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Evaluation of the Beckman Coulter Access Anti-SARS-CoV-2 IgG assay for the detection of anti-SARS-CoV-2 antibodies

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Executive summary

This document sets out the evaluation of the Beckman Coulter Access SARS-CoV-2 IgG 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 between 24 June 2020 and 2 July 2020. 100 serum samples from convalescent patients and 499 negative samples were included in the assessment.

The assay gave a **specificity** of 99.3% (95% confidence interval 97.8-99.8). The manufacturer reported a specificity of 99.8% (95%CI 99.4-99.9).

The assay gave an overall **sensitivity** of 69.0% (95%CI 59.0-77.9), with a sensitivity ≥14 days of 76.5% (95%CI 66.0-85.0). The sensitivity of the assay at ≥21 days' post symptom onset is 79.2% (95%CI 68.5-87.6). The manufacturer reported a sensitivity of 100.0% (95%CI 93.8-100.0) for samples taken >18 days between the positive PCR test and the blood sample draw.

Introduction

The Access SARS-CoV-2 IgG assay, manufactured by Beckman Coulter, is intended for the detection of IgG antibody to SARS-CoV-2 in human serum and plasma. The assay is a chemiluminescent assay and can be processed on an automatic analyser. The assay constitutes a supplement to direct pathogen detection and can also be used to collect epidemiological data. This report details an evaluation of the assay conducted at PHE Porton Down between 24 June 2020 and 2 July 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.

Access SARS-CoV-2 IgG assay

Test principle

The Access SARS-CoV-2 IgG assay is a paramagnetic particle, chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum, serum separator tubes and plasma (EDTA, citrate and heparin) using the Access Immunoassay Systems.

The Access SARS-CoV-2 IgG assay is a 2-step enzyme immunoassay. A sample is added to a reaction vessel with buffer, and paramagnetic particles coated with recombinant SARS-CoV-2 protein specific for the receptor binding domain (RBD) of the S1 protein. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. A monoclonal anti-human IgG alkaline phosphatase conjugate is added and the conjugate binds to the IgG antibodies captured on the particles. A second separation and wash step remove unbound conjugate. A chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is compared to the cut-off value defined during calibration of the instrument.

This assay is listed as CE marked.

Interpretation of the result

Test results are determined automatically by the system software. Detection of analyte in the sample is determined from the measured light production by means of the stored calibration data. Results are reported as reactive or non-reactive. Results located 20% below the cut-off are interpreted as equivocal, and should be carefully reviewed. For samples in the equivocal zone, the manufacturer recommends a new sample should be collected and tested approximately 1 to 2 weeks later using the Access SARS-CoV-2 IgG assay. A conversion from equivocal to reactive for IgG antibody should be considered as evidence of seroconversion due to recent infection.

Table 1: Manufacturer's result interpretation and reporting.

Result	Interpretation	Reporting instructions
≤ 0.80 S/CO SARS-CoV-2 IgG	Non-reactive	Report result as non-reactive for SARS-CoV-2 IgG antibodies
> 0.80 to < 1.00 S/CO SARS-CoV-2 IgG	Equivocal	Report as equivocal for SARS-CoV-2 IgG antibodies. Collect a new sample 1 or 2 weeks later and retest.
≥ 1.00 S/CO SARS-CoV-2 IgG	Reactive	Report result as reactive for SARS-CoV-2 IgG antibodies

Manufacturer's listed limitations

These limitations include:

- do not dilute samples as this could lead to incorrect results
- for assays that employ antibodies, the possibility exists for interference by heterophile antibodies in the test sample. Patients who are regularly exposed to animals, or are subjected to medical treatments that utilize immunoglobulins or immunoglobulin fragments, may produce human anti-animal antibodies, for example HAMA, that interfere with immunoassays – these interfering antibodies may cause erroneous results
- other potential interferences could be present in the sample and may cause erroneous results in immunoassays. Some examples that are documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase – carefully evaluate results if the sample is suspected of having these types of interferences
- the Access SARS-CoV-2 IgG assay results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information
- negative results do not preclude acute SARS-CoV-2 infection – if acute infection is suspected, direct testing for SARS-CoV-2 is necessary
- false positive test results for IgG antibodies can occur due to cross-reactivity with pre-existing antibodies or from other possible causes
- this test cannot be used to diagnose an acute SARS-CoV-2 infection

Manufacturer's performance characteristics

Sensitivity

The clinical sensitivity of the Access SARS-CoV-2 IgG assay was evaluated in 192 individual patients in a study of 247 serum and plasma samples from symptomatic individuals diagnosed with SARS-CoV-2 by PCR methods from France and the United States. The results are presented in the following table, classified by days between the positive PCR test and the blood sample draw. The evaluation was determined by the Wilson Score method. Two samples recorded as equivocal (from >0.80 to < 1.00 S/CO) were excluded from clinical sensitivity calculation.

Table 2: Manufacturer's reported sensitivity

Days between positive PCR and sample collection	Total samples	Number non-reactive	Number reactive	Number equivocal	Clinical sensitvity (95% CI)
0-6	47	14	33	0	70.2% (56.0-81.3)
7-14	88	4	84	0	95.5% (88.9-98.2)
>14	112	1	109	2	99.1% (95.0-99.8)
>18	58	0	58	0	100.0% (93.8-100.0)

Specificity

The clinical specificity of the Access SARS-CoV-2 IgG assay was evaluated in a study of 1,400 samples collected prior to December 2019 in France and the United States. This total includes 1,000 samples from blood donors in France and 200 samples each from routine clinical laboratory diagnostic samples in France and the United States. Based on this evaluation, the overall clinical specificity of the Access SARS-CoV-2 IgG assay is 99.8% (1395/1398), with a 95% confidence interval of 99.4% - 99.9% determined by the Wilson Score method. Two samples with equivocal results (from > 0.80 to < 1.00 S/CO) were excluded from clinical specificity calculation.

Table 3: Manufacturer's reported specificity

Population	Total samples	Number non-reactive	Number reactive	Number equivocal	Clinical specificity (95% CI)
Blood donors (France)	1,000	997	2	1	99.8% (99.3-99.9)
Diagnostic samples (France)	200	199	1	0	99.5% (97.2-99.9)
Diagnostic samples (USA)	200	199	0	1	100% (98.1-100.0)
Total	1,400	1,395	3	2	99.8% (99.4-99.9)

Interferences

High concentrations of endogenous serum components were assessed for interference in the Access SARS-CoV-2 IgG assay. The test protocol was based on CLSI EP07, Interference Testing in Clinical Chemistry, 3rd Edition. Human serum was spiked with a patient sample containing SARS-CoV-2 IgG antibodies to achieve a positive reactivity in the Access SARS- CoV-2 IgG assay. None of the substances tested demonstrated significant interference in the Access SARS-CoV-2 IgG assay as defined by a shift is concentration greater than 20% using the test concentrations indicated in the table below.

Table 4: Manufacturer's reported interferences

Substance	Interferent concentration tested
Bilirubin (conjugated)	43 mg/dL
Bilirubin (unconjugated)	43 mg/dL
Haemoglobin	300 mg/dL
Triglycerides (Triolein)	1,500 mg/dL

Cross-reactions

Cross-reactivity of the Access SARS-CoV-2 IgG assay was evaluated by testing serum and plasma samples for each of the potentially cross-reacting conditions listed in the following table. No cross-reactivity was observed for the Access SARS-CoV-2 IgG assay.

Table 5: Manufacturer's reported cross-reactions

Category of samples	Number of samples	Number reactive	Number non-reactive
Anti-influenza A	5	0	5
Anti-influenza B	5	0	5
Anti-hepatitis C virus (HCV)	5	0	5
Anti-hepatitis B virus (HBV)	5	0	5
Anti-HIV	10	0	10
Anti-nuclear antibodies (ANA)	5	0	5
Adenovirus positive IgG	2	0	2
Cytomegalovirus positive IgG	7	0	7
Anti-rheumatoid factor	5	0	5

Testing of Access SARS-CoV-2 IgG assay by PHE

Lot 971197 of Access SARS-CoV-2 IgG assay was received from Beckman Coulter. The evaluation took place on an Access 2 instrument at PHE Porton Down between 24 June 2020 and 2 July 2020.

Procedure for testing

Research operators from DSP performed testing of kits using the following sample sets. All testing was performed per the manufacturer's instructions:

- 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 and samples were taken from patients with a range of disease severities
- 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, 313 historic negative samples from PHE Immunoassay Group (IAG)
- Manchester negative samples: 86 historic samples from the SEU

This sample set was constructed to cover the same range as other evaluations but some individual samples were changed as the original sample was exhausted.

Testing results

Sensitivity

Table 6: Overall sensitivity of the Access SARS-CoV-2 IgG assay from the PHE assessment

No. Samples	Positive	Negative	Sensitivity (95% CI)
100	69	31	69.0% (59.0-77.9)

Three equivocal samples (see Table 7 below) were considered to be negative in line with other evaluations. The manufacturer recommends to report as equivocal for SARS-CoV-2 IgG antibodies and to collect a new sample 1 or 2 weeks later and retest.

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

Table 7: Sensitivity of the Access SARS-CoV-2 IgG assay by interval

Group	Interval (days)	Positive	Equivocal	Negative	Total	Sensitivity (95% CI)
Reported onset to sample date	<= 10	2	0	9	11	18.2% (2.3-51.8)
	11 to 20	6	1	5	12	50.0% (21.1-78.9)
	21 to 30	30	0	7	37	81.1% (64.8-92.0)
	31 to 40	25	1	5	31	80.6% (62.5-92.5)
	41 to 50	6	1	2	9	66.7% (29.9-92.5)
	From 14 days	65	3	17	85	76.5% (66.0-85.0)
	From 21 days	61	2	14	77	79.2% (68.5-87.6)

Specificity

Three negative sample sets were used to determine the specificity of the assay

Table 8: Specificity of the Access SARS-CoV-2 IgG assay from the PHE assessment

Category	n	Positive	Negative	Specificity (95% CI)
Confounder + RIPL samples	100	3	97	97.0% (91.5-99.4)
Negative samples	399	3	396	99.3% (97.8-99.8)

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 76.5% (65/85) and specificity calculated at 99.2% (396/399).

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

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	91.9% (79.6-98.2)	97.4% (96.3-98.3)

Precision testing

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 shows that the assay performed within acceptable parameters for precision with inter-assay %CV of <7 for each sample pool tested. The results are presented in Table 10.

Table 10: Precision data for Access SARS-CoV-2 IgG Assay.

	Mean/SD/%CV	Date of Testing					Inter-assay Mean	Inter-assay SD	Inter-assay % CV
		Day 1 24/06/20	Day 2 25/06/20	Day 3 26/06/20	Day 4 30/06/20	Day 5 01/07/20			
Pool 1	Mean	14.44	16.48	16.04	15.48	16.51	15.79	0.98	6.19
	SD	0.37	0.78	0.41	0.73	0.76			
	% CV	2.58	4.73	2.58	4.72	4.61			
Pool 2	Mean	8.19	8.19	7.99	8.03	8.06	8.00	0.29	3.58
	SD	0.21	0.21	0.17	0.29	0.40			
	% CV	2.51	2.51	2.17	3.55	5.00			
Pool 3	Mean	3.37	3.31	3.12	3.04	3.37	3.24	0.18	5.52
	SD	0.14	0.12	0.15	0.08	0.14			
	% CV	4.16	5.11	4.76	2.70	4.10			
Pool 4	Mean	1.28	1.35	1.30	1.28	1.27	1.30	0.08	5.96
	SD	0.07	0.07	0.10	0.09	0.04			
	% CV	5.65	5.11	7.65	7.18	2.99			
Pool 5	Mean	0.50	0.51	0.49	0.46	0.50	0.49	0.02	4.92
	SD	0.02	0.02	0.01	0.02	0.01			
	% CV	3.46	4.69	1.70	3.27	5.26			

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). There is a tighter grouping of samples in the negative sample sets with the positive samples showing a wider distribution of assay results.

Figure 1: Scatterplot of results by sample category

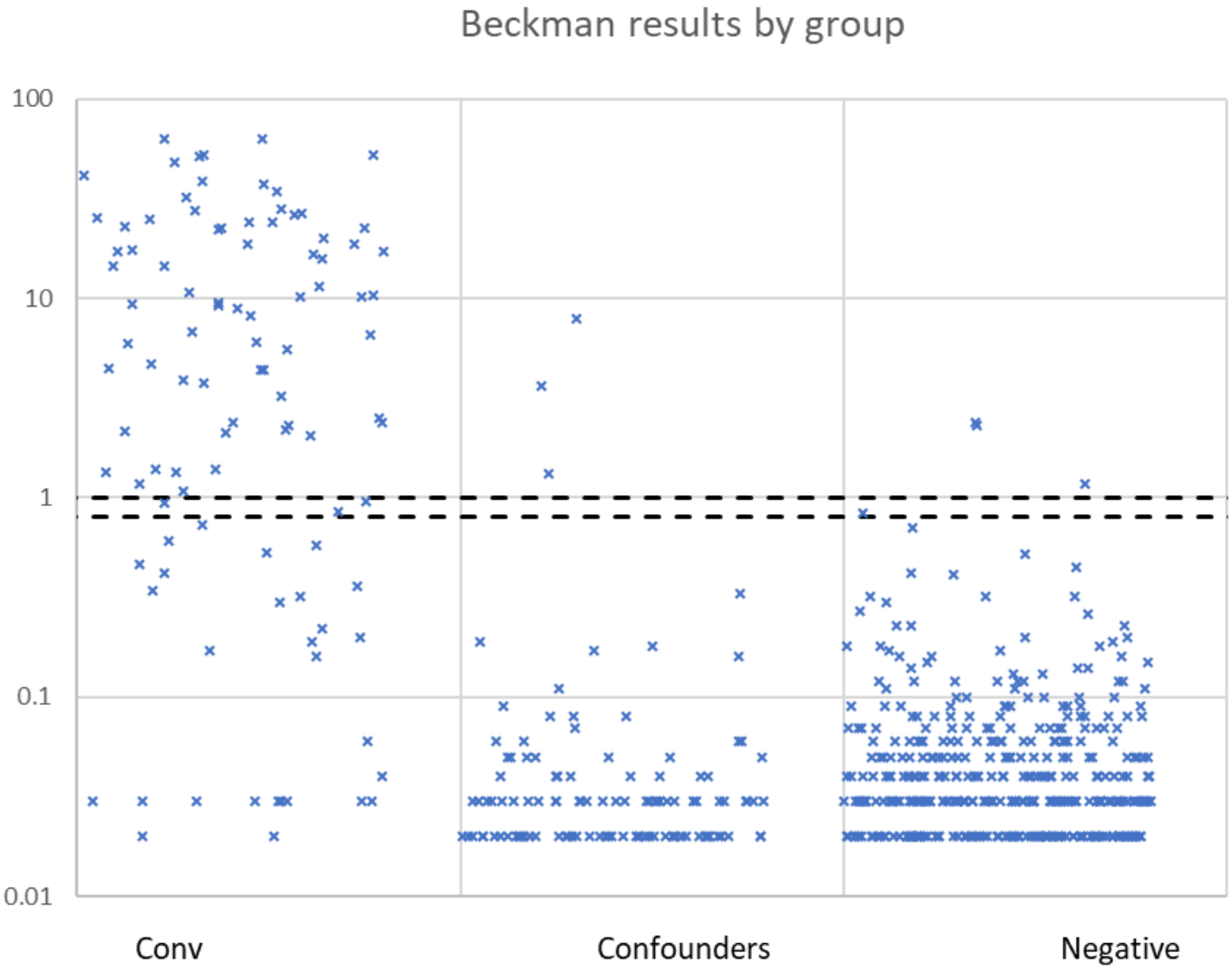


Figure 2: Scatterplot analysis of samples according to their time since symptom onset

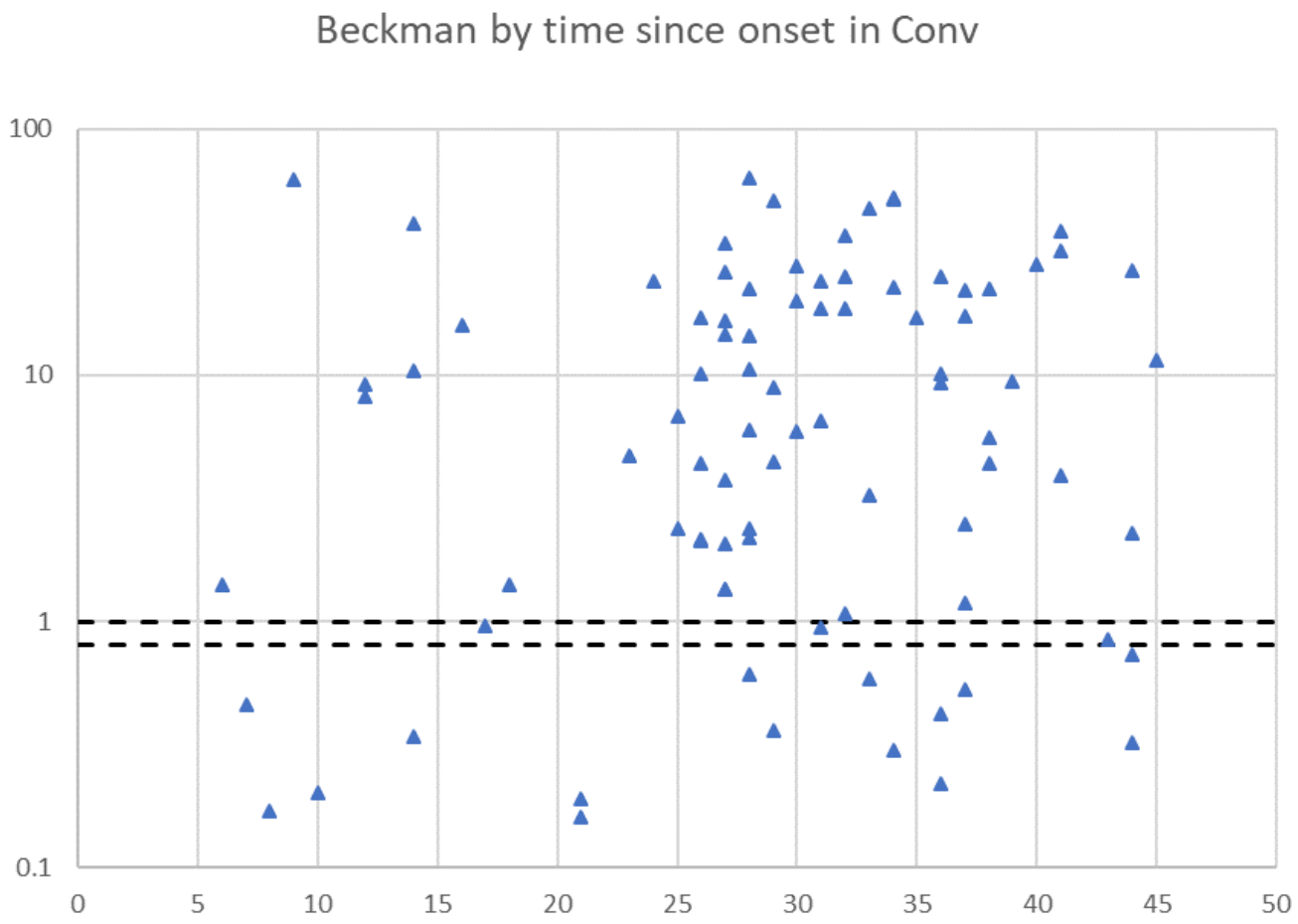


Figure 3 shows the distribution of antibodies against the manufacturer's cut-off. To assess the cut-off for the assay, the distribution of the assay units in the negative samples are assessed (see Figure 4). It is usually desirable that a cut-off is set at least about 3 standard deviations (SD) above the mean of the negatives. This calculation assumes the negative samples are normally distributed (usually on a log-scale) but for the COVID-19 assays it is apparent that the negative distribution is often positively skewed. In addition, some negatives are clearly outliers from the main negative distribution so should be excluded. Therefore, to identify a +3SD cut-point, clear outliers were dropped (clearly above assay cut-offs if any existed) and only the right-hand tail of the negative distribution was used to fit a half-normal distribution using all results above an appropriate cut-point that ideally gives a reasonable fit for the half-normal. This can then be used to identify a 3SD cut-point from this distribution as well as obtain a z-score and theoretical specificity of the manufacturer cut-off. Looking at those with results <2 the mean was 0.043 (-1.37 log10) and the half-normal standard deviation was 0.431 (log10) (right hand part of the distribution above a value of 0.05). 0.05 + 2.58 SD = 0.67 (anti-logged) and 0.05 + 3SD = 1.02 (anti-logged). So a cut-off of mean + 3 SD of 1.02 is close to the manufacturer's cut-off. The manufatcurer cut-off gives a theoretical specificity of 99.8% ignoring outlier false positives.

Figure 3: Antibody distribution on a logarithmic scale. The light blue line denotes the manufacturer's cut-off between 0.8 and 1.0 S/C

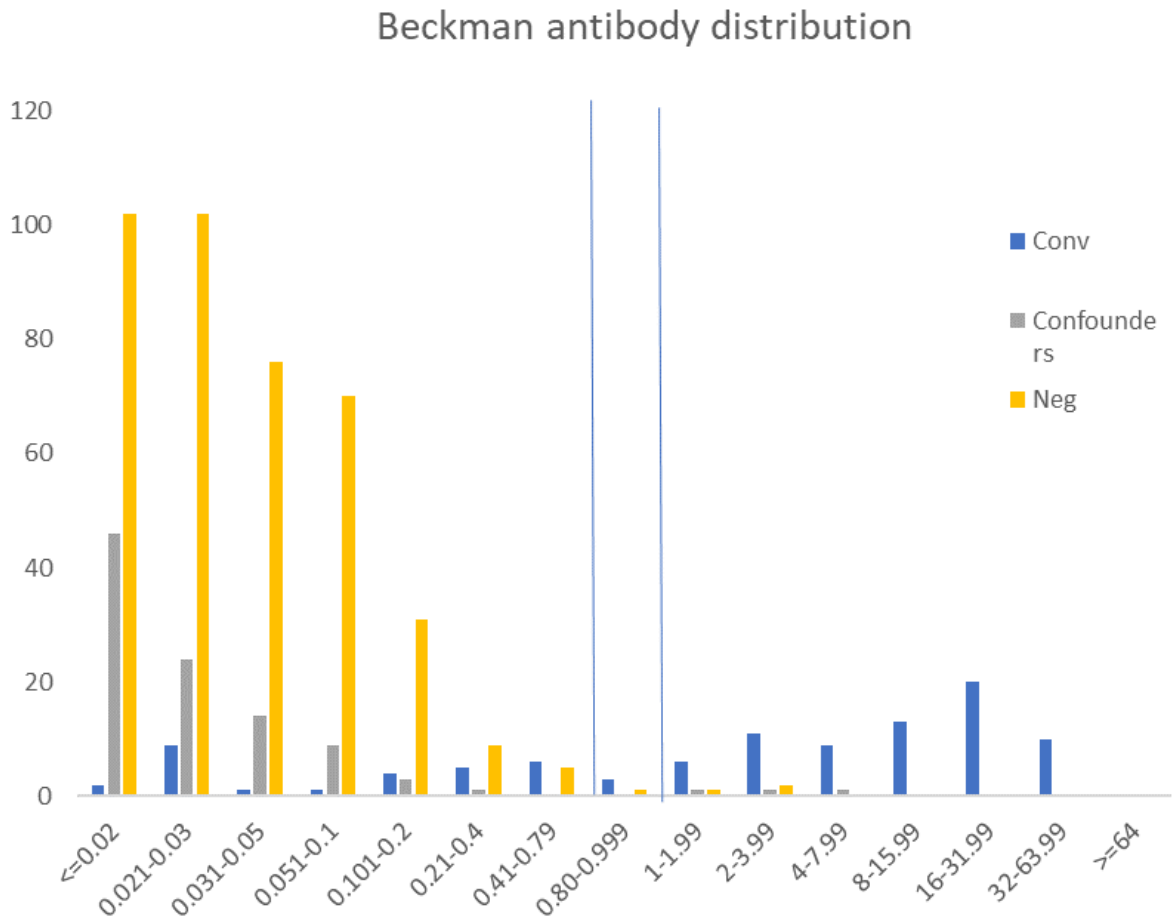
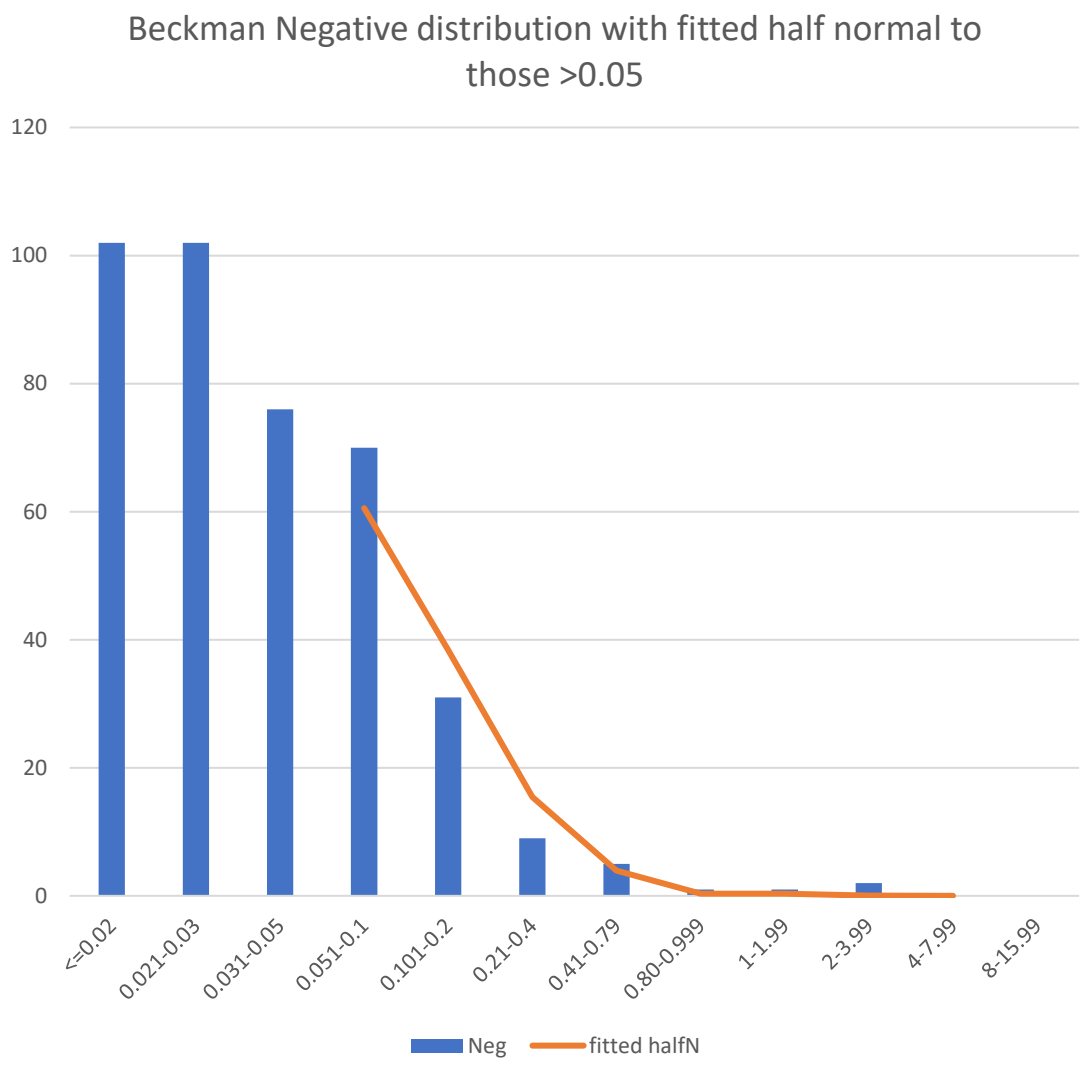


Figure 4: Negative distribution with a fitted half normal



Conclusions

In conclusion, the Access SARS-CoV-2 IgG assay gave a specificity of 99.3% (95%CI 97.8-99.8) in this evaluation; the reported specificity of the manufacturer is 99.8% (95%CI 99.4-99.9).

In this evaluation, the sensitivity of the Access SARS-CoV-2 IgG assay increased from 76.5% (95%CI 66.0-85.0) for samples collected ≥14 days' post symptom onset to 79.2% (95%CI 68.5-87.6) for samples collected ≥21 days' post symptom onset. For all samples, the sensitivity was 69.0% (95%CI 59.0-77.9). The manufacturer reported a sensitivity of 99.1% (95.0-99.8) for samples >14 days' post symptom onset and a sensitivity of 100.0% (95%CI 93.8-100.0) for samples taken >18 days between the positive PCR test and the blood sample draw.