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Evaluation of DiaSorin LIAISON SARS-CoV-2 S1/S2 IgG serology assay for the detection of anti-SARS-CoV-2 antibodies

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Executive summary

This document sets out the evaluation of the DiaSorin LIAISON SARS-CoV-2 S1/S2 IgG serology assay for the detection of anti-SARS-CoV-2 in serum samples.

The assessment was conducted by the Diagnostic Support Group (DSP) at PHE Porton on 28 May 2020; precision testing took place 2-10 June 2020. 100 serum samples from convalescent patients and 472 negative samples were included in the assessment.

The assay gave a specificity of 97.7% (95% confidence interval 95.8-99.0) in the evaluation. The manufacturer reported a specificity of 98.5% (95%CI 97.6% - 99.1%).

The assay gave an overall sensitivity of 64.0% (95%CI 53.8-73.4), with a sensitivity of 69.4% (95%CI 58.5-79.0) at ≥14 days post symptom onset. The sensitivity of the assay at ≥21 days post symptom onset was 71.4% (95%CI 60.0-81.2). The manufacturer reported a sensitivity of 97.4% (95%CI 86.8-99.5) for samples ≥15 days post RT-PCR confirmation.

Introduction

LIAISON SARS-CoV-2 S1/S2 IgG assay is intended for the detection of IgG antibodies to SARS-CoV-2 in human serum and plasma. The assay is a chemiluminescent immunoassay (CLIA) for the quantitative determination of anti-S1and anti-S2 specific IgG antibodies. The assay is intended for use on the LIAISON XL immunoassay analysers. This report details an evaluation of the assay conducted at PHE Porton Down on 28 May 2020 with precision testing taking place 2-10 June 2020, to inform a decision by the Department of Health and Social Care on use of the assay by NHS laboratories for the detection of anti-SARS-CoV-2 antibodies in patient samples. Please note that the DiaSorin assay includes an equivocal zone which reflects the biological variation of any ELISA between different runs. The equivocal zone correctly allows for the variation between assay runs, but the interpretation is left to the user, usually resulting in a second sample being requested. The sensitivity therefore for varies according to whether the equivocal results are scored as positive or negative, increasing or decreasing sensitivity, respectively. In this evaluation, borderline samples were included in the negative data set for consistency with other evaluations –note the instructions detail a repeat and/or second test process which the manufacturer recommends for an equivocal result; this was not possible due to limited sample volume and absence of a second sample.

LIAISON SARS-CoV-2 S1/S2 IgG Assay

The LIAISON SARS-CoV-2 S1/S2 IgG assay is a CLIA assay manufactured by DiaSorin S.p.A. The assay is listed as CE marked.

As per the manufacturer's information, the assay uses recombinant proteins representing the spike 1 (S1) and spike 2 (S2) proteins of SARS-CoV-2.

Test principle

The method for quantitative determination of IgG anti-S1 and IgG anti-S2 specific antibodies to SARS-CoV-2 is an indirect chemiluminescence immunoassay (CLIA). The specific recombinant S1 and S2 antigens are used for coating magnetic particles (solid phase) and mouse monoclonal antibodies to human IgG are linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, the SARS-CoV-2 IgG antibodies present in calibrators, samples or controls bind to the solid phase through the recombinant S1 and S2 antigens. During the second incubation the antibody conjugate reacts with IgG to SARS-CoV-2 already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of IgG to SARS-CoV2 concentration present in calibrators, samples or controls.

Interpretation of the result

The analyser automatically calculates SARS-CoV-2 S1/S2 IgG antibody concentrations expressed as arbitrary units (AU/mL) and grades the results. The assay range is up to 400 AU/mL.

Table 1: Manufacturer's interpretation of the results

AU/ml	Results	Retest rules and interpretation
<12.0	Negative	No retest is required. A negative result may indicate the absence or a very low level of IgG antibodies to the pathogen. The test could score negative in infected patients during the incubation period and in the early stages of infection.
12.0 ≤ x < 15.0	Equivocal	Retest the same specimen in duplicate with the LIAISON SARS-CoV-2 S1/S2 IgG assay. Samples having at least 2 out of the 3 results equal to or above 15.0 AU/mL should be graded positive. Samples having at least 2 out of the 3 results less than 12.0 AU/mL should be graded negative. A second sample should be collected and tested no less than one to 2 weeks later when the result is repeatedly equivocal.
≥ 15.0	Positive	No retest is required. A positive result generally indicates exposure of the subject to the pathogen

Manufacturer's listed limitations

The limitations of the assay are:

- a skilful technique and strict adherence to the instructions are necessary to obtain reliable results
- bacterial contamination or heat inactivation of the specimens may affect the test results
- specimens from patients receiving therapeutic doses of Biotin (Vitamin H, B7 or B8) may interfere in immunoassays based on biotinylated reagents – interference was not observed testing Biotin serum concentration up to 3500 ng/mL with LIAISON SARS-CoV-2 S1/S2 IgG assay
- detection of neutralising IgG antibodies against SARS-CoV-2 at present is not yet established to determine long term immunity to the virus or to protect the patient against re-infection by the virus
- the results obtained with this test should only be interpreted in conjunction with clinical findings, and the results from other laboratory tests and evaluations

Manufacturer's performance characteristics

Sensitivity

The sensitivity was determined by investigating 135 samples collected over time from 76 European patients. Infection with SARS-CoV-2 was confirmed by RT-PCR test at the time of the diagnosis.

The LIAISON SARS-CoV-2 S1/S2 IgG test was performed on samples collected at the time of admission and thereafter up to 36 days for 44 patients hospitalised with moderate symptoms and 11 admitted to the ICU with severe symptoms.
Twenty-one single samples were from patients affected by COVID-19, who were confirmed RT-PCR positive and admitted to the ICU with known time frame from PCR testing to sample collection.

The following table describes diagnostic sensitivity in 3 groups, i.e. the early samples (≤ 5 days after diagnosis), the samples between 5 and 15 days after diagnosis, and the later samples (> 15 days after diagnosis).

Table 2: Sensitivity of the assay according to the manufacturer

Days post RT-PCR diagnosis	<12 AU/mL (negative)	12-15 AU/mL (equivocal)	>15 AU/mL (positive)	Total	Sensitivity, % (95% CI)
≤5 days	31	2	11	44	25.0% (14.6-39.4)
5-15 days	4	1	47	52	90.4% (79.4-95.8)
>15 days	1	0	38	39	97.4% (86.8-99.5)

Specificity

One thousand ninety presumed SARS-CoV-2 negative samples from a European laboratory routine (n=90) and European blood donors (n=1000) were tested resulting in 98.5% clinical specificity (95%CI: 97.6% – 99.1%).

Table 3: Specificity of the assay according to the manufacturer

Samples	<12 AU/mL (negative)	12-15 AU/mL (equivocal)	>15 AU/mL (positive)	Total	Sensitivity, % (95% CI)
Laboratory routine	89	0	1	90	98.9% (94.0-99.8)
Blood donors	985	8	7	1000	98.5% (97.5-99.2)

Interferences

Controlled studies of potentially interfering substances showed no interference to each substance listed below in the LIAISON SARS-CoV-2 S1/S2 IgG assay, at the indicated concentration.

Table 4: Interferences and their tested concentrations according to the manufacturer

Substances	**Tested concentrations**
Biotin	3500ng/mL
Triglycerides	3000mg/dL
Haemoglobin	1000mg/dL
Unconjugated bilirubin	40 mg/dL
Conjugated bilirubin	40 mg/dL
Cholesterol total	400 mg/dL
Paracetamol	500 mg/dL
Ibuprofen	500mg/dL

Cross-reactions

The cross-reactivity study for the LIAISON SARS-CoV-2 S1/S2 IgG assay was designed to evaluate potential interference from antibodies to other viruses that may cause symptoms similar to SARS-CoV-2 infection, other organisms that may cause infectious diseases, as well as from other conditions that may result from atypical immune system activity. Samples for the evaluation were collected before October 2019, prior to the SARS-CoV-2 pandemic. Three (3) specimens out of 168 assessed specimens resulted Positive with the LIAISON SARS-CoV-2 S1/S2 IgG assay. The observed specificity for potentially cross-reactive specimens is comparable to that of open populations.

Table 5: Cross-reactions according to the manufacturer

Condition	**Number of samples tested**	**LIAISON XL positive results**
Anti-nuclear autoantibodies (ANA)	10	0
Anti-HBV	10	1
Anti-HCV	10	0
Anti-Influenza A	10	1
Anti-Influenza B	10	0
Anti-respiratory syncytial virus	10	0
Anti-Borrelia burgdorferii	10	0
Anti-Mycoplasma pneumoniae	10	0
Anti-EBV antibodies	10	0
Anti-CMV	10	0
Anti-HIV ½	10	0
HAMA	10	0
Anti-Parvovirus B19	10	0
Rheumatoid factor	10	1
Anti-Rubella	10	0
Anti-VZV	10	0
Anti-Human CoV OC53	3	0
Anti-Human CoV HKU1	1	0
Anti-Human CoV unknown strain	4	0
Total	168	3

Testing of LIAISON SARS-CoV-2 S1/S2 IgG assay by PHE

Kits from batch 354011, exp date 01/11/2020 were used for the evaluation outlined below. The evaluation took place at PHE Porton Down on 28 May 2020; precision testing took place 2-10 June 2020.

In this evaluation, equivocal samples were included in the negative data set for consistency with other evaluations – note the instructions detail a repeat and/or second test process which the manufacturer recommends for an equivocal result; this was not possible due to limited sample volume and absence of a second sample.

Procedure for testing

Research operators from DSP and RIPL performed testing of kits using sample sets. All testing was performed per the manufacturer's instructions on a DiaSorin LIAISON XL instrument. The sample sets were:

- positive samples – 100 convalescent samples defined by a positive PCR from a swab sample for that patient; the interval (symptom onset date to sample collection date) is known for all samples
- confounder negative samples – 50 samples from the Sero-Epidemiology Unit (SEU), Manchester that are rheumatoid factor (12 samples), CMV (6 samples), EBV (19 samples) or VZV (13 samples) positive
- Porton negative samples – 50 samples from the RIPL 2015 Lyme disease negative sample collection and 313 historic negative samples from the Immunoassay group at Porton
- Manchester negative samples – 86 historic samples from the SEU

Testing results

Sensitivity

The overall sensitivity of the LIAISON assay was measured as being 64.0% (95%CI 53.8-73.4).

Table 6: Overall sensitivity of the LIAISON SARS-CoV-2 S1/S2 IgG assay from the PHE assessment

No. Samples	**Positive**	**Equivocal**	**Negative**	**Sensitivity (95% CI)**
100	64	6	30	64.0% (53.8-73.4)

The number of positive samples based on interval is given in Table 7 below.

Table 7: Assay sensitivity of the LIAISON SARS-CoV-2 S1/S2 IgG assay by interval when tested with PHE's sample set

Group	Interval (days)	Positive	Equivocal	Negative	Total	Sensitivity
Reported onset to sample date	<=10	3	0	8	11	27.3% (6.0-61.0)
	11-20	6	1	5	12	50.0% (21.1-78.9)
	21-30	25	3	9	37	67.6% (50.2-82.0)
	31-40	23	1	7	31	74.2% (55.4-88.1)
	41-50	7	1	1	9	77.8% (40.0-97.2)
	From 14 days	59	6	20	85	69.4% (58.5-79.0)
	From 21 days	55	5	17	77	71.4% (60.0-81.2)

Specificity

Three sample sets were used to determine the specificity of the assay, 50 confounder samples, 50 RIPL Lyme disease negative samples and 399 negative historical samples.

Table 8: Specificity of the LIAISON SARS-CoV-2 S1/S2 IgG assay from the PHE assessment

Category	n	Positive	Negative	Specificity (95% CI)
Negative samples	399	9	390	97.7% (95.8-99.0)
Confounder + RIPL samples	100	1	99	99.0% (94.6-100)

Positive and negative predictive values

The table below shows the positive predictive value (PPV) and negative predictive value (NPV), assuming a 10% seroprevalence in samples collected ≥14 days following onset of symptoms, with sensitivity calculated at 69.4% (59/85) and specificity calculated at 97.7% (390/399).

Table 9: Positive and negative predictive values assuming 10% seroprevalence

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	77.4% (64.5-88.2)	96.6% (95.5-97.7)

Precision

To demonstrate the repeatability of the assay, 5 sample pools representing a dilution series of SARS-CoV-2 antibody positive samples were run on 5 days with 5 runs per sample per day. The data in Table 10 below shows that the assay performed within acceptable parameters for precision with inter-assay %CV of <5 for 4 of the 5 sample pools tested.

Table 10: Precision data for Liaison SARS-CoV-2 S1/S2 IgG Serology Assay

	Mean/SD/%CV	Date of Testing					Inter-Assay Mean	Inter-Assay SD	Inter-Assay % CV
		Day 1 02/06/20	Day 2 04/06/20	Day 3 05/06/20	Day 4 08/06/20	Day 5 10/06/20			
Pool 1	Mean	79.62	77.98	77.60	75.14	78.62	77.79	2.83	3.64
	SD	2.51	1.61	1.75	2.44	4.03			
	% CV	3.16	2.07	2.26	3.24	5.13			
Pool 2	Mean	49.68	49.70	49.08	47.10	48.90	48.89	2.04	4.16
	SD	1.58	0.98	1.76	2.54	2.50			
	% CV	3.17	1.97	3.59	5.39	5.11			
Pool 3	Mean	29.18	30.14	29.12	28.96	29.30	29.34	1.05	3.57
	SD	1.07	0.75	1.12	1.18	1.07			
	% CV	3.66	2.50	3.85	4.09	3.64			
Pool 4	Mean	16.36	16.58	15.76	16.60	17.24	16.51	0.90	5.46
	SD	0.74	0.78	0.85	1.20	0.36			
	% CV	4.55	4.70	5.40	7.24	2.08			
Pool 5	Mean	8.29	8.52	8.28	8.30	7.80	8.24	0.36	4.39
	SD	0.36	0.20	0.27	0.34	0.29			
	% CV	4.36	2.36	3.34	4.11	3.66			

Statistical analysis

The plots below show the statistical analysis on the data obtained.

The scatterplot in Figure 1 shows the distribution of the samples by group (convalescent, confounder + RIPL samples and negative samples). The plot includes 2 cut off values at 12 AU/mL and 15 AU/mL. Results in this range are considered equivocal according to the manufacturer. For the purpose of this evaluation, a cut-off of 15 AU/mL was used to denote positive results.

Figure 1: Scatterplot of results by sample category

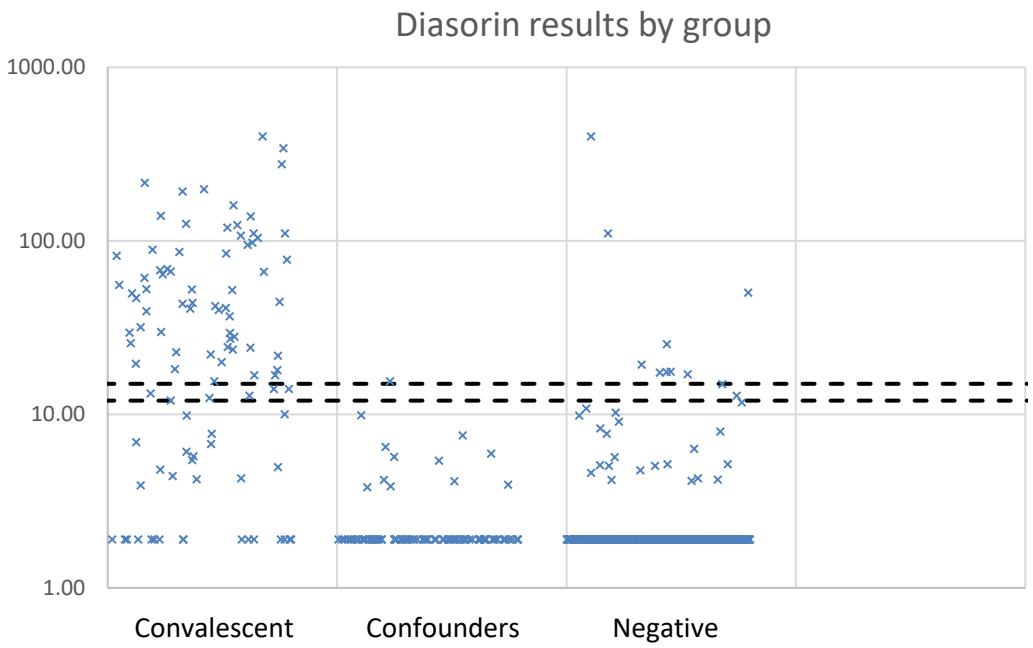
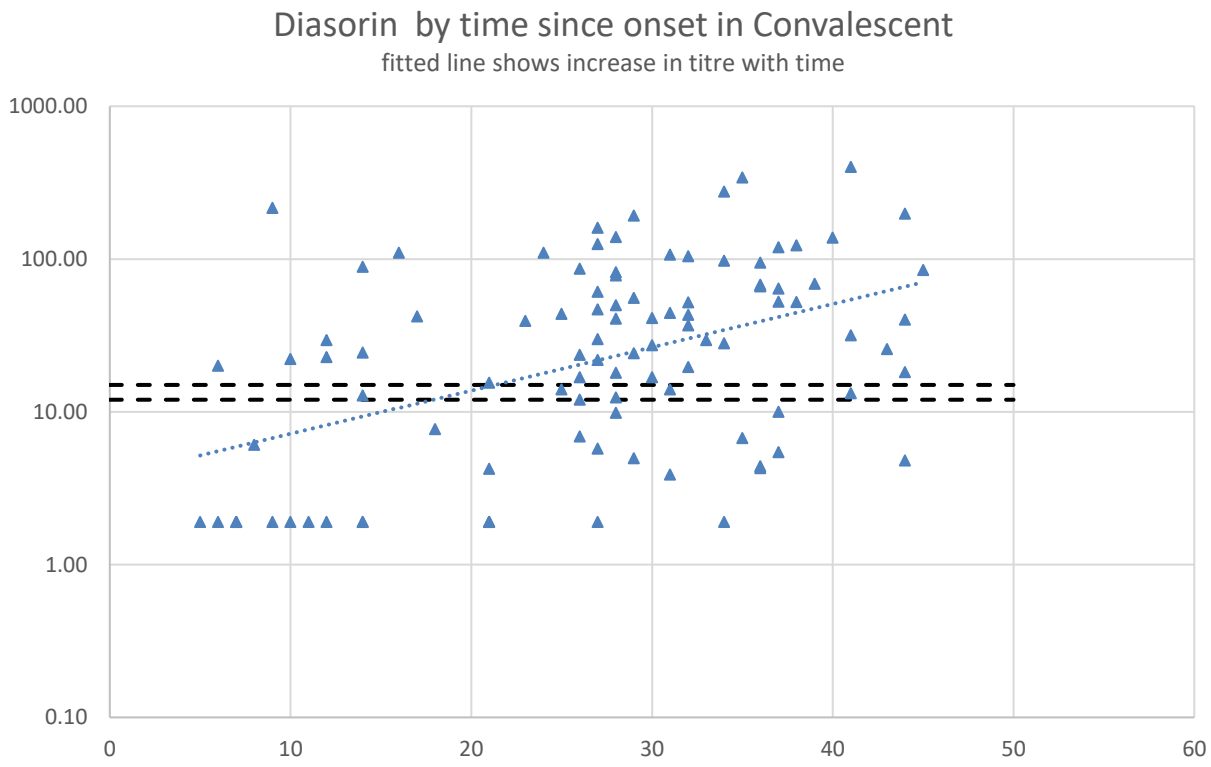


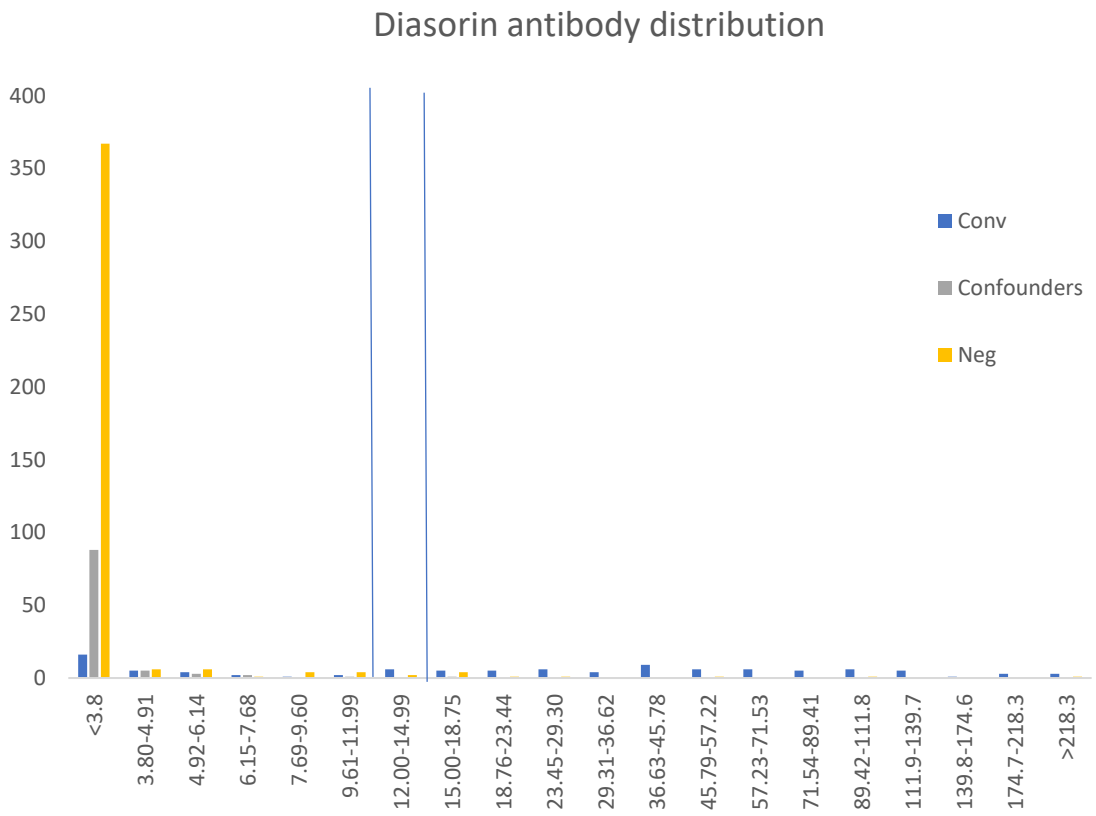
Figure 2 shows a scatterplot analysis of samples according to their time since symptom onset. The diagonal line in the plot shows the increase in antibody titre over time.

Figure 2: Scatterplot of time since symptom onset



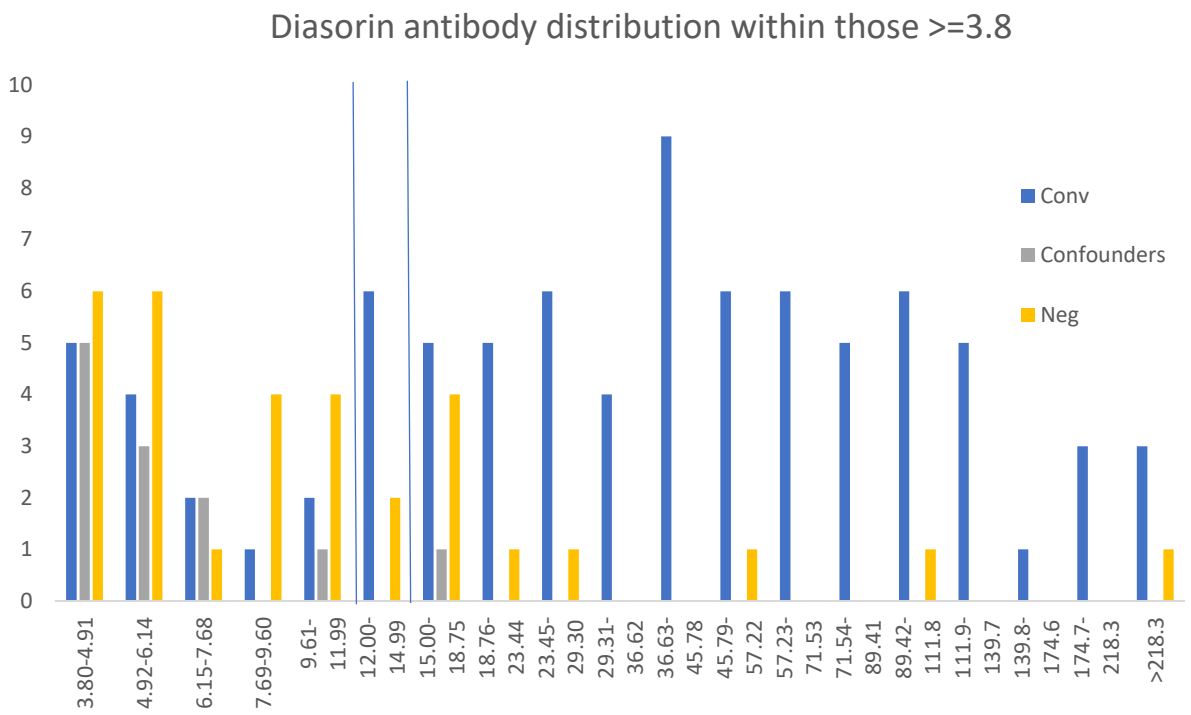
Figures 3a and b shows the distribution of antibodies as well as the manufacturer's cut-offs at 12 AU/mL and 15 AU/mL. It was not possible to assess the suitability of the cut-offs due to the large number of negative samples that gave results towards the lower limit of the assay.

Figure 3a: Antibody distribution on a logarithmic scale



The light blue lines denote the manufacturer's 2 cut off values at 12 AU/mL and 15 AU/mL

Figure 3b: Antibody distribution on a logarithmic scale, for samples ≥3.8 AU/mL



The light blue lines denote the manufacturer's 2 cut off values at 12 AU/mL and 15 AU/mL.

Conclusions

In conclusion, the DiaSorin Liaison SARS-CoV-2 S1/S2 IgG assay gave a specificity of 97.7% (95%CI 95.8-99.0) in this evaluation; the reported specificity of the manufacturer is 98.5% (95%CI 97.6-99.1).

In this evaluation, the sensitivity of the DiaSorin Liaison SARS-CoV-2 S1/S2 IgG assay measured 69.4% (95%CI 58.5-79.0) for samples collected ≥14 post symptom onset and 71.4% (95%CI 60.0-81.2) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity was 64.0% (95%CI 53.8-73.4). The manufacturer reported a sensitivity of 90.4% (95%CI 79.4-95.8) for samples 5-15 days and a sensitivity of 97.4% (95%CI 86.8-99.5) for samples taken >15 days' post RT-PCR diagnosis.