL4	80	80	1920
CAL5	40	40	1960
CAL6	20	20	1980
CAL7	10	10	1990

Table 3: Calibrant standards

Swirl or mix gently to obtain a homogeneous solution.

11.1.3 OmniPATH Max 384 SARS-CoV-2 N-Protein IgG ELISA Assay Procedure

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and control standards. Each sample, cut-off control and blank should be assayed in duplicate.
2. Pre-dilute samples in sample diluent buffer according to specific study requirements.
3. Open the foil pouch (extra microplate strips should be returned to the foil pouch with the desiccant provided, sealed tightly and returned to storage at 2°C to 8°C).
4. Pipette **20µL of samples to sample wells**.
5. Pipette **20uL controls** (Cut-off, negative control/sample diluent and or calibrant) to control wells.
6. **Incubate at room temperature (18°C to 25°C) for 1 hour**.
7. It is recommended to use a microplate washer. Shake out or aspirate the contents of the wells. Wash the microwell strips **5 times with approximately 100-125 µL Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the wash buffer to sit in the wells for about 10–15 seconds before aspiration. Take care not to scratch the surface of the microwells. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash buffer. Continue to the next step immediately after washing
8. Pipette **20µL of detector conjugate** to each well, incubate for a further **1 hour at room temperature** (15-25^{o}C). Place adhesive strip or lid on the microplate.
9. Remove adhesive film and empty wells. Wash microwell strips **5 times** according to point 3. of the test protocol. Proceed immediately to the next step.
10. Pipette **20µL of TMB Substrate Solution to all wells**.
11. Incubate at **room temperature** (15-25^{o}C) for **15 minutes**. Avoid direct exposure to intense light.
12. Stop the enzyme reaction by quickly pipetting **5 µL of Stop Solution** into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme - gently tap the plate frame until the yellow colour is uniform to ensure thorough mixing.
13. Read optical density of each microwell on a microplate reader using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the optical density (OD) of

both the samples and the standards. The assay must be read within 30 minutes from the addition of the stop solution.

Interpretation of Qualitative Result

1. Calculate the average optical density values for samples and cut-off control. Duplicate OD should be within 20% of the mean OD value, if not this should be noted within the deviations section of the study report.
2. Subtract the mean OD negative control (sample diluent) as blank from that of test samples and cut-off control mean values to provide the blanked value for each.
3. Determine the Sample index ratio as follows:

$$Sample\ Index\ Ratio = \frac{Sample\ OD}{Cutoff\ OD}$$

4. Interpret results as follows:

Interpretation	Sample Index Ratio	Result
Positive	>1	The sample contains Anti-SARS-CoV-2 N-Protein IgG related antibody
Negative	$\leq$ 1	The sample does not contain Anti-SARS-CoV-2 N-Protein IgG related antibody

13.2 Study Results Raw Data

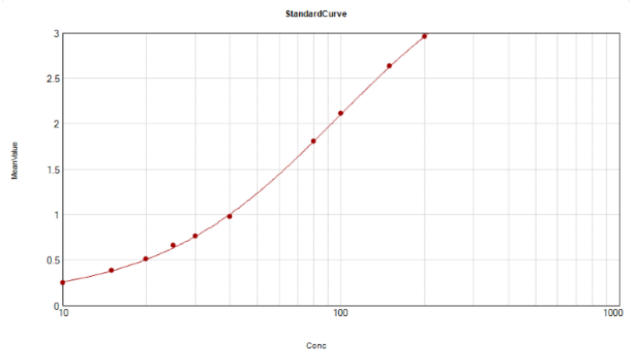
13.2.1 Study 1

13.2.1.1 Linearity

Study Results Section:	10.1.1
Parameter	Linearity

Raw Data

Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Back Calc Conc ng / mL	Mean OD Value - Blank	SD	CV %
200	B1	2.954	2.948	197.637	2.955	0.009	0.3
	B2	2.967	2.961	200.048			
150	C1	2.707	2.701	159.153	2.635	0.093	3.5
	C2	2.575	2.569	142.917			
100	D1	2.221	2.215	108.752	2.114	0.144	6.8
	D2	2.018	2.012	93.384			
80	E1	1.84	1.834	81.653	1.802	0.045	2.5
	E2	1.777	1.771	77.819			
40	F1	0.976	0.97	38.541	0.975	0.007	0.7
	F2	0.986	0.98	38.958			
30	G1	0.758	0.752	29.703	0.762	0.014	1.9
	G2	0.778	0.772	30.497			
25	H1	0.703	0.697	27.528	0.662	0.049	7.4
	H2	0.634	0.628	24.818			
20	A3	0.514	0.508	20.116	0.507	0.001	0.3
	A4	0.512	0.506	20.038			
15	B3	0.379	0.373	14.746	0.383	0.014	3.7
	B4	0.399	0.393	15.553			
10	C3	0.255	0.249	9.542	0.246	0.004	1.7
	C4	0.249	0.243	9.277			
Blank	D3	0.007	0.006		0		
	D4	0.005					



● Std (Standards@Time 1: MeanValue vs Concentration) Weighting: Fixed

Curve Fit Results ▲

Curve Fit : 4-Parameter Logistic $y = D + \frac{A-D}{1+\left(\frac{x}{C}\right)^B}$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Std R^2 = 1.000 EC50 = 97.63	A	0.080	0.038	[-0.013, 0.173]
	B	1.340	0.070	[1.169, 1.511]
	C	97.63	5.535	[84.09, 111.2]
	D	4.063	0.159	[3.673, 4.452]

13.2.1.2 Trueness

Study Results Section:	10.1.2
Parameter	Trueness

Raw Data

Cut-off concentration Anti-SARS-CoV-2 IgG ng mL	Wells	OD (650-450nm)	OD - Blank (650-450nm)	Mean - Blank OD
30	G1	0.675	0.669	0.692
	G2	0.721	0.715	
25	H1	0.557	0.551	0.556
	H2	0.568	0.562	
20	A3	0.446	0.44	0.442
	A4	0.45	0.444	
15	B3	0.347	0.341	0.341
	B4	0.347	0.341	
10	C3	0.361	0.355	0.278
	C4	0.207	0.201	
Blank	D3	0.005	0	
	D4	0.007		

NIBSC Positive Samples

NIBSC Sample Number	Wells	OD Value - Blank	Sample Index Ratio at Cut-off anti-SARS-CoV-2 N-Protein levels ng mL				
			10	15	20	25	30
1	A5	1.283	4.62	3.76	2.90	2.31	1.85
	A6	1.337	4.81	3.92	3.02	2.40	1.93
2	B5	1.121	4.03	3.29	2.54	2.02	1.62
	B6	1.121	4.03	3.29	2.54	2.02	1.62
3	C5	1.032	3.71	3.03	2.33	1.86	1.49
	C6	1.255	4.51	3.68	2.84	2.26	1.81
4	D5	2.597	9.34	7.62	5.88	4.67	3.75
	D6	2.631	9.46	7.72	5.95	4.73	3.80
5	E5	1.742	6.27	5.11	3.94	3.13	2.52
	E6	1.958	7.04	5.74	4.43	3.52	2.83
6	F5	1.439	5.18	4.22	3.26	2.59	2.08
	F6	1.629	5.86	4.78	3.69	2.93	2.35
7	G5	1.392	5.01	4.08	3.15	2.50	2.01
	G6	1.524	5.48	4.47	3.45	2.74	2.20
8	H5	2.381	8.56	6.98	5.39	4.28	3.44
	H6	1.948	7.01	5.71	4.41	3.50	2.82
9	A7	0.92	3.31	2.70	2.08	1.65	1.33
	A8	0.965	3.47	2.83	2.18	1.74	1.39
10	B7	1.189	4.28	3.49	2.69	2.14	1.72
	B8	1.23	4.42	3.61	2.78	2.21	1.78
11	C7	0.623	2.24	1.83	1.41	1.12	0.90
	C8	0.614	2.21	1.80	1.39	1.10	0.89
12	D7	2.373	8.54	6.96	5.37	4.27	3.43
	D8	2.265	8.15	6.64	5.12	4.07	3.27
13	E7	2.021	7.27	5.93	4.57	3.63	2.92
	E8	2.47	8.88	7.24	5.59	4.44	3.57

14	F7	1.83	6.58	5.37	4.14	3.29	2.64
	F8	1.906	6.86	5.59	4.31	3.43	2.75
15	G7	2.804	10.09	8.22	6.34	5.04	4.05
	G8	2.902	10.44	8.51	6.57	5.22	4.19
16	H7	1.642	5.91	4.82	3.71	2.95	2.37
	H8	1.559	5.61	4.57	3.53	2.80	2.25
17	A9	1.789	6.44	5.25	4.05	3.22	2.59
	A10	1.842	6.63	5.40	4.17	3.31	2.66
18	B9	1.161	4.18	3.40	2.63	2.09	1.68
	B10	1.032	3.71	3.03	2.33	1.86	1.49
19	C9	1.719	6.18	5.04	3.89	3.09	2.48
	C10	1.834	6.60	5.38	4.15	3.30	2.65
20	D9	1.829	6.58	5.36	4.14	3.29	2.64
	D10	1.94	6.98	5.69	4.39	3.49	2.80
21	E9	1.438	5.17	4.22	3.25	2.59	2.08
	E10	1.493	5.37	4.38	3.38	2.69	2.16
22	F9	2.471	8.89	7.25	5.59	4.44	3.57
	F10	1.997	7.18	5.86	4.52	3.59	2.89
23	G9	2.319	8.34	6.80	5.25	4.17	3.35
	G10	2.311	8.31	6.78	5.23	4.16	3.34

NIBSC Negative Samples

			Sample Index Ratio at Cut-off anti-SARS-CoV-2 N-Protein levels ng mL				
NIBSC Sample Number	**Wells**	**OD Value - Blank**	**10**	**15**	**20**	**25**	**30**
24	A11	0.03	0.11	0.09	0.07	0.05	0.04
	A12	0.031	0.11	0.09	0.07	0.06	0.04
25	B11	0.047	0.17	0.14	0.11	0.08	0.07
	B12	0.054	0.19	0.16	0.12	0.10	0.08
26	C11	0.059	0.21	0.17	0.13	0.11	0.09
	C12	0.059	0.21	0.17	0.13	0.11	0.09
27	D11	0.034	0.12	0.10	0.08	0.06	0.05
	D12	0.039	0.14	0.11	0.09	0.07	0.06
28	E11	0.012	0.04	0.04	0.03	0.02	0.02
	E12	0.011	0.04	0.03	0.02	0.02	0.02
29	F11	0.02	0.07	0.06	0.05	0.04	0.03
	F12	0.021	0.08	0.06	0.05	0.04	0.03
30	G11	0.051	0.18	0.15	0.12	0.09	0.07
	G12	0.052	0.19	0.15	0.12	0.09	0.08
31	H11	0.076	0.27	0.22	0.17	0.14	0.11
	H12	0.081	0.29	0.24	0.18	0.15	0.12
32	A13	0.051	0.18	0.15	0.12	0.09	0.07
	A14	0.044	0.16	0.13	0.10	0.08	0.06
33	B13	0.057	0.21	0.17	0.13	0.10	0.08
	B14	0.058	0.21	0.17	0.13	0.10	0.08
34	C13	0.077	0.28	0.23	0.17	0.14	0.11
	C14	0.072	0.26	0.21	0.16	0.13	0.10
35	D13	0.018	0.06	0.05	0.04	0.03	0.03
	D14	0.017	0.06	0.05	0.04	0.03	0.02
36	E13	0.054	0.19	0.16	0.12	0.10	0.08
	E14	0.054	0.19	0.16	0.12	0.10	0.08
37	F13	0.01	0.04	0.03	0.02	0.02	0.01
	F14	0.011	0.04	0.03	0.02	0.02	0.02

13.2.1.3 Precision

Study Results Section:	10.1.3
Parameter	Precision – Repeatability (intra-assay precision) and Reproducibility (inter-assay precision)

Raw Data

A) Summary

- ***Inter-Assay (Run 1,2,3; Day1,2, 3; Operator 1,2)***

			Intra-assay			
Sample Matrix	**Reactivity**	**N**	**Results**	**Average OD**	**SD**	**% CV**
Serum	High	48	48/48 are positive	2.72	0.07	2.89
	Medium	48	48/48 are positive	1.92	0.08	4.35
	Low	48	48/48 are positive	1.12	0.05	4.29
	Normal	48	48/48 are negative	0.1	0.01	10.36
Plasma	High	48	48/48 are positive	2.73	0.07	2.65
	Medium	48	48/48 are positive	1.87	0.06	3.25
	Low	48	48/48 are positive	1.08	0.03	2.92
	Normal	48	48/48 are negative	0.05	0.01	23.27

- ***Average intra-assay (Run 1, Day 1, operator 1)***

			Average Intra-assay			
Sample Matrix	**Reactivity**	**N**	**Results**	**Average OD**	**SD**	**% CV**
Serum	High	16	16/16 are positive	2.63	0.05	1.97
	Medium	16	16/16 are positive	1.83	0.07	3.64
	Low	16	16/16 are positive	1.07	0.03	3.19
	Normal	16	16/16 are positive	0.11	0.003	3.06
Plasma	High	16	16/16 are positive	2.65	0.05	2.05
	Medium	16	16/16 are positive	1.81	0.04	2.2
	Low	16	16/16 are positive	1.07	0.03	2.55
	Normal	16	16/16 are positive	0.07	0.003	4

B) Run 1, Day 1, Operator 1

Summary

			Intra-assay			
Sample Matrix	**Reactivity**	**N**	**Results**	**Average OD**	**SD**	**% CV**
Serum	High	16	16/16 are positive	2.63	0.05	1.97
	Medium	16	16/16 are positive	1.83	0.07	3.64
	Low	16	16/16 are positive	1.07	0.03	3.19
	Normal	16	16/16 are positive	0.11	0.003	3.06
Plasma	High	16	16/16 are positive	2.65	0.05	2.05
	Medium	16	16/16 are positive	1.81	0.04	2.2
	Low	16	16/16 are positive	1.07	0.03	2.55
	Normal	16	16/16 are positive	0.07	0.003	4

Cut-off Controls

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	G1	0.758	0.752	0.762	0.014	1.9
	G2	0.778	0.772			
25	H1	0.703	0.697	0.662	0.049	7.4
	H2	0.634	0.628			
20	A3	0.514	0.508	0.507	0.001	0.3
	A4	0.512	0.506			
15	B3	0.379	0.373	0.383	0.014	3.7
	B4	0.399	0.393			
10	C3	0.255	0.249	0.246	0.004	1.7
	C4	0.249	0.243			
Blank	D3	0.007	0	0		
	D4	0.005				

Spiked and un-spiked plasma and serum samples

Plasma Samples

Sample	Wells	OD Value - Blank	Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
			10	15	20	25	30	Mean OD	SD	CV %	10	15	20	25	30
High spiked human plasma	E5	2.661	10.82	6.95	5.25	4.02	3.49	2.65	0.05	2.05	10.76	6.91	5.22	4.00	3.47
	E6	2.645	10.75	6.91	5.22	4.00	3.47								
	E7	2.531	10.29	6.61	4.99	3.82	3.32								
	E8	2.654	10.79	6.93	5.23	4.01	3.48								
	E9	2.547	10.35	6.65	5.02	3.85	3.34								
	E10	2.728	11.09	7.12	5.38	4.12	3.58								
	E11	2.72	11.06	7.10	5.36	4.11	3.57								
	E12	2.642	10.74	6.90	5.21	3.99	3.47								
	E13	2.643	10.74	6.90	5.21	3.99	3.47								
	E14	2.645	10.75	6.91	5.22	4.00	3.47								
	E15	2.613	10.62	6.82	5.15	3.95	3.43								
	E16	2.698	10.97	7.04	5.32	4.08	3.54								
	E17	2.632	10.70	6.87	5.19	3.98	3.45								
	E18	2.685	10.91	7.01	5.30	4.06	3.52								
	E19	2.62	10.65	6.84	5.17	3.96	3.44								
	E20	2.695	10.96	7.04	5.32	4.07	3.54								
Low spiked human plasma	G5	1.066	4.33	2.78	2.10	1.61	1.40	1.07	0.03	2.55	4.33	2.78	2.10	1.61	1.40
	G6	1.078	4.38	2.81	2.13	1.63	1.41								
	G7	1.058	4.30	2.76	2.09	1.60	1.39								
	G8	1.099	4.47	2.87	2.17	1.66	1.44								
	G9	1.061	4.31	2.77	2.09	1.60	1.39								
	G10	1.091	4.43	2.85	2.15	1.65	1.43								
	G11	1.092	4.44	2.85	2.15	1.65	1.43								
	G12	1.072	4.36	2.80	2.11	1.62	1.41								
	G13	1.065	4.33	2.78	2.10	1.61	1.40								
	G14	1.041	4.23	2.72	2.05	1.57	1.37								
	G15	1.068	4.34	2.79	2.11	1.61	1.40								
	G16	1.078	4.38	2.81	2.13	1.63	1.41								
	G17	1.069	4.35	2.79	2.11	1.61	1.40								
	G18	1.085	4.41	2.83	2.14	1.64	1.42								
	G19	0.982	3.99	2.56	1.94	1.48	1.29								
	G20	1.051	4.27	2.74	2.07	1.59	1.38								
Medium spiked human plasma	F5	1.782	7.24	4.65	3.51	2.69	2.34	1.81	0.04	2.20	7.36	4.73	3.57	2.74	2.38
	F6	1.86	7.56	4.86	3.67	2.81	2.44								
	F7	1.749	7.11	4.57	3.45	2.64	2.30								
	F8	1.785	7.26	4.66	3.52	2.70	2.34								
	F9	1.761	7.16	4.60	3.47	2.66	2.31								
	F10	1.843	7.49	4.81	3.64	2.78	2.42								
	F11	1.771	7.20	4.62	3.49	2.68	2.32								
	F12	1.834	7.46	4.79	3.62	2.77	2.41								

	F13	1.859	7.56	4.85	3.67	2.81	2.44								
	F14	1.833	7.45	4.79	3.62	2.77	2.41								
	F15	1.856	7.54	4.85	3.66	2.80	2.44								
	F16	1.849	7.52	4.83	3.65	2.79	2.43								
	F17	1.802	7.33	4.70	3.55	2.72	2.36								
	F18	1.803	7.33	4.71	3.56	2.72	2.37								
	F19	1.754	7.13	4.58	3.46	2.65	2.30								
	F20	1.839	7.48	4.80	3.63	2.78	2.41								
Normal, unspiked human plasma	H5	0.068	0.28	0.18	0.13	0.10	0.09	0.07	0.003	4.00	0.27	0.17	0.13	0.10	0.09
	H6	0.067	0.27	0.17	0.13	0.10	0.09								
	H7	0.065	0.26	0.17	0.13	0.10	0.09								
	H8	0.068	0.28	0.18	0.13	0.10	0.09								
	H9	0.066	0.27	0.17	0.13	0.10	0.09								
	H10	0.068	0.28	0.18	0.13	0.10	0.09								
	H11	0.066	0.27	0.17	0.13	0.10	0.09								
	H12	0.073	0.30	0.19	0.14	0.11	0.10								
	H13	0.063	0.26	0.16	0.12	0.10	0.08								
	H14	0.065	0.26	0.17	0.13	0.10	0.09								
	H15	0.062	0.25	0.16	0.12	0.09	0.08								
	H16	0.069	0.28	0.18	0.14	0.10	0.09								
	H17	0.067	0.27	0.17	0.13	0.10	0.09								
	H18	0.069	0.28	0.18	0.14	0.10	0.09								
	H19	0.07	0.28	0.18	0.14	0.11	0.09								
	H20	0.066	0.27	0.17	0.13	0.10	0.09								

Serum Samples

Sample	Wells	OD Value - Blank	Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
			10	15	20	25	30	Mean OD	SD	CV %	10	15	20	25	30
High spiked human Serum	A5	2.59	10.53	6.76	5.11	3.91	3.40	2.63	0.05	1.97	10.68	6.86	5.18	3.97	3.45
	A6	2.675	10.87	6.98	5.28	4.04	3.51								
	A7	2.625	10.67	6.85	5.18	3.97	3.44								
	A8	2.667	10.84	6.96	5.26	4.03	3.50								
	A9	2.612	10.62	6.82	5.15	3.95	3.43								
	A10	2.58	10.49	6.74	5.09	3.90	3.39								
	A11	2.712	11.02	7.08	5.35	4.10	3.56								
	A12	2.613	10.62	6.82	5.15	3.95	3.43								
	A13	2.721	11.06	7.10	5.37	4.11	3.57								
	A14	2.637	10.72	6.89	5.20	3.98	3.46								
	A15	2.591	10.53	6.77	5.11	3.91	3.40								
	A16	2.695	10.96	7.04	5.32	4.07	3.54								
	A17	2.6	10.57	6.79	5.13	3.93	3.41								
	A18	2.588	10.52	6.76	5.10	3.91	3.40								
	A19	2.541	10.33	6.63	5.01	3.84	3.33								
	A20	2.6	10.57	6.79	5.13	3.93	3.41								
Low spiked human serum	C5	1.107	4.50	2.89	2.18	1.67	1.45	1.07	0.03	3.19	4.35	2.80	2.11	1.62	1.40
	C6	1.037	4.22	2.71	2.05	1.57	1.36								
	C7	1.077	4.38	2.81	2.12	1.63	1.41								
	C8	1.088	4.42	2.84	2.15	1.64	1.43								
	C9	1.125	4.57	2.94	2.22	1.70	1.48								
	C10	1.075	4.37	2.81	2.12	1.62	1.41								
	C11	1.084	4.41	2.83	2.14	1.64	1.42								
	C12	1.041	4.23	2.72	2.05	1.57	1.37								
	C13	1.043	4.24	2.72	2.06	1.58	1.37								
	C14	1.035	4.21	2.70	2.04	1.56	1.36								
	C15	1.112	4.52	2.90	2.19	1.68	1.46								
	C16	1.078	4.38	2.81	2.13	1.63	1.41								
	C17	1.08	4.39	2.82	2.13	1.63	1.42								
	C18	1.008	4.10	2.63	1.99	1.52	1.32								
	C19	1.032	4.20	2.69	2.04	1.56	1.35								
	C20	1.107	4.50	2.89	2.18	1.67	1.45								
Medium spiked human serum	B5	1.727	7.02	4.51	3.41	2.61	2.27	1.83	0.07	3.64	7.43	4.77	3.60	2.76	2.40
	B6	1.933	7.86	5.05	3.81	2.92	2.54								
	B7	1.776	7.22	4.64	3.50	2.68	2.33								
	B8	1.845	7.50	4.82	3.64	2.79	2.42								
	B9	1.817	7.39	4.74	3.58	2.74	2.38								
	B10	1.79	7.28	4.67	3.53	2.70	2.35								
	B11	1.864	7.58	4.87	3.68	2.82	2.45								
	B12	1.763	7.17	4.60	3.48	2.66	2.31								
	B13	1.868	7.59	4.88	3.68	2.82	2.45								
	B14	1.795	7.30	4.69	3.54	2.71	2.36								

	B15	1.857	7.55	4.85	3.66	2.81	2.44								
	B16	1.842	7.49	4.81	3.63	2.78	2.42								
	B17	1.807	7.35	4.72	3.56	2.73	2.37								
	B18	1.843	7.49	4.81	3.64	2.78	2.42								
	B19	1.977	8.04	5.16	3.90	2.99	2.59								
	B20	1.736	7.06	4.53	3.42	2.62	2.28								
Unspiked normal human serum	D5	0.102	0.41	0.27	0.20	0.15	0.13	0.11	0.003	3.06	0.43	0.28	0.21	0.16	0.14
	D6	0.105	0.43	0.27	0.21	0.16	0.14								
	D7	0.106	0.43	0.28	0.21	0.16	0.14								
	D8	0.107	0.43	0.28	0.21	0.16	0.14								
	D9	0.105	0.43	0.27	0.21	0.16	0.14								
	D10	0.099	0.40	0.26	0.20	0.15	0.13								
	D11	0.109	0.44	0.28	0.21	0.16	0.14								
	D12	0.109	0.44	0.28	0.21	0.16	0.14								
	D13	0.111	0.45	0.29	0.22	0.17	0.15								
	D14	0.102	0.41	0.27	0.20	0.15	0.13								
	D15	0.108	0.44	0.28	0.21	0.16	0.14								
	D16	0.109	0.44	0.28	0.21	0.16	0.14								
	D17	0.109	0.44	0.28	0.21	0.16	0.14								
	D18	0.104	0.42	0.27	0.21	0.16	0.14								
	D19	0.105	0.43	0.27	0.21	0.16	0.14								
	D20	0.104	0.42	0.27	0.21	0.16	0.14								

C) Run 2, Day 2, Operator 2

Summary

			Intra-assay			
Sample Matrix	**Reactivity**	**N**	**Results**	**Average OD**	**SD**	**% CV**
Serum	High	16	16/16 are positive	2.77	0.04	1.49
	Medium	16	16/16 are positive	1.94	0.04	2.29
	Low	16	16/16 are positive	1.15	0.03	2.93
	Normal	16	16/16 are positive	0.1	0.001	2.6
Plasma	High	16	16/16 are positive	2.76	0.04	1.48
	Medium	16	16/16 are positive	1.89	0.05	2.43
	Low	16	16/16 are positive	1.08	0.03	3.21
	Normal	16	16/16 are positive	0.05	0.001	2.6

Cut-off Controls

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	G1	0.786	0.782	0.78	0.003	0.4
	G2	0.782	0.778			
25	H1	0.661	0.657	0.629	0.04	6.3
	H2	0.605	0.601			
20	A3	0.517	0.513	0.526	0.018	3.5
	A4	0.543	0.539			
15	B3	0.384	0.38	0.387	0.011	2.7
	B4	0.399	0.395			
10	C3	0.259	0.255	0.256	0.002	0.8
	C4	0.262	0.258			
Blank	D3	0.005	0.0045	0		
	D4	0.004				

Spiked and un-spiked plasma and serum samples

Plasma Samples

Sample	Wells	OD Value - Blank	Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
			10	15	20	25	30	Mean OD	SD	CV %	10	15	20	25	30
High spiked human plasma	E5	2.74	10.72	7.09	5.22	4.36	3.52	2.76	0.04	1.48	10.78	7.13	5.25	4.39	3.54
	E6	2.74	10.72	7.09	5.22	4.36	3.52								
	E7	2.7	10.55	6.98	5.13	4.29	3.46								
	E8	2.73	10.65	7.04	5.18	4.33	3.49								
	E9	2.72	10.63	7.03	5.17	4.33	3.49								
	E10	2.75	10.75	7.11	5.23	4.38	3.53								
	E11	2.83	11.07	7.32	5.39	4.50	3.63								
	E12	2.74	10.69	7.07	5.20	4.35	3.51								
	E13	2.72	10.64	7.04	5.18	4.33	3.49								
	E14	2.77	10.80	7.15	5.26	4.40	3.55								
	E15	2.73	10.67	7.06	5.19	4.34	3.50								
	E16	2.83	11.07	7.32	5.39	4.50	3.63								
	E17	2.77	10.81	7.15	5.26	4.40	3.55								
	E18	2.82	11.02	7.29	5.36	4.48	3.62								
	E19	2.75	10.73	7.10	5.22	4.37	3.52								
	E20	2.79	10.91	7.22	5.31	4.44	3.58								
Low spiked human plasma	G5	1.1	4.29	2.84	2.09	1.75	1.41	1.08	0.03	3.21	4.21	2.78	2.05	1.71	1.38
	G6	1.1	4.31	2.85	2.10	1.75	1.41								
	G7	1.05	4.12	2.72	2.00	1.68	1.35								
	G8	1.12	4.36	2.88	2.12	1.77	1.43								
	G9	1.04	4.05	2.68	1.97	1.65	1.33								
	G10	1.1	4.31	2.85	2.10	1.76	1.42								
	G11	1.06	4.14	2.74	2.02	1.69	1.36								
	G12	1.11	4.34	2.87	2.11	1.77	1.42								
	G13	1.08	4.20	2.78	2.05	1.71	1.38								
	G14	1.08	4.21	2.79	2.05	1.71	1.38								
	G15	1.09	4.25	2.81	2.07	1.73	1.40								
	G16	1.1	4.30	2.84	2.09	1.75	1.41								
	G17	1.04	4.08	2.70	1.98	1.66	1.34								
	G18	1.11	4.34	2.87	2.11	1.77	1.43								
	G19	0.99	3.86	2.55	1.88	1.57	1.27								
	G20	1.07	4.18	2.77	2.04	1.70	1.37								
Medium spiked human plasma	F5	1.85	7.24	4.79	3.52	2.95	2.38	1.89	0.05	2.43	7.37	4.87	3.59	3.00	2.42
	F6	1.95	7.61	5.03	3.70	3.10	2.50								
	F7	1.86	7.25	4.80	3.53	2.95	2.38								
	F8	1.93	7.54	4.98	3.67	3.07	2.47								
	F9	1.84	7.19	4.75	3.50	2.93	2.36								
	F10	1.94	7.58	5.01	3.69	3.08	2.49								
	F11	1.78	6.95	4.59	3.38	2.83	2.28								
	F12	1.89	7.38	4.88	3.59	3.00	2.42								

	F13	1.91	7.46	4.93	3.63	3.03	2.45								
	F14	1.92	7.48	4.95	3.64	3.04	2.46								
	F15	1.92	7.49	4.95	3.64	3.05	2.46								
	F16	1.93	7.53	4.98	3.66	3.06	2.47								
	F17	1.86	7.28	4.82	3.54	2.96	2.39								
	F18	1.9	7.43	4.91	3.61	3.02	2.44								
	F19	1.84	7.17	4.74	3.49	2.92	2.35								
	F20	1.88	7.34	4.86	3.57	2.99	2.41								
Normal, unspiked human plasma	H5	0.05	0.21	0.14	0.10	0.09	0.07	0.05	0.00	2.60	0.20	0.14	0.10	0.08	0.07
	H6	0.05	0.20	0.13	0.10	0.08	0.07								
	H7	0.05	0.20	0.13	0.10	0.08	0.07								
	H8	0.05	0.21	0.14	0.10	0.09	0.07								
	H9	0.05	0.20	0.13	0.10	0.08	0.07								
	H10	0.05	0.21	0.14	0.10	0.09	0.07								
	H11	0.05	0.20	0.13	0.10	0.08	0.07								
	H12	0.06	0.21	0.14	0.10	0.09	0.07								
	H13	0.05	0.20	0.13	0.10	0.08	0.07								
	H14	0.05	0.21	0.14	0.10	0.08	0.07								
	H15	0.05	0.20	0.13	0.10	0.08	0.06								
	H16	0.05	0.21	0.14	0.10	0.08	0.07								
	H17	0.05	0.21	0.14	0.10	0.08	0.07								
	H18	0.05	0.20	0.13	0.10	0.08	0.07								
	H19	0.05	0.20	0.13	0.10	0.08	0.07								
	H20	0.05	0.20	0.13	0.10	0.08	0.07								

Serum Samples

Sample	Wells	OD Value - Blank	Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
			10	15	20	25	30	Mean OD	SD	CV %	10	15	20	25	30
High spiked human Serum	A5	2.752	10.75	7.11	5.23	4.38	3.53	2.77	0.04	1.49	10.82	7.16	5.27	4.40	3.55
	A6	2.821	11.02	7.29	5.36	4.48	3.62								
	A7	2.775	10.84	7.17	5.28	4.41	3.56								
	A8	2.819	11.01	7.28	5.36	4.48	3.61								
	A9	2.766	10.80	7.15	5.26	4.40	3.55								
	A10	2.734	10.68	7.06	5.20	4.35	3.51								
	A11	2.791	10.90	7.21	5.31	4.44	3.58								
	A12	2.813	10.99	7.27	5.35	4.47	3.61								
	A13	2.834	11.07	7.32	5.39	4.51	3.63								
	A14	2.767	10.81	7.15	5.26	4.40	3.55								
	A15	2.736	10.69	7.07	5.20	4.35	3.51								
	A16	2.809	10.97	7.26	5.34	4.47	3.60								
	A17	2.741	10.71	7.08	5.21	4.36	3.51								
	A18	2.724	10.64	7.04	5.18	4.33	3.49								
	A19	2.694	10.52	6.96	5.12	4.28	3.45								
	A20	2.737	10.69	7.07	5.20	4.35	3.51								
Low spiked human serum	C5	1.201	4.69	3.10	2.28	1.91	1.54	1.15	0.03	2.93	4.47	2.96	2.18	1.82	1.47
	C6	1.114	4.35	2.88	2.12	1.77	1.43								
	C7	1.157	4.52	2.99	2.20	1.84	1.48								
	C8	1.169	4.57	3.02	2.22	1.86	1.50								
	C9	1.19	4.65	3.07	2.26	1.89	1.53								
	C10	1.145	4.47	2.96	2.18	1.82	1.47								
	C11	1.183	4.62	3.06	2.25	1.88	1.52								
	C12	1.112	4.34	2.87	2.11	1.77	1.43								
	C13	1.149	4.49	2.97	2.18	1.83	1.47								
	C14	1.147	4.48	2.96	2.18	1.82	1.47								
	C15	1.169	4.57	3.02	2.22	1.86	1.50								
	C16	1.117	4.36	2.89	2.12	1.78	1.43								
	C17	1.145	4.47	2.96	2.18	1.82	1.47								
	C18	1.077	4.21	2.78	2.05	1.71	1.38								
	C19	1.107	4.32	2.86	2.10	1.76	1.42								
	C20	1.139	4.45	2.94	2.17	1.81	1.46								
Medium spiked human serum	B5	1.919	7.50	4.96	3.65	3.05	2.46	1.94	0.04	2.29	7.57	5.01	3.68	3.08	2.48
	B6	1.899	7.42	4.91	3.61	3.02	2.43								
	B7	1.875	7.32	4.84	3.56	2.98	2.40								
	B8	1.915	7.48	4.95	3.64	3.04	2.46								
	B9	1.903	7.43	4.92	3.62	3.03	2.44								
	B10	1.945	7.60	5.03	3.70	3.09	2.49								
	B11	1.994	7.79	5.15	3.79	3.17	2.56								
	B12	1.955	7.64	5.05	3.72	3.11	2.51								
	B13	2.023	7.90	5.23	3.85	3.22	2.59								

	B14	1.933	7.55	4.99	3.67	3.07	2.48								
	B15	1.909	7.46	4.93	3.63	3.03	2.45								
	B16	1.943	7.59	5.02	3.69	3.09	2.49								
	B17	1.969	7.69	5.09	3.74	3.13	2.52								
	B18	1.963	7.67	5.07	3.73	3.12	2.52								
	B19	1.993	7.79	5.15	3.79	3.17	2.56								
	B20	1.863	7.28	4.81	3.54	2.96	2.39								
Unspiked normal human serum	D5	0.101	0.39	0.26	0.19	0.16	0.13	0.10	0.00	2.59	0.39	0.26	0.19	0.16	0.13
	D6	0.099	0.39	0.26	0.19	0.16	0.13								
	D7	0.103	0.40	0.27	0.20	0.16	0.13								
	D8	0.103	0.40	0.27	0.20	0.16	0.13								
	D9	0.101	0.39	0.26	0.19	0.16	0.13								
	D10	0.096	0.38	0.25	0.18	0.15	0.12								
	D11	0.103	0.40	0.27	0.20	0.16	0.13								
	D12	0.097	0.38	0.25	0.18	0.15	0.12								
	D13	0.105	0.41	0.27	0.20	0.17	0.13								
	D14	0.099	0.39	0.26	0.19	0.16	0.13								
	D15	0.1	0.39	0.26	0.19	0.16	0.13								
	D16	0.103	0.40	0.27	0.20	0.16	0.13								
	D17	0.1	0.39	0.26	0.19	0.16	0.13								
	D18	0.098	0.38	0.25	0.19	0.16	0.13								
	D19	0.098	0.38	0.25	0.19	0.16	0.13								
	D20	0.098	0.38	0.25	0.19	0.16	0.13								

D) Run 3, Day 3, Operator 3

Summary

			Intra-assay			
Sample Matrix	**Reactivity**	**N**	**Results**	**Average OD**	**SD**	**% CV**
Serum	High	16	16/16 are positive	2.75	0.05	1.7
	Medium	16	16/16 are positive	1.98	0.04	2.22
	Low	16	16/16 are positive	1.15	0.03	2.33
	Normal	16	16/16 are positive	0.08	0.003	2.6
Plasma	High	16	16/16 are positive	2.78	0.03	3.58
	Medium	16	16/16 are positive	1.91	0.05	2.5
	Low	16	16/16 are positive	1.09	0.03	2.72
	Normal	16	16/16 are positive	0.04	0.003	9.02

Cut-off Controls

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	G1	0.766	0.741	0.766	0.036	4.7
	G2	0.817	0.791			
25	H1	0.628	0.603	0.609	0.009	1.5
	H2	0.641	0.616			
20	A3	0.545	0.52	0.512	0.011	2.2
	A4	0.529	0.504			
15	B3	0.394	0.368	0.368	0	0
	B4	0.394	0.368			
10	C3	0.282	0.256	0.244	0.018	7.2
	C4	0.257	0.232			
Blank	D3	0.043	0.026	0		
	D4	0.008				

Spiked and un-spiked plasma and serum samples

Plasma Samples

			Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
Sample	**Wells**	**OD Value - Blank**	**10**	**15**	**20**	**25**	**30**	**Mean OD**	**SD**	**CV %**	**10**	**15**	**20**	**25**	**30**
High spiked human plasma	E5	2.761	11.32	7.50	5.39	4.53	3.60	2.78	0.03	0.97	11.40	7.56	5.43	4.57	3.63
	E6	2.765	11.33	7.51	5.40	4.54	3.61								
	E7	2.756	11.30	7.49	5.38	4.53	3.60								
	E8	2.813	11.53	7.64	5.49	4.62	3.67								
	E9	2.776	11.38	7.54	5.42	4.56	3.62								
	E10	2.78	11.39	7.55	5.43	4.56	3.63								
	E11	2.732	11.20	7.42	5.34	4.49	3.57								
	E12	2.833	11.61	7.70	5.53	4.65	3.70								
	E13	2.797	11.46	7.60	5.46	4.59	3.65								
	E14	2.797	11.46	7.60	5.46	4.59	3.65								
	E15	2.757	11.30	7.49	5.38	4.53	3.60								
	E16	2.806	11.50	7.63	5.48	4.61	3.66								
	E17	2.784	11.41	7.57	5.44	4.57	3.63								
	E18	2.817	11.55	7.65	5.50	4.63	3.68								
	E19	2.757	11.30	7.49	5.38	4.53	3.60								
	E20	2.782	11.40	7.56	5.43	4.57	3.63								
Low spiked human plasma	G5	1.05	4.30	2.85	2.05	1.72	1.37	1.09	0.03	2.72	4.46	2.96	2.13	1.79	1.42
	G6	1.095	4.49	2.98	2.14	1.80	1.43								
	G7	1.063	4.36	2.89	2.08	1.75	1.39								
	G8	1.129	4.63	3.07	2.21	1.85	1.47								
	G9	1.091	4.47	2.96	2.13	1.79	1.42								
	G10	1.1	4.51	2.99	2.15	1.81	1.44								
	G11	1.119	4.59	3.04	2.19	1.84	1.46								
	G12	1.077	4.41	2.93	2.10	1.77	1.41								
	G13	1.032	4.23	2.80	2.02	1.69	1.35								
	G14	1.119	4.59	3.04	2.19	1.84	1.46								
	G15	1.091	4.47	2.96	2.13	1.79	1.42								
	G16	1.093	4.48	2.97	2.13	1.79	1.43								
	G17	1.075	4.41	2.92	2.10	1.77	1.40								
	G18	1.117	4.58	3.04	2.18	1.83	1.46								
	G19	1.048	4.30	2.85	2.05	1.72	1.37								
	G20	1.126	4.61	3.06	2.20	1.85	1.47								
Medium spiked human plasma	F5	1.79	7.34	4.86	3.50	2.94	2.34	1.91	0.05	2.50	7.82	5.19	3.73	3.13	2.49
	F6	1.922	7.88	5.22	3.75	3.16	2.51								
	F7	1.862	7.63	5.06	3.64	3.06	2.43								
	F8	1.924	7.89	5.23	3.76	3.16	2.51								
	F9	1.912	7.84	5.20	3.73	3.14	2.50								
	F10	1.954	8.01	5.31	3.82	3.21	2.55								
	F11	1.891	7.75	5.14	3.69	3.11	2.47								
	F12	1.914	7.84	5.20	3.74	3.14	2.50								

	F13	1.891	7.75	5.14	3.69	3.11	2.47								
	F14	2.012	8.25	5.47	3.93	3.30	2.63								
	F15	1.865	7.64	5.07	3.64	3.06	2.43								
	F16	1.954	8.01	5.31	3.82	3.21	2.55								
	F17	1.909	7.82	5.19	3.73	3.13	2.49								
	F18	1.903	7.80	5.17	3.72	3.12	2.48								
	F19	1.917	7.86	5.21	3.74	3.15	2.50								
	F20	1.921	7.87	5.22	3.75	3.15	2.51								
Normal, unspiked human plasma	H5	0.034	0.14	0.09	0.07	0.06	0.04	0.04	0.00	9.02	0.16	0.10	0.07	0.06	0.05
	H6	0.031	0.13	0.08	0.06	0.05	0.04								
	H7	0.032	0.13	0.09	0.06	0.05	0.04								
	H8	0.039	0.16	0.11	0.08	0.06	0.05								
	H9	0.045	0.18	0.12	0.09	0.07	0.06								
	H10	0.037	0.15	0.10	0.07	0.06	0.05								
	H11	0.037	0.15	0.10	0.07	0.06	0.05								
	H12	0.04	0.16	0.11	0.08	0.07	0.05								
	H13	0.041	0.17	0.11	0.08	0.07	0.05								
	H14	0.04	0.16	0.11	0.08	0.07	0.05								
	H15	0.039	0.16	0.11	0.08	0.06	0.05								
	H16	0.039	0.16	0.11	0.08	0.06	0.05								
	H17	0.038	0.16	0.10	0.07	0.06	0.05								
	H18	0.039	0.16	0.11	0.08	0.06	0.05								
	H19	0.038	0.16	0.10	0.07	0.06	0.05								
	H20	0.04	0.16	0.11	0.08	0.07	0.05								

Serum Samples

			Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
Sample	**Wells**	**OD Value - Blank**	**10**	**15**	**20**	**25**	**30**	**Mean OD**	**SD**	**CV %**	**10**	**15**	**20**	**25**	**30**
High spiked human Serum	A5	2.704	11.08	7.35	5.28	4.44	3.53	2.75	0.05	1.70	11.28	7.48	5.37	4.52	3.59
	A6	2.772	11.36	7.53	5.41	4.55	3.62								
	A7	2.773	11.36	7.54	5.42	4.55	3.62								
	A8	2.692	11.03	7.32	5.26	4.42	3.51								
	A9	2.752	11.28	7.48	5.38	4.52	3.59								
	A10	2.777	11.38	7.55	5.42	4.56	3.63								
	A11	2.752	11.28	7.48	5.38	4.52	3.59								
	A12	2.699	11.06	7.33	5.27	4.43	3.52								
	A13	2.772	11.36	7.53	5.41	4.55	3.62								
	A14	2.695	11.05	7.32	5.26	4.43	3.52								
	A15	2.736	11.21	7.43	5.34	4.49	3.57								
	A16	2.788	11.43	7.58	5.45	4.58	3.64								
	A17	2.836	11.62	7.71	5.54	4.66	3.70								
	A18	2.776	11.38	7.54	5.42	4.56	3.62								
	A19	2.822	11.57	7.67	5.51	4.63	3.68								
	A20	2.683	11.00	7.29	5.24	4.41	3.50								
Low spiked human serum	C5	1.162	4.76	3.16	2.27	1.91	1.52	1.15	0.03	2.33	4.72	3.13	2.25	1.89	1.50
	C6	1.104	4.52	3.00	2.16	1.81	1.44								
	C7	1.12	4.59	3.04	2.19	1.84	1.46								
	C8	1.123	4.60	3.05	2.19	1.84	1.47								
	C9	1.161	4.76	3.15	2.27	1.91	1.52								
	C10	1.159	4.75	3.15	2.26	1.90	1.51								
	C11	1.15	4.71	3.13	2.25	1.89	1.50								
	C12	1.179	4.83	3.20	2.30	1.94	1.54								
	C13	1.173	4.81	3.19	2.29	1.93	1.53								
	C14	1.166	4.78	3.17	2.28	1.91	1.52								
	C15	1.19	4.88	3.23	2.32	1.95	1.55								
	C16	1.095	4.49	2.98	2.14	1.80	1.43								
	C17	1.163	4.77	3.16	2.27	1.91	1.52								
	C18	1.143	4.68	3.11	2.23	1.88	1.49								
	C19	1.159	4.75	3.15	2.26	1.90	1.51								
	C20	1.161	4.76	3.15	2.27	1.91	1.52								
Medium spiked human serum	B5	2.043	8.37	5.55	3.99	3.35	2.67	1.98	0.04	2.22	8.12	5.38	3.87	3.25	2.59
	B6	1.944	7.97	5.28	3.80	3.19	2.54								
	B7	1.982	8.12	5.39	3.87	3.25	2.59								
	B8	1.892	7.75	5.14	3.70	3.11	2.47								
	B9	1.986	8.14	5.40	3.88	3.26	2.59								
	B10	1.886	7.73	5.13	3.68	3.10	2.46								
	B11	2.032	8.33	5.52	3.97	3.34	2.65								
	B12	1.995	8.18	5.42	3.90	3.28	2.60								
	B13	2.02	8.28	5.49	3.95	3.32	2.64								

	B14	1.976	8.10	5.37	3.86	3.24	2.58								
	B15	2.022	8.29	5.49	3.95	3.32	2.64								
	B16	1.99	8.16	5.41	3.89	3.27	2.60								
	B17	1.998	8.19	5.43	3.90	3.28	2.61								
	B18	1.996	8.18	5.42	3.90	3.28	2.61								
	B19	1.978	8.11	5.38	3.86	3.25	2.58								
	B20	1.964	8.05	5.34	3.84	3.22	2.56								
Unspiked normal human serum	D5	0.083	0.34	0.23	0.16	0.14	0.11	0.08	0.00	3.58	0.34	0.23	0.16	0.14	0.11
	D6	0.086	0.35	0.23	0.17	0.14	0.11								
	D7	0.085	0.35	0.23	0.17	0.14	0.11								
	D8	0.079	0.32	0.21	0.15	0.13	0.10								
	D9	0.086	0.35	0.23	0.17	0.14	0.11								
	D10	0.087	0.36	0.24	0.17	0.14	0.11								
	D11	0.087	0.36	0.24	0.17	0.14	0.11								
	D12	0.081	0.33	0.22	0.16	0.13	0.11								
	D13	0.082	0.34	0.22	0.16	0.13	0.11								
	D14	0.081	0.33	0.22	0.16	0.13	0.11								
	D15	0.082	0.34	0.22	0.16	0.13	0.11								
	D16	0.09	0.37	0.24	0.18	0.15	0.12								
	D17	0.081	0.33	0.22	0.16	0.13	0.11								
	D18	0.082	0.34	0.22	0.16	0.13	0.11								
	D19	0.084	0.34	0.23	0.16	0.14	0.11								
	D20	0.081	0.33	0.22	0.16	0.13	0.11								

E) Combined, Run 1,2,3

Plasma Sample	OD Value – Blank (n = 48)			Mean Sample Index at given Cut-off level ng mL (n = 48)				
	Mean OD	SD	CV %	10	15	20	25	30
High spiked human plasma	2.73	0.07	2.65	10.98	7.20	5.30	4.32	3.55
Medium spiked human plasma	1.87	0.06	3.25	7.52	4.93	3.63	2.96	2.43
Low spiked human plasma	1.08	0.03	2.92	4.34	2.84	2.09	1.70	1.40
Un-spiked normal human plasma	0.05	0.01	23.27	0.21	0.14	0.10	0.08	0.07

Serum Sample	OD Value – Blank (n = 48)			Mean Sample Index at given Cut-off level ng mL (n = 48)				
	Mean OD	SD	CV %	10	15	20	25	30
High spiked human plasma	2.72	0.08	2.89	10.93	7.17	5.27	4.30	3.53
Medium spiked human plasma	1.92	0.08	4.35	7.71	5.05	3.72	3.03	2.49
Low spiked human plasma	1.12	0.05	4.29	4.51	2.96	2.18	1.78	1.46
Un-spiked normal human plasma	0.10	0.01	10.36	0.39	0.26	0.19	0.16	0.13

13.2.1.4 Robustness

Study Results Section:	10.1.4
Parameter	Robustness

Raw Data

A) End-point stability

A1) Time Zero, Cut-off Controls

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	G1	0.758	0.752	0.762	0.014	1.9
	G2	0.778	0.772			
25	H1	0.703	0.697	0.662	0.049	7.4
	H2	0.634	0.628			
20	A3	0.514	0.508	0.507	0.001	0.3
	A4	0.512	0.506			
15	B3	0.379	0.373	0.383	0.014	3.7
	B4	0.399	0.393			
10	C3	0.255	0.249	0.246	0.004	1.7
	C4	0.249	0.243			
Blank	D3	0.007	0	0		
	D4	0.005				

A2) Spiked and un-spiked plasma and serum samples at time zero

Plasma Samples

			Sample Index at given Cut-off level ng mL					OD Value - Blank			Sample Index at given Cut-off level ng mL				
Sample	Wells	OD Value - Blank	10	15	20	25	30	Mean OD	SD	CV %	10	15	20	25	30
High spiked human plasma	E5	2.661	10.82	6.95	5.25	4.02	3.49	2.65	0.05	2.05	10.76	6.91	5.22	4.00	3.47
	E6	2.645	10.75	6.91	5.22	4.00	3.47								
	E7	2.531	10.29	6.61	4.99	3.82	3.32								
	E8	2.654	10.79	6.93	5.23	4.01	3.48								
	E9	2.547	10.35	6.65	5.02	3.85	3.34								
	E10	2.728	11.09	7.12	5.38	4.12	3.58								
	E11	2.72	11.06	7.10	5.36	4.11	3.57								
	E12	2.642	10.74	6.90	5.21	3.99	3.47								
	E13	2.643	10.74	6.90	5.21	3.99	3.47								
	E14	2.645	10.75	6.91	5.22	4.00	3.47								
	E15	2.613	10.62	6.82	5.15	3.95	3.43								
	E16	2.698	10.97	7.04	5.32	4.08	3.54								
	E17	2.632	10.70	6.87	5.19	3.98	3.45								
	E18	2.685	10.91	7.01	5.30	4.06	3.52								
	E19	2.62	10.65	6.84	5.17	3.96	3.44								
	E20	2.695	10.96	7.04	5.32	4.07	3.54								
Low spiked human plasma	G5	1.066	4.33	2.78	2.10	1.61	1.40	1.07	0.03	2.55	4.33	2.78	2.10	1.61	1.40
	G6	1.078	4.38	2.81	2.13	1.63	1.41								
	G7	1.058	4.30	2.76	2.09	1.60	1.39								
	G8	1.099	4.47	2.87	2.17	1.66	1.44								
	G9	1.061	4.31	2.77	2.09	1.60	1.39								
	G10	1.091	4.43	2.85	2.15	1.65	1.43								
	G11	1.092	4.44	2.85	2.15	1.65	1.43								
	G12	1.072	4.36	2.80	2.11	1.62	1.41								
	G13	1.065	4.33	2.78	2.10	1.61	1.40								
	G14	1.041	4.23	2.72	2.05	1.57	1.37								
	G15	1.068	4.34	2.79	2.11	1.61	1.40								
	G16	1.078	4.38	2.81	2.13	1.63	1.41								
	G17	1.069	4.35	2.79	2.11	1.61	1.40								
	G18	1.085	4.41	2.83	2.14	1.64	1.42								
	G19	0.982	3.99	2.56	1.94	1.48	1.29								
	G20	1.051	4.27	2.74	2.07	1.59	1.38								
Medium spiked human plasma	F5	1.782	7.24	4.65	3.51	2.69	2.34	1.81	0.04	2.20	7.36	4.73	3.57	2.74	2.38
	F6	1.86	7.56	4.86	3.67	2.81	2.44								
	F7	1.749	7.11	4.57	3.45	2.64	2.30								
	F8	1.785	7.26	4.66	3.52	2.70	2.34								
	F9	1.761	7.16	4.60	3.47	2.66	2.31								
	F10	1.843	7.49	4.81	3.64	2.78	2.42								
	F11	1.771	7.20	4.62	3.49	2.68	2.32								

	F12	1.834	7.46	4.79	3.62	2.77	2.41								
	F13	1.859	7.56	4.85	3.67	2.81	2.44								
	F14	1.833	7.45	4.79	3.62	2.77	2.41								
	F15	1.856	7.54	4.85	3.66	2.80	2.44								
	F16	1.849	7.52	4.83	3.65	2.79	2.43								
	F17	1.802	7.33	4.70	3.55	2.72	2.36								
	F18	1.803	7.33	4.71	3.56	2.72	2.37								
	F19	1.754	7.13	4.58	3.46	2.65	2.30								
	F20	1.839	7.48	4.80	3.63	2.78	2.41								
Normal, unspiked human plasma	H5	0.068	0.28	0.18	0.13	0.10	0.09	0.07	0.003	4.00	0.27	0.17	0.13	0.10	0.09
	H6	0.067	0.27	0.17	0.13	0.10	0.09								
	H7	0.065	0.26	0.17	0.13	0.10	0.09								
	H8	0.068	0.28	0.18	0.13	0.10	0.09								
	H9	0.066	0.27	0.17	0.13	0.10	0.09								
	H10	0.068	0.28	0.18	0.13	0.10	0.09								
	H11	0.066	0.27	0.17	0.13	0.10	0.09								
	H12	0.073	0.30	0.19	0.14	0.11	0.10								
	H13	0.063	0.26	0.16	0.12	0.10	0.08								
	H14	0.065	0.26	0.17	0.13	0.10	0.09								
	H15	0.062	0.25	0.16	0.12	0.09	0.08								
	H16	0.069	0.28	0.18	0.14	0.10	0.09								
	H17	0.067	0.27	0.17	0.13	0.10	0.09								
	H18	0.069	0.28	0.18	0.14	0.10	0.09								
	H19	0.07	0.28	0.18	0.14	0.11	0.09								
	H20	0.066	0.27	0.17	0.13	0.10	0.09								

Serum Samples

			Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
Sample	**Wells**	**OD Value - Blank**	**10**	**15**	**20**	**25**	**30**	**Mean OD**	**SD**	**CV %**	**10**	**15**	**20**	**25**	**30**
High spiked human Serum	A5	2.59	10.53	6.76	5.11	3.91	3.40	2.63	0.05	1.97	10.68	6.86	5.18	3.97	3.45
	A6	2.675	10.87	6.98	5.28	4.04	3.51								
	A7	2.625	10.67	6.85	5.18	3.97	3.44								
	A8	2.667	10.84	6.96	5.26	4.03	3.50								
	A9	2.612	10.62	6.82	5.15	3.95	3.43								
	A10	2.58	10.49	6.74	5.09	3.90	3.39								
	A11	2.712	11.02	7.08	5.35	4.10	3.56								
	A12	2.613	10.62	6.82	5.15	3.95	3.43								
	A13	2.721	11.06	7.10	5.37	4.11	3.57								
	A14	2.637	10.72	6.89	5.20	3.98	3.46								
	A15	2.591	10.53	6.77	5.11	3.91	3.40								
	A16	2.695	10.96	7.04	5.32	4.07	3.54								
	A17	2.6	10.57	6.79	5.13	3.93	3.41								
	A18	2.588	10.52	6.76	5.10	3.91	3.40								
	A19	2.541	10.33	6.63	5.01	3.84	3.33								
	A20	2.6	10.57	6.79	5.13	3.93	3.41								
Low spiked human serum	C5	1.107	4.50	2.89	2.18	1.67	1.45	1.07	0.03	3.19	4.35	2.80	2.11	1.62	1.40
	C6	1.037	4.22	2.71	2.05	1.57	1.36								
	C7	1.077	4.38	2.81	2.12	1.63	1.41								
	C8	1.088	4.42	2.84	2.15	1.64	1.43								
	C9	1.125	4.57	2.94	2.22	1.70	1.48								
	C10	1.075	4.37	2.81	2.12	1.62	1.41								
	C11	1.084	4.41	2.83	2.14	1.64	1.42								
	C12	1.041	4.23	2.72	2.05	1.57	1.37								
	C13	1.043	4.24	2.72	2.06	1.58	1.37								
	C14	1.035	4.21	2.70	2.04	1.56	1.36								
	C15	1.112	4.52	2.90	2.19	1.68	1.46								
	C16	1.078	4.38	2.81	2.13	1.63	1.41								
	C17	1.08	4.39	2.82	2.13	1.63	1.42								
	C18	1.008	4.10	2.63	1.99	1.52	1.32								
	C19	1.032	4.20	2.69	2.04	1.56	1.35								
	C20	1.107	4.50	2.89	2.18	1.67	1.45								
Medium spiked human serum	B5	1.727	7.02	4.51	3.41	2.61	2.27	1.83	0.07	3.64	7.43	4.77	3.60	2.76	2.40
	B6	1.933	7.86	5.05	3.81	2.92	2.54								
	B7	1.776	7.22	4.64	3.50	2.68	2.33								
	B8	1.845	7.50	4.82	3.64	2.79	2.42								
	B9	1.817	7.39	4.74	3.58	2.74	2.38								
	B10	1.79	7.28	4.67	3.53	2.70	2.35								
	B11	1.864	7.58	4.87	3.68	2.82	2.45								
	B12	1.763	7.17	4.60	3.48	2.66	2.31								
	B13	1.868	7.59	4.88	3.68	2.82	2.45								

	B14	1.795	7.30	4.69	3.54	2.71	2.36								
	B15	1.857	7.55	4.85	3.66	2.81	2.44								
	B16	1.842	7.49	4.81	3.63	2.78	2.42								
	B17	1.807	7.35	4.72	3.56	2.73	2.37								
	B18	1.843	7.49	4.81	3.64	2.78	2.42								
	B19	1.977	8.04	5.16	3.90	2.99	2.59								
	B20	1.736	7.06	4.53	3.42	2.62	2.28								
Unspiked normal human serum	D5	0.102	0.41	0.27	0.20	0.15	0.13	0.11	0.003	3.06	0.43	0.28	0.21	0.16	0.14
	D6	0.105	0.43	0.27	0.21	0.16	0.14								
	D7	0.106	0.43	0.28	0.21	0.16	0.14								
	D8	0.107	0.43	0.28	0.21	0.16	0.14								
	D9	0.105	0.43	0.27	0.21	0.16	0.14								
	D10	0.099	0.40	0.26	0.20	0.15	0.13								
	D11	0.109	0.44	0.28	0.21	0.16	0.14								
	D12	0.109	0.44	0.28	0.21	0.16	0.14								
	D13	0.111	0.45	0.29	0.22	0.17	0.15								
	D14	0.102	0.41	0.27	0.20	0.15	0.13								
	D15	0.108	0.44	0.28	0.21	0.16	0.14								
	D16	0.109	0.44	0.28	0.21	0.16	0.14								
	D17	0.109	0.44	0.28	0.21	0.16	0.14								
	D18	0.104	0.42	0.27	0.21	0.16	0.14								
	D19	0.105	0.43	0.27	0.21	0.16	0.14								
	D20	0.104	0.42	0.27	0.21	0.16	0.14								

A3) Cut-off controls, Time ≥ 30 minutes after assay stopped / first reading

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	G1	0.69	0.684	0.69	0.008	1.1
	G2	0.701	0.695			
25	H1	0.631	0.625	0.593	0.045	7.5
	H2	0.588	0.562			
20	A3	0.447	0.441	0.444	0.004	1
	A4	0.453	0.447			
15	B3	0.329	0.323	0.328	0.007	2.2
	B4	0.339	0.333			
10	C3	0.216	0.21	0.207	0.004	1.7
	C4	0.211	0.205			
Blank	D3	0.007		0		
	D4	0.005				

A4) Spiked and un-spiked plasma and serum samples Time ≥ 30 minutes after assay stopped / first reading

Plasma Samples

			Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
Sample	**Wells**	**OD Value - Blank**	**10**	**15**	**20**	**25**	**30**	**Mean OD**	**SD**	**CV %**	**10**	**15**	**20**	**25**	**30**
High spiked human plasma	E5	2.44	11.79	7.44	5.50	4.11	3.54	2.33	0.19	7.98	11.24	7.09	5.24	3.92	3.37
	E6	2.453	11.85	7.48	5.52	4.14	3.56								
	E7	2.075	10.02	6.33	4.67	3.50	3.01								
	E8	2.483	12.00	7.57	5.59	4.19	3.60								
	E9	2.17	10.48	6.62	4.89	3.66	3.14								
	E10	2.526	12.20	7.70	5.69	4.26	3.66								
	E11	2.345	11.33	7.15	5.28	3.95	3.40								
	E12	2.388	11.54	7.28	5.38	4.03	3.46								
	E13	2.23	10.77	6.80	5.02	3.76	3.23								
	E14	2.39	11.55	7.29	5.38	4.03	3.46								
	E15	2.253	10.88	6.87	5.07	3.80	3.27								
	E16	2.559	12.36	7.80	5.76	4.32	3.71								
	E17	2.31	11.16	7.04	5.20	3.90	3.35								
	E18	2.509	12.12	7.65	5.65	4.23	3.64								
	E19	1.862	9.00	5.68	4.19	3.14	2.70								
	E20	2.226	10.75	6.79	5.01	3.75	3.23								
Low spiked human plasma	G5	0.977	4.72	2.98	2.20	1.65	1.42	0.98	0.03	2.82	4.72	2.98	2.20	1.65	1.42
	G6	0.997	4.82	3.04	2.25	1.68	1.44								
	G7	0.968	4.68	2.95	2.18	1.63	1.40								
	G8	1.014	4.90	3.09	2.28	1.71	1.47								
	G9	0.968	4.68	2.95	2.18	1.63	1.40								
	G10	1.005	4.86	3.06	2.26	1.69	1.46								
	G11	0.994	4.80	3.03	2.24	1.68	1.44								
	G12	0.985	4.76	3.00	2.22	1.66	1.43								
	G13	0.975	4.71	2.97	2.20	1.64	1.41								
	G14	0.953	4.60	2.91	2.15	1.61	1.38								
	G15	0.981	4.74	2.99	2.21	1.65	1.42								
	G16	0.995	4.81	3.03	2.24	1.68	1.44								
	G17	0.972	4.70	2.96	2.19	1.64	1.41								
	G18	0.999	4.83	3.05	2.25	1.68	1.45								
	G19	0.895	4.32	2.73	2.02	1.51	1.30								
	G20	0.964	4.66	2.94	2.17	1.63	1.40								
Medium spiked human plasma	F5	1.652	7.98	5.04	3.72	2.79	2.39	1.68	0.04	2.21	8.14	5.13	3.79	2.84	2.44
	F6	1.728	8.35	5.27	3.89	2.91	2.50								
	F7	1.632	7.88	4.98	3.68	2.75	2.37								
	F8	1.668	8.06	5.09	3.76	2.81	2.42								
	F9	1.637	7.91	4.99	3.69	2.76	2.37								
	F10	1.719	8.30	5.24	3.87	2.90	2.49								
	F11	1.629	7.87	4.97	3.67	2.75	2.36								

	F12	1.724	8.33	5.26	3.88	2.91	2.50								
	F13	1.72	8.31	5.24	3.87	2.90	2.49								
	F14	1.723	8.32	5.25	3.88	2.91	2.50								
	F15	1.704	8.23	5.20	3.84	2.87	2.47								
	F16	1.715	8.29	5.23	3.86	2.89	2.49								
	F17	1.681	8.12	5.13	3.79	2.83	2.44								
	F18	1.675	8.09	5.11	3.77	2.82	2.43								
	F19	1.638	7.91	4.99	3.69	2.76	2.37								
	F20	1.703	8.23	5.19	3.84	2.87	2.47								
Normal, unspiked human plasma	H5	0.052	0.25	0.16	0.12	0.09	0.08	0.05	0.00	4.02	0.25	0.16	0.12	0.09	0.08
	H6	0.052	0.25	0.16	0.12	0.09	0.08								
	H7	0.05	0.24	0.15	0.11	0.08	0.07								
	H8	0.052	0.25	0.16	0.12	0.09	0.08								
	H9	0.051	0.25	0.16	0.11	0.09	0.07								
	H10	0.052	0.25	0.16	0.12	0.09	0.08								
	H11	0.052	0.25	0.16	0.12	0.09	0.08								
	H12	0.057	0.28	0.17	0.13	0.10	0.08								
	H13	0.049	0.24	0.15	0.11	0.08	0.07								
	H14	0.05	0.24	0.15	0.11	0.08	0.07								
	H15	0.048	0.23	0.15	0.11	0.08	0.07								
	H16	0.053	0.26	0.16	0.12	0.09	0.08								
	H17	0.052	0.25	0.16	0.12	0.09	0.08								
	H18	0.053	0.26	0.16	0.12	0.09	0.08								
	H19	0.054	0.26	0.16	0.12	0.09	0.08								
	H20	0.051	0.25	0.16	0.11	0.09	0.07								

Serum Samples

			Sample Index at given Cut-off level ng mL					OD Value - Blank			Mean Sample Index at given Cut-off level ng mL				
Sample	**Wells**	**OD Value - Blank**	**10**	**15**	**20**	**25**	**30**	**Mean OD**	**SD**	**CV %**	**10**	**15**	**20**	**25**	**30**
High spiked human Serum	A5	2.35	11.35	7.16	5.29	3.96	3.41	2.15	0.16	7.28	10.36	6.54	4.83	3.62	3.11
	A6	2.174	10.50	6.63	4.90	3.67	3.15								
	A7	2.298	11.10	7.01	5.18	3.88	3.33								
	A8	2.407	11.63	7.34	5.42	4.06	3.49								
	A9	2.101	10.15	6.41	4.73	3.54	3.04								
	A10	2.256	10.90	6.88	5.08	3.80	3.27								
	A11	2.145	10.36	6.54	4.83	3.62	3.11								
	A12	2.235	10.80	6.81	5.03	3.77	3.24								
	A13	2.017	9.74	6.15	4.54	3.40	2.92								
	A14	2.01	9.71	6.13	4.53	3.39	2.91								
	A15	2.291	11.07	6.98	5.16	3.86	3.32								
	A16	2.076	10.03	6.33	4.68	3.50	3.01								
	A17	2.183	10.55	6.66	4.92	3.68	3.16								
	A18	1.938	9.36	5.91	4.36	3.27	2.81								
	A19	1.956	9.45	5.96	4.41	3.30	2.83								
	A20	1.885	9.11	5.75	4.25	3.18	2.73								
Low spiked human serum	C5	1.007	4.86	3.07	2.27	1.70	1.46	0.98	0.03	3.14	4.75	3.00	2.21	1.66	1.42
	C6	0.949	4.58	2.89	2.14	1.60	1.38								
	C7	0.993	4.80	3.03	2.24	1.67	1.44								
	C8	1	4.83	3.05	2.25	1.69	1.45								
	C9	1.038	5.01	3.16	2.34	1.75	1.50								
	C10	0.991	4.79	3.02	2.23	1.67	1.44								
	C11	1	4.83	3.05	2.25	1.69	1.45								
	C12	0.956	4.62	2.91	2.15	1.61	1.39								
	C13	0.968	4.68	2.95	2.18	1.63	1.40								
	C14	0.957	4.62	2.92	2.16	1.61	1.39								
	C15	1.022	4.94	3.12	2.30	1.72	1.48								
	C16	0.994	4.80	3.03	2.24	1.68	1.44								
	C17	0.983	4.75	3.00	2.21	1.66	1.42								
	C18	0.924	4.46	2.82	2.08	1.56	1.34								
	C19	0.943	4.56	2.88	2.12	1.59	1.37								
	C20	1.005	4.86	3.06	2.26	1.69	1.46								
Medium spiked human serum	B5	1.619	7.82	4.94	3.65	2.73	2.35	1.68	0.05	3.11	8.14	5.14	3.79	2.84	2.44
	B6	1.672	8.08	5.10	3.77	2.82	2.42								
	B7	1.621	7.83	4.94	3.65	2.73	2.35								
	B8	1.71	8.26	5.21	3.85	2.88	2.48								
	B9	1.685	8.14	5.14	3.80	2.84	2.44								
	B10	1.658	8.01	5.05	3.73	2.80	2.40								
	B11	1.733	8.37	5.28	3.90	2.92	2.51								
	B12	1.642	7.93	5.01	3.70	2.77	2.38								

	B13	1.737	8.39	5.30	3.91	2.93	2.52									
	B14	1.679	8.11	5.12	3.78	2.83	2.43									
	B15	1.729	8.35	5.27	3.89	2.92	2.51									
	B16	1.7	8.21	5.18	3.83	2.87	2.46									
	B17	1.673	8.08	5.10	3.77	2.82	2.42									
	B18	1.701	8.22	5.19	3.83	2.87	2.47									
	B19	1.8	8.70	5.49	4.05	3.04	2.61									
	B20	1.593	7.70	4.86	3.59	2.69	2.31									
Unspiked normal human serum	D5	0.081	0.39	0.25	0.18	0.14	0.12	0.08	0.00	3.23	0.40	0.25	0.19	0.14	0.12	
	D6	0.082	0.40	0.25	0.18	0.14	0.12									
	D7	0.084	0.41	0.26	0.19	0.14	0.12									
	D8	0.084	0.41	0.26	0.19	0.14	0.12									
	D9	0.084	0.41	0.26	0.19	0.14	0.12									
	D10	0.079	0.38	0.24	0.18	0.13	0.11									
	D11	0.087	0.42	0.27	0.20	0.15	0.13									
	D12	0.085	0.41	0.26	0.19	0.14	0.12									
	D13	0.087	0.42	0.27	0.20	0.15	0.13									
	D14	0.079	0.38	0.24	0.18	0.13	0.11									
	D15	0.084	0.41	0.26	0.19	0.14	0.12									
	D16	0.086	0.42	0.26	0.19	0.15	0.12									
	D17	0.086	0.42	0.26	0.19	0.15	0.12									
	D18	0.08	0.39	0.24	0.18	0.13	0.12									
	D19	0.082	0.40	0.25	0.18	0.14	0.12									
	D20	0.081	0.39	0.25	0.18	0.14	0.12									

A5) Difference in OD signal

Plasma Sample	Time Zero OD Value – Blank (n = 16)		
	Time Zero Mean OD	Time 30 Mean OD	Difference %
High spiked human plasma	2.65	2.33	12.13
Medium spiked human plasma	1.81	1.68	7.01
Low spiked human plasma	1.066	0.978	8.29
Un-spiked normal human plasma	0.07	0.105	22.76

Serum Sample	Time Zero OD Value – Blank (n = 16)		
	Time Zero Mean OD	Time 30 Mean OD	Difference %
High spiked human serum	2.63	2.15	18.37
Medium spiked human serum	1.828	1.685	7.82
Low spiked human serum	1.07	0.983	8.17
Un-spiked normal human serum	0.11	0.08	21.43

B) Temperature

B1) Cut-off controls @ 15oC

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	M1	0.358	0.353	0.377	0.034	9
	M2	0.406	0.401			
25	O1	0.297	0.292	0.309	0.025	8
	O2	0.332	0.327			
20	A3	0.245	0.24	0.242	0.004	1.5
	A4	0.25	0.245			
15	C3	0.19	0.185	0.186	0.002	2.1
	C4	0.193	0.188			
10	E3	0.132	0.127	0.126	0.001	1.1
	E4	0.13	0.125			
Blank	G3	0.006		0		
	G4	0.005				

B2) Plasma and serum samples @ 15oC

Plasma Sample	Well	OD - Blank	Mean OD - Blank	Sample Index Ratio at:				
				10ng/mL	15ng/mL	20ng/mL	25ng/mL	30ng/mL
High spiked human serum	A5	1.557	1.638	12.980	8.793	6.758	5.29	4.338
	A6	1.714						
Medium spiked human serum	B5	1.036	1.035	8.214	5.565	4.277	3.35	2.745
	B6	1.034						
Low spiked human serum	C5	0.584	0.58	4.603	3.118	2.397	1.88	1.538
	C6	0.576						
Un-spiked normal human serum	D5	0.077	0.078	0.615	0.417	0.320	0.25	0.206
	D6	0.078						

Plasma Sample	Well	OD - Blank	Mean OD - Blank	Sample Index Ratio at:				
				10ng/mL	15ng/mL	20ng/mL	25ng/mL	30ng/mL
High spiked human plasma	A7	1.634	1.588	12.603	8.538	6.562	5.14	4.212
	A8	1.542						
Medium spiked human plasma	B7	0.994	0.972	7.710	5.223	4.014	3.14	2.577
	B8	0.949						
Low spiked human plasma	C7	0.556	0.553	4.385	2.970	2.283	1.79	1.466
	C8	0.549						
Un-spiked normal human plasma	D7	0.05	0.05	0.393	0.266	0.205	0.16	0.131
	D8	0.049						

B3) Cut-off controls @ 25oC

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
30	M1	0.737	0.731	0.771	0.057	7.3
	M2	0.817	0.811			
25	O1	0.628	0.622	0.629	0.01	1.6
	O2	0.642	0.636			
20	A3	0.529	0.523	0.512	0.016	3.2
	A4	0.506	0.5			
15	C3	0.392	0.386	0.381	0.008	2
	C4	0.381	0.375			
10	E3	0.26	0.254	0.253	0.002	0.8
	E4	0.257	0.251			
Blank	G3	0.006		0		
	G4	0.006				

B4) Plasma and serum samples @ 25oC

Plasma Sample	Well	OD - Blank	Mean OD - Blank	Sample Index Ratio at:				
				10ng/mL	15ng/mL	20ng/mL	25ng/mL	30ng/mL
High spiked human serum	A5	2.712	2.741	10.834	7.194	5.354	4.36	3.555
	A6	2.77						
Medium spiked human serum	B5	1.992	1.9865	7.852	5.214	3.880	3.16	2.577
	B6	1.981						
Low spiked human serum	C5	1.215	1.184	4.680	3.108	2.313	1.88	1.536
	C6	1.153						
Un-spiked normal human serum	D5	0.098	0.099	0.391	0.260	0.193	0.16	0.128
	D6	0.1						

Plasma Sample	Well	OD - Blank	Mean OD - Blank	Sample Index Ratio at:				
				10ng/mL	15ng/mL	20ng/mL	25ng/mL	30ng/mL
High spiked human plasma	A7	2.734	2.7535	10.883	7.227	5.378	4.38	3.571
	A8	2.773						
Medium spiked human plasma	B7	1.955	1.8975	7.500	4.980	3.706	3.02	2.461
	B8	1.84						
Low spiked human plasma	C7	1.095	1.092	4.316	2.866	2.133	1.74	1.416
	C8	1.089						
Un-spiked normal human plasma	D7	0.055	0.0515	0.204	0.135	0.101	0.08	0.067
	D8	0.048						

13.2.1.5 Hook Effect

Study Results Section:	10.1.5
Parameter	Hook Effect

Raw Data

Cut-off controls

Anti-SARS-CoV-2 N protein IgG Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value - Blank	Mean OD Value - Blank
200	B1	2.436	2.431	2.724
	B2	3.022	3.017	
150	C1	2.818	2.813	2.785
	C2	2.761	2.756	
100	D1	2.277	2.272	2.283
	D2	2.298	2.293	
80	E1	1.947	1.942	1.947
	E2	1.957	1.952	
40	F1	1.097	1.092	1.102
	F2	1.116	1.111	
30	G1	0.79	0.79	0.809
	G2	0.838	0.838	
25	H1	0.659	0.659	0.661
	H2	0.674	0.674	
20	A3	0.562	0.557	0.554
	A4	0.555	0.55	
15	B3	0.423	0.418	0.409
	B4	0.405	0.4	
10	C3	0.29	0.285	0.28
	C4	0.28	0.275	
Blank	G3	0.005	0	0
	G4	0.005		

Sample	Wells	Dilution Factor	Conc.ng mL	OD - Blank	Ave OD	Sample Index Ratio at:				
						10ng/mL	15ng/mL	20ng/mL	25ng/mL	30ng/mL
Spiked Human Plasma	B7	50	1280	2.397	2.116	7.56	5.17	3.82	3.20	2.62
	B8			1.835						
	C7	100	640	3.274	3.1725	11.33	7.76	5.73	4.80	3.92
	C8			3.071						
	D7	200	320	3.199	3.189	11.39	7.80	5.76	4.82	3.94
	D8			3.179						
	E7	400	160	2.94	2.964	10.59	7.25	5.35	4.48	3.66
	E8			2.988						
	F7	800	80	2.04	2.05	7.32	5.01	3.70	3.10	2.53
	F8			2.06						
	G7	1600	40	1.107	1.086	3.88	2.66	1.96	1.64	1.34
	G8			1.065						
	H7	3200	20	0.523	0.544	1.94	1.33	0.98	0.82	0.67
	H8			0.565						
Spiked Human Serum	B5	50	1280	2.792	2.9465	10.52	7.20	5.32	4.46	3.64
	B6			3.101						
	C5	100	640	3.18	3.1925	11.40	7.81	5.76	4.83	3.95
	C6			3.205						
	D5	200	320	2.721	3.1725	11.33	7.76	5.73	4.80	3.92
	D6			3.221						
	E5	400	160	2.977	2.9685	10.60	7.26	5.36	4.49	3.67
	E6			2.96						
	F5	800	80	1.989	2.0615	7.36	5.04	3.72	3.12	2.55
	F6			2.134						
	G5	1600	40	1.083	1.121	4.00	2.74	2.02	1.70	1.39
	G6			1.159						
	H5	3200	20	0.568	0.5495	1.96	1.34	0.99	0.83	0.68
	H6			0.531						

13.2.2 Study 2

Study Results section:	10.2
Parameter	Study 2: Seasonal coronavirus samples

Raw Data

Summary

NIBSC Anti-SARS-CoV-2 Verification Panel for Serology NIBSC 20/B770 (n = 37)						
Preliminary Cut-off level (ng mL Anti-SARS-CoV-2 N Protein IgG)	Positive samples (n = 23)		Negative Samples (n = 14)		Sensitivity %	Specificity %
	Sample index ratio > 1 (True Positive)	Sample index ratio ≤ 1 (False Negative)	Sample index ratio ≤ 1 (True Negative)	Sample index ratio > 1 (False Positive)		
10	23	0	14	0	100	100
15	22	1	14	0	95.65	100
20	22	1	14	0	95.65	100
25	22	1	14	0	95.65	100
30	22	1	14	0	95.65	100

Cut-off controls

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
10	A2	0.14	0.135	0.147	0.016	11.1
	B2	0.163	0.158			
15	C2	0.243	0.238	0.223	0.02	9.1
	D2	0.214	0.209			
20	E2	0.329	0.324	0.301	0.032	10.6
	F2	0.284	0.278			
25	G2	0.327	0.322	0.353	0.045	12.7
	H2	0.39	0.385			
30	I2	0.471	0.466	0.491	0.035	7.1
	J2	0.521	0.516			
Blank	O2	0.009	0.057	0		
	P2	0.009	0.058			

Seasonal Coronavirus samples

Preliminary Cut-off level (ng mL Anti-SARS-CoV-2 N Protein IgG)	Samples (n = 17)	
	Sample index ratio > 1 (Positive)	Sample index ratio < 1 (Negative)
10	2	15
15	0	17
20	0	17
25	0	17
30	0	17

Sample	Wells	OD - Blank	Cut-off level ng mL				
			10	15	20	25	30
SCOV0001	A6	0.031	0.21	0.14	0.10	0.09	0.06
SCOV0002	B6	0.07	0.48	0.31	0.23	0.20	0.14
SCOV0003	C6	0.212	1.44	0.95	0.70	0.60	0.43
SCOV0004	D6	0.038	0.26	0.17	0.13	0.11	0.08
SCOV0005	E6	0.035	0.24	0.16	0.12	0.10	0.07
SCOV0006	F6	0.005	0.03	0.02	0.02	0.01	0.01
SCOV0007	G6	0.051	0.35	0.23	0.17	0.14	0.10
SCOV0008	H6	0.088	0.60	0.39	0.29	0.25	0.18
SCOV0009	I6	0.021	0.14	0.09	0.07	0.06	0.04
SCOV0010	J6	0.107	0.73	0.48	0.36	0.30	0.22
SCOV0011	K6	0.075	0.51	0.34	0.25	0.21	0.15
SCOV0012	L6	0.181	1.23	0.81	0.60	0.51	0.37
SCOV0013	M6	0.092	0.63	0.41	0.31	0.26	0.19
SCOV0014	N6	0.059	0.40	0.26	0.20	0.17	0.12
SCOV0015	O6	0.011	0.07	0.05	0.04	0.03	0.02
SCOV0016	P6	0.085	0.58	0.38	0.28	0.24	0.17
SCOV0017	A7	0.053	0.36	0.24	0.18	0.15	0.11

13.2.3 Study 3

13.2.3.1 Positive and Negative samples

Study Results Section:	10.3
Parameter	Study 3 – Positive and Negative samples

Raw Data

A) Positive samples

Summary

- *With 8 false negatives removed (independently found to be S antigen and N antigen negative by assay)*

Cut-off level ng mL									
10		**15**		**20**		**25**		**30**	
Total (n = 399)									
Positive (n = 192)									
True positive	189	True positive	188	True positive	187	True positive	185	True positive	181
False negative	3	False negative	4	False negative	5	False negative	7	False negative	11
Negative (n = 207)									
False positive	80	False positive	44	False positive	31	False positive	22	False positive	15
True negative	127	True negative	163	True negative	176	True negative	185	True negative	192
Results									
Sensitivity	98.44%	Sensitivity	97.92%	Sensitivity	97.40%	Sensitivity	96.35%	Sensitivity	**94.27%**
Specificity	61%	Specificity	78.74%	Specificity	85%	Specificity	89%	Specificity	**92.75%**

- *All samples (without 8 false negatives removed above)*

Cut-off level ng mL									
10		**15**		**20**		**25**		**30**	
Total n = 407									
Positive (n = 200)									
True positive	191	True positive	189	True positive	187	True positive	185	True positive	181
False negative	9	False negative	11	False negative	13	False negative	15	False negative	19
Negative (n = 207)									
False positive	72	False positive	43	False positive	28	False positive	22	False positive	15
True negative	127	True negative	163	True negative	176	True negative	185	True negative	192
Results									
Sensitivity	95.50%	Sensitivity	94.50%	Sensitivity	93.50%	Sensitivity	92.50%	Sensitivity	**90.50%**
Specificity	64%	Specificity	79.13%	Specificity	86%	Specificity	89%	Specificity	**92.75%**

(Note also, 1 negative sample, pipetting error removed in above)

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
10	A2	0.21	0.214	0.219	0.008	3.7
	B2	0.222	0.225			
15	C2	0.305	0.308	0.309	0.001	0.3
	D2	0.306	0.309			
20	E2	0.396	0.399	0.416	0.024	5.7
	F2	0.43	0.433			
25	G2	0.491	0.494	0.501	0.01	2.1
	H2	0.505	0.509			
30	I2	0.598	0.601	0.626	0.035	5.6
	J2	0.647	0.651			
Blank	M2	0.073	0	0		
	M24	0.058				
	N2	0.05				
	N24	0.056				
	O1	0.048				
	O2	0.048				
	O23	0.05				
	O24	0.056				
	P1	0.05				
	P2	0.051				
	P23	0.055				
	P24	0.056				

Sample	Wells	OD - Blank	Cut-off level				
			10 ng mL	15 ng mL	20 ng mL	25 ng mL	30 ng mL
GI-5063-49-Serum-Conv	A3	2.777	12.68	8.99	6.68	5.54	4.44
GI-5144-56-Serum-Conv	B3	2.754	12.58	8.91	6.62	5.5	4.4
GI-5119-53-Serum-Conv	C3	0.697	3.18	2.26	1.68	1.39	1.11
GI-5220-28-Serum-Conv	D3	2.367	10.81	7.66	5.69	4.72	3.78
GI-5109-55-Serum-Conv	E3	2.691	12.29	8.71	6.47	5.37	4.3
GI-5221-28-Serum-Conv	F3	2.755	12.58	8.92	6.62	5.5	4.4
GI-5192-28-Serum-Conv	G3	1.022	4.67	3.31	2.46	2.04	1.63
GI-5232-28-Serum-Conv	H3	2.624	11.98	8.49	6.31	5.24	4.19
GI-5193-29-Serum-Conv	I3	2.588	11.82	8.38	6.22	5.17	4.13
GI-5271-30-Serum-Conv	J3	2.727	12.45	8.83	6.56	5.44	4.36
GI-5194-30-Serum-Conv	K3	2.614	11.94	8.46	6.28	5.22	4.18
GI-5272-34-Serum-Conv	L3	1.641	7.49	5.31	3.94	3.28	2.62
GI-5126-56-Serum-Conv	M3	1.031	4.71	3.34	2.48	2.06	1.65
GI-5169-28-Serum-Conv	N3	2.203	10.06	7.13	5.3	4.4	3.52
GI-5243-56-Serum-Conv	O3	2.559	11.68	8.28	6.15	5.11	4.09
GI-5072-53-Serum-Conv	P3	0.865	3.95	2.8	2.08	1.73	1.38
GI-5274-33-Serum-Conv	A4	2.399	10.95	7.76	5.77	4.79	3.83
GI-5131—Serum-Conv	B4	0.866	3.95	2.8	2.08	1.73	1.38
GI-5291-56-Serum-Conv	C4	1.757	8.02	5.69	4.22	3.51	2.81
GI-5406-44-Serum-Conv	D4	2.144	9.79	6.94	5.15	4.28	3.42
GI-5157-56-Serum-Conv	E4	1.417	6.47	4.59	3.41	2.83	2.26
GI-5146-56-Serum-Conv	F4	0.743	3.39	2.4	1.79	1.48	1.19
GI-5289-52-Serum-Conv	G4	2.79	12.74	9.03	6.71	5.57	4.46
GI-5097-56-Serum-Conv	H4	2.707	12.36	8.76	6.51	5.4	4.32
GI-5130-55-Serum-Conv	I4	2.739	12.51	8.86	6.58	5.47	4.38
GI-5118-56-Serum-Conv	J4	0.214	0.98	0.69	0.51	0.43	0.34
GI-5228-53-Serum-Conv	K4	2.682	12.25	8.68	6.45	5.35	4.28
GI-5061-56-Serum-Conv	L4	2.697	12.32	8.73	6.48	5.38	4.31
GI-5170-56-Serum-Conv	M4	2.708	12.37	8.76	6.51	5.41	4.33
GI-5111-56-Serum-Conv	N4	2.74	12.51	8.87	6.59	5.47	4.38
GI-5292-49-Serum-Conv	O4	2.714	12.39	8.78	6.52	5.42	4.34
GI-5413-31-Serum-Conv	P4	0.183	0.84	0.59	0.44	0.37	0.29
GI-5076-56-Serum-Conv	A5	1.12	5.11	3.62	2.69	2.24	1.79
GI-5166-56-Serum-Conv	B5	2.751	12.56	8.9	6.61	5.49	4.39
GI-5201-28-Serum-Conv	C5	1.087	4.96	3.52	2.61	2.17	1.74

GI-5060-56-Serum-Conv	D5	2.339	10.68	7.57	5.62	4.67	3.74
GI-4225-56-Serum-Conv	E5	2.107	9.62	6.82	5.06	4.21	3.37
GI-5296-50-Serum-Conv	F5	2.733	12.48	8.84	6.57	5.46	4.37
GI-5082-56-Serum-Conv	G5	2.728	12.46	8.83	6.56	5.45	4.36
GI-5419-33-Serum-Conv	H5	2.005	9.16	6.49	4.82	4	3.2
GI-5065-54-Serum-Conv	I5	0.651	2.97	2.11	1.56	1.3	1.04
GI-5143-56-Serum-Conv	J5	2.7	12.33	8.74	6.49	5.39	4.31
GI-5415-29-Serum-Conv	K5	0.366	1.67	1.18	0.88	0.73	0.58
GI-5114-56-Serum-Conv	L5	0.794	3.63	2.57	1.91	1.58	1.27
GI-5416-57-Serum-Conv	M5	0.167	0.76	0.54	0.4	0.33	0.27
GI-5263-29-Serum-Conv	N5	0.122	0.56	0.39	0.29	0.24	0.19
GI-5417-28-Serum-Conv	O5	2.348	10.72	7.6	5.64	4.69	3.75
GI-5122-56-Serum-Conv	P5	0.115	0.53	0.37	0.28	0.23	0.18
GI-5071-56-Serum-Conv	A6	2.331	10.64	7.54	5.6	4.65	3.72
GI-5167-51-Serum-Conv	B6	2.279	10.41	7.38	5.48	4.55	3.64
GI-5120-56-Serum-Conv	C6	1.059	4.84	3.43	2.55	2.11	1.69
GI-5427-33-Serum-Conv	D6	1.207	5.51	3.91	2.9	2.41	1.93
GI-5104-56-Serum-Conv	E6	2.76	12.6	8.93	6.63	5.51	4.41
GI-5121-56-Serum-Conv	F6	1.883	8.6	6.09	4.53	3.76	3.01
GI-5077-56-Serum-Conv	G6	2.456	11.21	7.95	5.9	4.9	3.92
GI-5178-56-Serum-Conv	H6	2.772	12.66	8.97	6.66	5.53	4.43
GI-5068-56-Serum-Conv	I6	2.749	12.55	8.9	6.61	5.49	4.39
GI-5168-56-Serum-Conv	J6	2.759	12.6	8.93	6.63	5.51	4.41
GI-5067-56-Serum-Conv	K6	2.647	12.09	8.57	6.36	5.28	4.23
GI-5161-28-Serum-Conv	L6	1.449	6.62	4.69	3.48	2.89	2.31
GI-5227-56-Serum-Conv	M6	2.726	12.45	8.82	6.55	5.44	4.35
GI-5162-56-Serum-Conv	N6	2.77	12.65	8.96	6.66	5.53	4.42
GI-5164-56-Serum-Conv	O6	2.681	12.24	8.68	6.44	5.35	4.28
GI-5075-56-Serum-Conv	P6	2.229	10.18	7.21	5.36	4.45	3.56
GI-5174-56-Serum-Conv	A7	1.208	5.52	3.91	2.9	2.41	1.93
GI-5281-52-Serum-Conv	B7	0.869	3.97	2.81	2.09	1.73	1.39
GI-5234-28-Serum-Conv	C7	1.948	8.89	6.3	4.68	3.89	3.11
GI-5280-32-Serum-Conv	D7	2.589	11.82	8.38	6.22	5.17	4.14
GI-5405-28-Serum-Conv	E7	0.495	2.26	1.6	1.19	0.99	0.79
GI-5222-32-Serum-Conv	F7	2.749	12.55	8.9	6.61	5.49	4.39
GI-5095-56-Serum-Conv	G7	2.255	10.3	7.3	5.42	4.5	3.6
GI-5141-54-Serum-Conv	H7	1.832	8.37	5.93	4.4	3.66	2.93
GI-5101-56-Serum-Conv	I7	2.586	11.81	8.37	6.22	5.16	4.13

GI-5198-30-Serum-Conv	J7	2.733	12.48	8.84	6.57	5.46	4.37
GI-5270-28-Serum-Conv	K7	2.264	10.34	7.33	5.44	4.52	3.62
GI-4245-33-Serum-Conv	L7	2.717	12.41	8.79	6.53	5.42	4.34
GI-5096-56-Serum-Conv	M7	2.497	11.4	8.08	6	4.98	3.99
GI-5062-56-Serum-Conv	N7	2.655	12.12	8.59	6.38	5.3	4.24
GI-5100-56-Serum-Conv	O7	2.731	12.47	8.84	6.56	5.45	4.36
GI-5093-56-Serum-Conv	P7	2.755	12.58	8.92	6.62	5.5	4.4
GI-5102-56-Serum-Conv	A8	2.75	12.56	8.9	6.61	5.49	4.39
GI-5269-28-Serum-Conv	B8	2.302	10.51	7.45	5.53	4.59	3.68
GI-5275-33-Serum-Conv	C8	0.953	4.35	3.08	2.29	1.9	1.52
GI-5079-56-Serum-Conv	D8	1.588	7.25	5.14	3.82	3.17	2.54
GI-5202-55-Serum-Conv	E8	2.724	12.44	8.82	6.55	5.44	4.35
GI-5208-28-Serum-Conv	F8	1.351	6.17	4.37	3.25	2.7	2.16
GI-5103-56-Serum-Conv	G8	2.143	9.79	6.94	5.15	4.28	3.42
GI-5203-56-Serum-Conv	H8	2.771	12.65	8.97	6.66	5.53	4.43
GI-5163-28-Serum-Conv	I8	1.577	7.2	5.1	3.79	3.15	2.52
GI-5235-55-Serum-Conv	J8	2.434	11.11	7.88	5.85	4.86	3.89
GI-5127-56-Serum-Conv	K8	2.741	12.52	8.87	6.59	5.47	4.38
GI-5409-28-Serum-Conv	L8	2.71	12.37	8.77	6.51	5.41	4.33
GI-5099-56-Serum-Conv	M8	2.738	12.5	8.86	6.58	5.47	4.37
GI-5418-28-Serum-Conv	N8	1.99	9.09	6.44	4.78	3.97	3.18
GI-5207-28-Serum-Conv	O8	2.206	10.07	7.14	5.3	4.4	3.52
GI-5098-56-Serum-Conv	P8	2.465	11.26	7.98	5.93	4.92	3.94
GI-5196-56-Serum-Conv	A9	0.402	1.84	1.3	0.97	0.8	0.64
GI-5241-28-Serum-Conv	B9	0.685	3.13	2.22	1.65	1.37	1.09
GI-5195-56-Serum-Conv	C9	2.558	11.68	8.28	6.15	5.11	4.09
GI-5158-56-Serum-Conv	D9	2.819	12.87	9.12	6.78	5.63	4.5
GI-5221-43-Serum-Conv	E9	2.546	11.63	8.24	6.12	5.08	4.07
GI-5128-56-Serum-Conv	F9	2.663	12.16	8.62	6.4	5.32	4.25
GI-5190-51-Serum-Conv	G9	2.507	11.45	8.11	6.03	5	4
GI-5280-29-Serum-Conv	H9	2.494	11.39	8.07	6	4.98	3.98
GI-5693-28-Serum-Conv	I9	1.61	7.35	5.21	3.87	3.21	2.57
GI-5272-56-Serum-Conv	J9	1.809	8.26	5.85	4.35	3.61	2.89
GI-5155-56-Serum-Conv	K9	2.749	12.55	8.9	6.61	5.49	4.39
GI-5284-28-Serum-Conv	L9	2.137	9.76	6.92	5.14	4.27	3.41
GI-5211-28-Serum-Conv	M9	1.603	7.32	5.19	3.85	3.2	2.56
GI-5189-56-Serum-Conv	N9	2.704	12.35	8.75	6.5	5.4	4.32
GI-5285-28-Serum-Conv	O9	2.343	10.7	7.58	5.63	4.68	3.74

GI-5237-28-Serum-Conv	P9	1.001	4.57	3.24	2.41	2	1.6
GI-5086-56-Serum-Conv	A10	2.719	12.42	8.8	6.54	5.43	4.34
GI-5434-32-Serum-Conv	B10	2.632	12.02	8.52	6.33	5.25	4.2
GI-5298-28-Serum-Conv	C10	2.184	9.97	7.07	5.25	4.36	3.49
GI-5191-56-Serum-Conv	D10	0.874	3.99	2.83	2.1	1.74	1.4
GI-5113-54-Serum-Conv	E10	2.255	10.3	7.3	5.42	4.5	3.6
GI-5169-56-Serum-Conv	F10	2.106	9.62	6.82	5.06	4.2	3.36
GI-5441-32-Serum-Conv	G10	2.698	12.32	8.73	6.49	5.39	4.31
GI-5478-30-Serum-Conv	H10	2.758	12.59	8.93	6.63	5.5	4.41
GI-5288-57-Serum-Conv	I10	2.339	10.68	7.57	5.62	4.67	3.74
GI-5194-56-Serum-Conv	J10	2.497	11.4	8.08	6	4.98	3.99
GI-5221-56-Serum-Conv	K10	2.745	12.53	8.88	6.6	5.48	4.38
GI-5220-56-Serum-Conv	L10	2.605	11.89	8.43	6.26	5.2	4.16
GI-5192-56-Serum-Conv	M10	1.49	6.8	4.82	3.58	2.97	2.38
GI-5198-56-Serum-Conv	N10	2.722	12.43	8.81	6.54	5.43	4.35
GI-5435-28-Serum-Conv	O10	2.592	11.84	8.39	6.23	5.17	4.14
GI-5413-56-Serum-Conv	P10	0.274	1.25	0.89	0.66	0.55	0.44
GI-5415-52-Serum-Conv	A11	0.294	1.34	0.95	0.71	0.59	0.47
GI-5245-54-Serum-Conv	B11	2.702	12.34	8.74	6.5	5.39	4.32
GI-5276-57-Serum-Conv	C11	0.531	2.42	1.72	1.28	1.06	0.85
GI-5250-34-Serum-Conv	D11	2.748	12.55	8.89	6.61	5.49	4.39
GI-5411-53-Serum-Conv	E11	0.09	0.41	0.29	0.22	0.18	0.14
GI-5275-56-Serum-Conv	F11	0.892	4.07	2.89	2.14	1.78	1.42
GI-5267-54-Serum-Conv	G11	2.614	11.94	8.46	6.28	5.22	4.18
GI-5417-54-Serum-Conv	H11	2.215	10.11	7.17	5.32	4.42	3.54
GI-5407-56-Serum-Conv	I11	2.392	10.92	7.74	5.75	4.77	3.82
GI-5234-56-Serum-Conv	J11	2.336	10.67	7.56	5.62	4.66	3.73
GI-5412-56-Serum-Conv	K11	0.381	1.74	1.23	0.92	0.76	0.61
GI-5270-56-Serum-Conv	L11	2.024	9.24	6.55	4.87	4.04	3.23
GI-5427-56-Serum-Conv	M11	1.086	4.96	3.51	2.61	2.17	1.73
GI-5405-56-Serum-Conv	N11	0.521	2.38	1.69	1.25	1.04	0.83
GI-5263-57-Serum-Conv	O11	0.184	0.84	0.6	0.44	0.37	0.29
GI-5240-49-Serum-Conv	P11	2.024	9.24	6.55	4.87	4.04	3.23
GI-5207-54-Serum-Conv	A12	2.219	10.13	7.18	5.33	4.43	3.54
GI-5152-56-Serum-Conv	B12	0.994	4.54	3.22	2.39	1.98	1.59
GI-5208-55-Serum-Conv	C12	1.065	4.86	3.45	2.56	2.13	1.7
GI-5176-56-Serum-Conv	D12	2.112	9.64	6.83	5.08	4.22	3.37
GI-5236-52-Serum-Conv	E12	2.612	11.93	8.45	6.28	5.21	4.17

GI-5264-64-Serum-Conv	F12	2.743	12.53	8.88	6.59	5.48	4.38
GI-5289-56-Serum-Conv	G12	2.673	12.21	8.65	6.43	5.34	4.27
GI-5238-60-Serum-Conv	H12	2.769	12.64	8.96	6.66	5.53	4.42
GI-5209-56-Serum-Conv	I12	2.284	10.43	7.39	5.49	4.56	3.65
GI-5147-56-Serum-Conv	J12	2.133	9.74	6.9	5.13	4.26	3.41
GI-5457-29-Serum-Conv	K12	2.436	11.12	7.88	5.86	4.86	3.89
GI-5242-69-Serum-Conv	L12	1.667	7.61	5.39	4.01	3.33	2.66
GI-5418-57-Serum-Conv	M12	2.371	10.83	7.67	5.7	4.73	3.79
GI-5273-61-Serum-Conv	N12	1.371	6.26	4.44	3.3	2.74	2.19
GI-5265-59-Serum-Conv	O12	2.128	9.72	6.89	5.12	4.25	3.4
GI-5262-62-Serum-Conv	P12	2.793	12.75	9.04	6.71	5.57	4.46
GI-5110-61-Serum-Conv	A13	1.157	5.28	3.74	2.78	2.31	1.85
GI-5410-78-Serum-Conv	B13	2.498	11.41	8.08	6	4.99	3.99
GI-5287-61-Serum-Conv	C13	2.759	12.6	8.93	6.63	5.51	4.41
GI-5294-64-Serum-Conv	D13	0.531	2.42	1.72	1.28	1.06	0.85
GI-5290-60-Serum-Conv	E13	0.451	2.06	1.46	1.08	0.9	0.72
GI-5188-58-Serum-Conv	F13	2.788	12.73	9.02	6.7	5.56	4.45
GI-5106-62-Serum-Conv	G13	2.87	13.11	9.29	6.9	5.73	4.58
GI-5414-59-Serum-Conv	H13	2.399	10.95	7.76	5.77	4.79	3.83
GI-5283-73-Serum-Conv	I13	2.653	12.11	8.59	6.38	5.3	4.24
GI-5173-59-Serum-Conv	J13	1.127	5.15	3.65	2.71	2.25	1.8
GI-5278-69-Serum-Conv	K13	1.838	8.39	5.95	4.42	3.67	2.94
GI-5073-58-Serum-Conv	L13	2.719	12.42	8.8	6.54	5.43	4.34
GI-5279-60-Serum-Conv	M13	2.475	11.3	8.01	5.95	4.94	3.95
GI-5107-58-Serum-Conv	N13	2.716	12.4	8.79	6.53	5.42	4.34
GI-5408—Serum-Conv	O13	2.688	12.27	8.7	6.46	5.37	4.29
GI-5142-63-Serum-Conv	P13	2.8	12.79	9.06	6.73	5.59	4.47
GI-5066-61-Serum-Conv	A14	2.716	12.4	8.79	6.53	5.42	4.34
GI-5424-61-Serum-Conv	B14	2.707	12.36	8.76	6.51	5.4	4.32
GI-5297-63-Serum-Conv	C14	2.832	12.93	9.17	6.81	5.65	4.52
GI-5423-86-Serum-Conv	D14	0.038	0.17	0.12	0.09	0.08	0.06
GI-5199-58-Serum-Conv	E14	2.792	12.75	9.04	6.71	5.57	4.46
GI-5425-50-Serum-Conv	F14	0.104	0.47	0.34	0.25	0.21	0.17
GI-5172-58-Serum-Conv	G14	2.716	12.4	8.79	6.53	5.42	4.34
GI-5224-43-Serum-Conv	H14	2.764	12.62	8.94	6.64	5.52	4.42
GI-5080-58-Serum-Conv	I14	1.687	7.7	5.46	4.06	3.37	2.69
GI-5426-61-Serum-Conv	J14	2.732	12.47	8.84	6.57	5.45	4.36
GI-5420-80-Serum-Conv	K14	2.751	12.56	8.9	6.61	5.49	4.39

GI-5433-84-Serum-Conv	L14	2.666	12.17	8.63	6.41	5.32	4.26
GI-5421-68-Serum-Conv	M14	2.721	12.42	8.81	6.54	5.43	4.35
GI-5299-90-Serum-Conv	N14	1.491	6.81	4.83	3.58	2.98	2.38
GI-5422-65-Serum-Conv	O14	2.471	11.28	8	5.94	4.93	3.95
GI-5129-94-Serum-Conv	P14	2.591	11.83	8.39	6.23	5.17	4.14
GI-5149-56-Serum-Conv	A15	0.893	4.08	2.89	2.15	1.78	1.43
GI-5145-58-Serum-Conv	C15	1.663	7.59	5.38	4	3.32	2.66
GI-5277-66-Serum-Conv	E15	2.799	12.78	9.06	6.73	5.59	4.47
GI-5092-59-Serum-Conv	G15	2.63	12.01	8.51	6.32	5.25	4.2
GI-5177-65-Serum-Conv	I15	0.544	2.48	1.76	1.31	1.09	0.87
GI-5094-62-Serum-Conv	K15	2.744	12.53	8.88	6.6	5.48	4.38
GI-5283-91-Serum-Conv	M15	2.334	10.66	7.55	5.61	4.66	3.73
GI-5437-91-Serum-Conv	O15	2.675	12.21	8.66	6.43	5.34	4.27

- *Independent testing of 12 samples for anti-SARS-CoV-2 Spike protein and anti-SARS-CoV-2-nucleoprotein IgG*

Sample	OmniPATH Spike protein reactivity	Abbott Nucleoprotein reactivity
GI-5118-56-Serum-Conv	+ve	low
GI-5413-31-Serum-Conv	-ve	-ve
GI-5416-57-Serum-Conv	+ve	-ve
GI-5263-29-Serum-Conv	-ve	-ve
GI-5122-56-Serum-Conv	+ve	low
GI-5413-56-Serum-Conv	-ve	-ve
GI-5415-52-Serum-Conv	+ve	+ve
GI-5411-53-Serum-Conv	-ve	-ve
GI-5412-56-Serum-Conv	-ve	-ve
GI-5263-57-Serum-Conv	-ve	-ve
GI-5423-86-Serum-Conv	-ve	-ve
GI-5425-50-Serum-Conv	-ve	-ve

8 Samples were confirmed as negative for both spike and nucleocapsid antigen.
2 samples positive for spike antigen were negative for nucleocapsid
2 samples positive for spike antigen were low for nucleocapsid
1 sample was positive for both spike and nucleocapsid.

Negative Samples, Sample Plate

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
10	A2	0.264	0.21	0.215	0.007	3.3
	B2	0.274	0.22			
15	C2	0.378	0.324	0.317	0.01	3
	D2	0.364	0.311			
20	E2	0.467	0.414	0.416	0.004	1
	F2	0.473	0.419			
25	G2	0.568	0.514	0.515	0.002	0.3
	H2	0.57	0.516			
30	I2	0.677	0.623	0.633	0.014	2.2
	J2	0.696	0.643			
Blank	M2	0.049	0	0		
	M24	0.049				
	N2	0.054				
	N24	0.052				
	O1	0.058				
	O2	0.051				
	O23	0.05				
	O24	0.048				
	P1	0.06				
	P2	0.058				
	P23	0.058				
	P24	0.057				

Negative samples, sample plate

Sample	Wells	Value	Cut-off level ng mL				
			10	15	20	25	30
OBB7361	A3	0.133	0.62	0.42	0.32	0.26	0.21
OBB7362	B3	0.211	0.98	0.67	0.51	0.41	0.33
OBB7375	C3	0.181	0.84	0.57	0.44	0.35	0.29
OBB7376	D3	0.19	0.88	0.60	0.46	0.37	0.30
OBB7389	E3	0.301	1.40	0.95	0.72	0.58	0.48
OBB7390	F3	0.19	0.88	0.60	0.46	0.37	0.30
OBB7401	G3	0.097	0.45	0.31	0.23	0.19	0.15

OBB7402	H3	1.287	5.99	4.06	3.09	2.50	2.03
OBB7413	I3	0.12	0.56	0.38	0.29	0.23	0.19
OBB7414	J3	0.119	0.55	0.38	0.29	0.23	0.19
OBB7425	K3	0.27	1.26	0.85	0.65	0.52	0.43
OBB7426	L3	0.071	0.33	0.22	0.17	0.14	0.11
OBB7440	M3	0.09	0.42	0.28	0.22	0.17	0.14
OBB7441	N3	0.544	2.53	1.72	1.31	1.06	0.86
OBB7453	O3	0.054	0.25	0.17	0.13	0.10	0.09
OBB7454	P3	0.033	0.15	0.10	0.08	0.06	0.05
OBB7363	A4	0.37	1.72	1.17	0.89	0.72	0.58
OBB7364	B4	0.347	1.61	1.09	0.83	0.67	0.55
OBB7377	C4	-0.003	-0.01	-0.01	-0.01	-0.01	0.00
OBB7378	D4	0.161	0.75	0.51	0.39	0.31	0.25
OBB7391	E4	0.377	1.75	1.19	0.91	0.73	0.60
OBB7392	F4	0.102	0.47	0.32	0.25	0.20	0.16
OBB7403	G4	0.189	0.88	0.60	0.45	0.37	0.30
OBB7404	H4	0.144	0.67	0.45	0.35	0.28	0.23
OBB7415	I4	1.172	5.45	3.70	2.82	2.28	1.85
OBB7416	J4	0.439	2.04	1.38	1.06	0.85	0.69
OBB7427	K4	0.184	0.86	0.58	0.44	0.36	0.29
OBB7428	L4	1.069	4.97	3.37	2.57	2.08	1.69
OBB7442	M4	0.115	0.53	0.36	0.28	0.22	0.18
OBB7443	N4	0.094	0.44	0.30	0.23	0.18	0.15
OBB7455	O4	0.142	0.66	0.45	0.34	0.28	0.22
OBB7456	P4	0.067	0.31	0.21	0.16	0.13	0.11
OBB7365	A5	0.246	1.14	0.78	0.59	0.48	0.39
OBB7366	B5	-0.003	-0.01	-0.01	-0.01	-0.01	0.00
OBB7379	C5	0.163	0.76	0.51	0.39	0.32	0.26
OBB7380	D5	0.405	1.88	1.28	0.97	0.79	0.64
OBB7393	E5	0.228	1.06	0.72	0.55	0.44	0.36
OBB7394	F5	0.186	0.87	0.59	0.45	0.36	0.29
OBB7405	G5	1.522	7.08	4.80	3.66	2.96	2.40
OBB7406	H5	0.112	0.52	0.35	0.27	0.22	0.18
OBB7417	I5	1.079	5.02	3.40	2.59	2.10	1.70
OBB7418	J5	0.096	0.45	0.30	0.23	0.19	0.15
OBB7429	K5	0.112	0.52	0.35	0.27	0.22	0.18
OBB7430	L5	0.252	1.17	0.79	0.61	0.49	0.40
OBB7444	M5	0.16	0.74	0.50	0.38	0.31	0.25
OBB7445	N5	0.114	0.53	0.36	0.27	0.22	0.18

OBB7457	O5	0.114	0.53	0.36	0.27	0.22	0.18
OBB7458	P5	0.043	0.20	0.14	0.10	0.08	0.07
OBB7368	A6	0.203	0.94	0.64	0.49	0.39	0.32
OBB7369	B6	0.137	0.64	0.43	0.33	0.27	0.22
OBB7382	C6	0.17	0.79	0.54	0.41	0.33	0.27
OBB7383	D6	0.105	0.49	0.33	0.25	0.20	0.17
OBB7395	E6	0.075	0.35	0.24	0.18	0.15	0.12
OBB7396	F6	0.208	0.97	0.66	0.50	0.40	0.33
OBB7407	G6	0.158	0.73	0.50	0.38	0.31	0.25
OBB7408	H6	0.104	0.48	0.33	0.25	0.20	0.16
OBB7419	I6	0.374	1.74	1.18	0.90	0.73	0.59
OBB7420	J6	0.106	0.49	0.33	0.25	0.21	0.17
OBB7433	K6	0.185	0.86	0.58	0.44	0.36	0.29
OBB7434	L6	0.072	0.33	0.23	0.17	0.14	0.11
OBB7446	M6	0.063	0.29	0.20	0.15	0.12	0.10
OBB7447	N6	0.154	0.72	0.49	0.37	0.30	0.24
OBB7459	O6	0.128	0.60	0.40	0.31	0.25	0.20
OBB7460	P6	0.107	0.50	0.34	0.26	0.21	0.17
OBB7370	A7	-0.005	-0.02	-0.02	-0.01	-0.01	-0.01
OBB7371	B7	0.257	1.20	0.81	0.62	0.50	0.41
OBB7385	C7	0.227	1.06	0.72	0.55	0.44	0.36
OBB7386	D7	0.264	1.23	0.83	0.63	0.51	0.42
OBB7397	E7	0.17	0.79	0.54	0.41	0.33	0.27
OBB7398	F7	0.157	0.73	0.50	0.38	0.30	0.25
OBB7409	G7	0.131	0.61	0.41	0.31	0.25	0.21
OBB7410	H7	0.206	0.96	0.65	0.50	0.40	0.33
OBB7421	I7	0.124	0.58	0.39	0.30	0.24	0.20
OBB7422	J7	0.147	0.68	0.46	0.35	0.29	0.23
OBB7435	K7	0.131	0.61	0.41	0.31	0.25	0.21
OBB7437	L7	0.202	0.94	0.64	0.49	0.39	0.32
OBB7448	M7	0.064	0.30	0.20	0.15	0.12	0.10
OBB7449	N7	0.09	0.42	0.28	0.22	0.17	0.14
OBB7461	O7	0.169	0.79	0.53	0.41	0.33	0.27
OBB7462	P7	0.056	0.26	0.18	0.13	0.11	0.09
OBB7373	A8	0.135	0.63	0.43	0.32	0.26	0.21
OBB7374	B8	0.189	0.88	0.60	0.45	0.37	0.30
OBB7387	C8	0.164	0.76	0.52	0.39	0.32	0.26
OBB7388	D8	0.191	0.89	0.60	0.46	0.37	0.30
OBB7399	E8	0.182	0.85	0.57	0.44	0.35	0.29

OBB7400	F8	0.177	0.82	0.56	0.43	0.34	0.28
OBB7411	G8	0.219	1.02	0.69	0.53	0.43	0.35
OBB7412	H8	0.092	0.43	0.29	0.22	0.18	0.15
OBB7423	I8	0.174	0.81	0.55	0.42	0.34	0.27
OBB7424	J8	0.34	1.58	1.07	0.82	0.66	0.54
OBB7438	K8	0.608	2.83	1.92	1.46	1.18	0.96
OBB7439	L8	0.095	0.44	0.30	0.23	0.18	0.15
OBB7450	M8	0.68	3.16	2.15	1.63	1.32	1.07
OBB7452	N8	1.302	6.06	4.11	3.13	2.53	2.06
OBB7463	O8	0.353	1.64	1.11	0.85	0.69	0.56
OBB7464	P8	0.152	0.71	0.48	0.37	0.30	0.24
OBB7255	A9	0.652	3.03	2.06	1.57	1.27	1.03
OBB7256	B9	0.004	0.02	0.01	0.01	0.01	0.01
OBB7268	C9	0.1	0.47	0.32	0.24	0.19	0.16
OBB7269	D9	0.112	0.52	0.35	0.27	0.22	0.18
OBB7280	E9	0.286	1.33	0.90	0.69	0.56	0.45
OBB7281	F9	0.196	0.91	0.62	0.47	0.38	0.31
OBB7292	G9	0.125	0.58	0.39	0.30	0.24	0.20
OBB7293	H9	0.271	1.26	0.85	0.65	0.53	0.43
OBB7306	I9	0.149	0.69	0.47	0.36	0.29	0.24
OBB7307	J9	0.888	4.13	2.80	2.13	1.72	1.40
OBB7318	K9	0.199	0.93	0.63	0.48	0.39	0.31
OBB7319	L9	0.173	0.80	0.55	0.42	0.34	0.27
OBB7332	M9	0.266	1.24	0.84	0.64	0.52	0.42
OBB7333	N9	0.108	0.50	0.34	0.26	0.21	0.17
OBB7346	O9	0.119	0.55	0.38	0.29	0.23	0.19
OBB7347	P9	0.155	0.72	0.49	0.37	0.30	0.24
OBB7257	A10	0.236	1.10	0.74	0.57	0.46	0.37
OBB7258	B10	0.14	0.65	0.44	0.34	0.27	0.22
OBB7270	C10	0.185	0.86	0.58	0.44	0.36	0.29
OBB7271	D10	0.222	1.03	0.70	0.53	0.43	0.35
OBB7282	E10	0.394	1.83	1.24	0.95	0.77	0.62
OBB7283	F10	0.217	1.01	0.68	0.52	0.42	0.34
OBB7294	G10	0.656	3.05	2.07	1.58	1.27	1.04
OBB7295	H10	0.22	1.02	0.69	0.53	0.43	0.35
OBB7308	I10	0.227	1.06	0.72	0.55	0.44	0.36
OBB7309	J10	0.189	0.88	0.60	0.45	0.37	0.30
OBB7320	K10	0.727	3.38	2.29	1.75	1.41	1.15
OBB7322	L10	0.187	0.87	0.59	0.45	0.36	0.30

OBB7334	M10	0.214	1.00	0.68	0.51	0.42	0.34
OBB7337	N10	0.092	0.43	0.29	0.22	0.18	0.15
OBB7348	O10	0.493	2.29	1.56	1.19	0.96	0.78
OBB7349	P10	0.401	1.87	1.26	0.96	0.78	0.63
OBB7259	A11	0.343	1.60	1.08	0.82	0.67	0.54
OBB7260	B11	0.148	0.69	0.47	0.36	0.29	0.23
OBB7272	C11	0.454	2.11	1.43	1.09	0.88	0.72
OBB7273	D11	0.103	0.48	0.32	0.25	0.20	0.16
OBB7284	E11	0.492	2.29	1.55	1.18	0.96	0.78
OBB7285	F11	0.299	1.39	0.94	0.72	0.58	0.47
OBB7297	G11	0.146	0.68	0.46	0.35	0.28	0.23
OBB7298	H11	0.154	0.72	0.49	0.37	0.30	0.24
OBB7310	I11	0.115	0.53	0.36	0.28	0.22	0.18
OBB7311	J11	0.634	2.95	2.00	1.52	1.23	1.00
OBB7323	K11	0.673	3.13	2.12	1.62	1.31	1.06
OBB7324	L11	0.139	0.65	0.44	0.33	0.27	0.22
OBB7338	M11	0.619	2.88	1.95	1.49	1.20	0.98
OBB7339	N11	0.253	1.18	0.80	0.61	0.49	0.40
OBB7350	O11	0.063	0.29	0.20	0.15	0.12	0.10
OBB7351	P11	0.057	0.27	0.18	0.14	0.11	0.09
OBB7261	A12	1.89	8.79	5.96	4.54	3.67	2.99
OBB7262	B12	0.196	0.91	0.62	0.47	0.38	0.31
OBB7274	C12	0.178	0.83	0.56	0.43	0.35	0.28
OBB7275	D12	0.197	0.92	0.62	0.47	0.38	0.31
OBB7286	E12	0.267	1.24	0.84	0.64	0.52	0.42
OBB7287	F12	0.303	1.41	0.96	0.73	0.59	0.48
OBB7299	G12	0.441	2.05	1.39	1.06	0.86	0.70
OBB7300	H12	0.11	0.51	0.35	0.26	0.21	0.17
OBB7312	I12	-0.001	0.00	0.00	0.00	0.00	0.00
OBB7313	J12	0.19	0.88	0.60	0.46	0.37	0.30
OBB7326	K12	0.235	1.09	0.74	0.56	0.46	0.37
OBB7327	L12	0.147	0.68	0.46	0.35	0.29	0.23
OBB7340	M12	-0.001	0.00	0.00	0.00	0.00	0.00
OBB7341	N12	0.002	0.01	0.01	0.00	0.00	0.00
OBB7352	O12	0.343	1.60	1.08	0.82	0.67	0.54
OBB7353	P12	0.33	1.53	1.04	0.79	0.64	0.52
OBB7263	A13	1.053	4.90	3.32	2.53	2.04	1.66
OBB7264	B13	0.354	1.65	1.12	0.85	0.69	0.56
OBB7276	C13	1.093	5.08	3.45	2.63	2.12	1.73

OBB7277	D13	0.227	1.06	0.72	0.55	0.44	0.36
OBB7288	E13	0.166	0.77	0.52	0.40	0.32	0.26
OBB7289	F13	0.281	1.31	0.89	0.68	0.55	0.44
OBB7301	G13	0.33	1.53	1.04	0.79	0.64	0.52
OBB7302	H13	0.52	2.42	1.64	1.25	1.01	0.82
OBB7314	I13	0.243	1.13	0.77	0.58	0.47	0.38
OBB7315	J13	0.236	1.10	0.74	0.57	0.46	0.37
OBB7328	K13	0.212	0.99	0.67	0.51	0.41	0.33
OBB7329	L13	0.173	0.80	0.55	0.42	0.34	0.27
OBB7342	M13	0.126	0.59	0.40	0.30	0.24	0.20
OBB7343	N13	0.1	0.47	0.32	0.24	0.19	0.16
OBB7355	O13	0.633	2.94	2.00	1.52	1.23	1.00
OBB7356	P13	0.1	0.47	0.32	0.24	0.19	0.16
OBB7266	A14	0.209	0.97	0.66	0.50	0.41	0.33
OBB7267	B14	0.256	1.19	0.81	0.62	0.50	0.40
OBB7278	C14	0.139	0.65	0.44	0.33	0.27	0.22
OBB7279	D14	0.153	0.71	0.48	0.37	0.30	0.24
OBB7290	E14	0.156	0.73	0.49	0.38	0.30	0.25
OBB7291	F14	0.39	1.81	1.23	0.94	0.76	0.62
OBB7303	G14	0.248	1.15	0.78	0.60	0.48	0.39
OBB7305	H14	0.328	1.53	1.03	0.79	0.64	0.52
OBB7316	I14	0.459	2.13	1.45	1.10	0.89	0.73
OBB7317	J14	-0.002	-0.01	-0.01	0.00	0.00	0.00
OBB7330	K14	0.165	0.77	0.52	0.40	0.32	0.26
OBB7331	L14	0.288	1.34	0.91	0.69	0.56	0.45
OBB7344	M14	0.601	2.80	1.90	1.44	1.17	0.95
OBB7345	N14	0.135	0.63	0.43	0.32	0.26	0.21
OBB7357	O14	0.181	0.84	0.57	0.44	0.35	0.29
OBB7360	P14	0.132	0.61	0.42	0.32	0.26	0.21
OBB7145	A15	0.266	1.24	0.84	0.64	0.52	0.42
OBB7146	B15	0.307	1.43	0.97	0.74	0.60	0.48
OBB7160	C15	0.188	0.87	0.59	0.45	0.37	0.30
OBB7162	D15	0.254	1.18	0.80	0.61	0.49	0.40
OBB7175	E15	0.198	0.92	0.62	0.48	0.38	0.31
OBB7176	F15	0.277	1.29	0.87	0.67	0.54	0.44
OBB7187	G15	0.119	0.55	0.38	0.29	0.23	0.19
OBB7188	H15	0.157	0.73	0.50	0.38	0.30	0.25
OBB7200	I15	0.294	1.37	0.93	0.71	0.57	0.46
OBB7201	J15	0.236	1.10	0.74	0.57	0.46	0.37

OBB7215	K15	0.304	1.41	0.96	0.73	0.59	0.48
OBB7216	L15	0.513	2.39	1.62	1.23	1.00	0.81
OBB7227	M15	0.066	0.31	0.21	0.16	0.13	0.10
OBB7228	N15	0.073	0.34	0.23	0.18	0.14	0.12
OBB7239	O15	0.062	0.29	0.20	0.15	0.12	0.10

13.2.3.2 NIBSC Verification Panel

Study Results Section:	10.3
Parameter	Study 3 – NIBSC Verification Panel

Raw Data

Summary

NIBSC Anti-SARS-CoV-2 Verification Panel for Serology NIBSC 20/B770 (n = 37)						
Preliminary Cut-off level (ng mL Anti-SARS-CoV-2 N Protein IgG)	Positive samples (n = 23)		Negative Samples (n = 14)		Sensitivity %	Specificity %
	Sample index ratio > 1 (True Positive)	Sample index ratio ≤ 1 (False Negative)	Sample index ratio ≤ 1 (True Negative)	Sample index ratio > 1 (False Positive)		
10	23	0	13	1	100	93
15	22	1	14	0	95.65	100
20	22	1	14	0	95.65	100
25	22	1	14	0	95.65	100
30	22	1	14	0	95.65	100

Cut-off controls

Cut-off control Concentration ng/mL	Wells	OD Value (450nm-650nm)	OD Value (450nm-650nm) - Blank	Mean OD Value - Blank	SD	CV %
10	A2	0.14	0.135	0.147	0.016	11.1
	B2	0.163	0.158			
15	C2	0.243	0.238	0.223	0.02	9.1
	D2	0.214	0.209			
20	E2	0.329	0.324	0.301	0.032	10.6
	F2	0.284	0.278			
25	G2	0.327	0.322	0.353	0.045	12.7
	H2	0.39	0.385			
30	I2	0.471	0.466	0.491	0.035	7.1
	J2	0.521	0.516			
Blank	O2	0.009	0.057	0		
	P2	0.009	0.058			

NIBSC Positive Samples

Sample	Wells	OD Value - Blank	Sample Index Ratio at Cut-off anti-SARS-CoV-2 N-Protein levels ng mL				
			10	10	10	10	10
NIBSC01	A3	2.042	13.89	9.16	6.78	5.78	4.16
NIBSC02	B3	1.59	10.82	7.13	5.28	4.50	3.24
NIBSC03	C3	2.792	18.99	12.52	9.28	7.91	5.69
NIBSC04	D3	2.703	18.39	12.12	8.98	7.66	5.51
NIBSC05	E3	2.715	18.47	12.17	9.02	7.69	5.53
NIBSC06	F3	1.63	11.09	7.31	5.42	4.62	3.32
NIBSC07	G3	1.532	10.42	6.87	5.09	4.34	3.12
NIBSC08	H3	2.596	17.66	11.64	8.62	7.35	5.29
NIBSC09	I3	2.729	18.56	12.24	9.07	7.73	5.56
NIBSC10	J3	2.745	18.67	12.31	9.12	7.78	5.59
NIBSC11	K3	0.676	4.60	3.03	2.25	1.92	1.38
NIBSC12	L3	1.434	9.76	6.43	4.76	4.06	2.92
NIBSC13	M3	2.269	15.44	10.17	7.54	6.43	4.62
NIBSC14	N3	1.948	13.25	8.74	6.47	5.52	3.97
NIBSC15	O3	0.213	1.45	0.96	0.71	0.60	0.43
NIBSC16	P3	1.622	11.03	7.27	5.39	4.59	3.30
NIBSC17	A4	2.484	16.90	11.14	8.25	7.04	5.06
NIBSC18	B4	2.75	18.71	12.33	9.14	7.79	5.60
NIBSC19	C4	2.777	18.89	12.45	9.23	7.87	5.66
NIBSC20	D4	2.297	15.63	10.30	7.63	6.51	4.68
NIBSC21	E4	2.613	17.78	11.72	8.68	7.40	5.32
NIBSC22	F4	2.641	17.97	11.84	8.77	7.48	5.38
NIBSC23	G4	2.413	16.41	10.82	8.02	6.84	4.91

NIBSC Negative Samples

Sample	Wells	OD Value - Blank	Sample Index Ratio at Cut-off anti-SARS-CoV-2 N-Protein levels ng mL				
			10	15	20	25	30
NIBSC24	H4	0.012	0.08	0.05	0.04	0.03	0.02
NIBSC25	I4	0.063	0.43	0.28	0.21	0.18	0.13
NIBSC26	J4	0.09	0.61	0.40	0.30	0.25	0.18
NIBSC27	K4	0.114	0.78	0.51	0.38	0.32	0.23
NIBSC28	L4	0.195	1.33	0.87	0.65	0.55	0.40
NIBSC29	M4	0.02	0.14	0.09	0.07	0.06	0.04
NIBSC30	N4	0.093	0.63	0.42	0.31	0.26	0.19
NIBSC31	O4	0.135	0.92	0.61	0.45	0.38	0.27
NIBSC32	P4	0.076	0.52	0.34	0.25	0.22	0.15
NIBSC33	A5	0.063	0.43	0.28	0.21	0.18	0.13
NIBSC34	B5	0.116	0.79	0.52	0.39	0.33	0.24
NIBSC35	C5	0.017	0.12	0.08	0.06	0.05	0.03
NIBSC36	D5	0.078	0.53	0.35	0.26	0.22	0.16
NIBSC37	E5	0.01	0.07	0.04	0.03	0.03	0.02

From: McBride, Jeffrey <jeffrey.mcbride@thermofisher.com>
Sent: Monday, March 22, 2021 10:46 AM
To: Stringer, James R. <james.r.stringer@thermofisher.com>
Subject: RE: Performance Evaluation Report

I have read and understood the attached document and I approve it's content.

Jeffrey

Dr. Jeffrey D. McBride
Biomolecular Lead

Microbiology - Dartford
Thermo Fisher Scientific
Clipper Boulevard West
Crossways, Dartford
Kent, DA2 6PT

Phone: +44 (0)1322-295687
jeffrey.mcbride@thermofisher.com | www.thermofisher.com/microbiology

Wed 24/03/2021 08:54

Donaldson, Matthew

RE: Performance Evaluation Report

To Stringer, James R.

Cc Ruscoe, Diane C.

I have read and understood the attached document and approve its content.

Dr Matt Donaldson
Manufacturing Sciences Manager
Microbiology

Thermo Fisher Scientific
Remel House, Clipper Boulevard West | Dartford Kent, DA2 6PT, United Kingdom
Mobile: + 44 (0)7827 845112
matthew.donaldson@thermofisher.com | thermofisher.com/microbiology

From: Brame, Philip <phil.brame@thermofisher.com>
Sent: Tuesday, March 23, 2021 9:01 AM
To: Stringer, James R. <james.r.stringer@thermofisher.com>
Subject: RE: Performance Evaluation Report

Hi James

I have read and understood the attached document and I approve its content.

Best regards, Phil

From: Curd, Fay C. <fay.curd@thermofisher.com>
Sent: Tuesday, March 23, 2021 8:36 AM
To: Stringer, James R. <james.r.stringer@thermofisher.com>
Subject: FW: Performance Evaluation Report

Dear James
I have reviewed the attached contents and approve its use.
Regards
Fay

From: Stringer, James R. <james.r.stringer@thermofisher.com>
Sent: Monday, March 22, 2021 10:21 AM
To: Stringer, James R. <james.r.stringer@thermofisher.com>
Subject: RE: Performance Evaluation Report

I have read and understood the attached document and I approve it's content.

James.

Issue Number	Date Created	Current Route Step
DF-IR-2355	30-Mar-2020	

Created By

Matthew Donaldson (MATTHEW.DONALDSON)

Signatures (T1) | Issue Details (T2) | **Risk Assessment (T3)** | Containment (T4) | Disposition (T5)

Gateway

Refined Issue Statement:

Due to the current COVID-19 outbreak there is a significant number of staff working remotely so approving documentation through the use of a physical signature is not possible in many cases. This process deviation will cover the approval of quality documentation through the use of email.

The new process will follow these three sequential steps:

1) The document owner:
- Document to be approved will be saved and scanned as a PDF file and emailed to approvers.
- The document reference should be used as the email subject.
- The text 'For approval' should be entered into the body of the email followed by the approvers name and role.

2) The approver:
- Read the document and if willing to approve respond to the document owner with the following text: 'I have read and understood the attached document and I approve it's content.' The approver should then insert their email e-signature after the statement.

3) The document owner:
- Print a copy of the document and write within the approval box 'Approved by email, sign and date'.
- Print all approval replies and attach them to the printed version of the document along with a copy of this IR.
- Document is then to be scanned with all attachments and held in accordance to local procedures