Public Health England

Protecting and improving the nation's health

Evaluation of Roche Elecsys Anti-SARS-CoV-2 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 Roche Elecsys Anti-SARS-CoV-2 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 between 5-7 May 2020. Ninety-three serum samples from convalescent patients and 472 negative samples were included in the assessment.

All negative samples tested negative by the assay, giving a specificity of 100%. This accords with the manufacturer's reported specificity of 100%.

The assay gave an overall sensitivity of 83.87%, with a sensitivity ≥14 days of 87.0%. This is lower than the manufacturer's reported sensitivity of 100% for samples taken ≥14 days post symptom onset. The sensitivity of the assay at ≥21 days post symptom onset is 87.7%, rising to 100% for samples >40 days after symptom onset.

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Introduction

Elecsys Anti-SARS-CoV-2 serology assay is intended for the detection of IgM and IgG antibodies to SARS-CoV-2 in human serum and plasma. The assay is an **e**lectro**c**hemi**l**uminescent **i**mmuno**a**ssay (ECLIA). The ECLIA assay is intended for use on the Roche Cobas E immunoassay analysers. This report details an evaluation of the ECLIA assay conducted at PHE Porton Down between 5-7 May 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.

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Roche Elecsys Anti-Sars-CoV-2 Assay

The Elecsys Anti-SARS-CoV-2 assay is an ECLIA assay manufactured by Roche Diagnostics GmbH. The assay is listed as CE marked.

As per the manufacturer's information, the assay uses a recombinant protein representing the nucleocapsid (N) protein of SARS-CoV-2.

Test Principle

The assay is a sandwich immunoassay with a total duration of 18 minutes from start to result per sample. There are four main steps in the assay which are:

- 1st incubation: 20 µL of sample, biotinylated SARS-CoV-2-specific recombinant antigen and SARS-CoV-2-specific recombinant antigen labelled with a ruthenium complex* form a sandwich complex
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin
- the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier
- results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cut off value previously obtained by calibration

* Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy))

The sample volume used in the assay is 20µL; the total minimum sample volume required to run the assay is 100µL.

Interpretation of the Result

The kits contain two controls: ACOV2 Cal1 containing human serum, non-reactive for anti-SARS-CoV-2 antibodies, and ACOV Cal2 containing human serum reactive for anti-SARS-CoV-2 antibodies. The analyser automatically calculates the cut off based on the measurement of ACOV2 Cal1 and ACOV2 Cal2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cut off index (COI; signal sample/cut off). The results can be interpreted as follows:

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Numeric Result	Result Message	Interpretation
COI <1.0	Non-reactive	Negative for anti-SARS-CoV-2 antibodies
COI ≥1.0	Reactive	Positive for anti-SARS-CoV-2 antibodies

Table 1: Manufacturer's interpretation of the results

Manufacturer's listed limitations

The limitations of the assay are:

- the magnitude of the measured result above the cut-off is not indicative of the total amount of antibody in the sample
- the individual immune response following SARS-CoV-2 infection varies considerably and might give different results with assays from different manufacturers. Results from different manufacturers should not be used interchangeably
- for diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings
- a negative test result does not completely rule out the possibility of an infection with SARS-CoV-2. Serum or plasma samples from the very early (pre-seroconversion) phase can yield negative findings. Therefore, this test cannot be used to diagnose an acute infection. Also, over time, titres may decline and eventually become negative

Sensitivity and Specificity

A total of 204 samples from 69 symptomatic patients with a PCR confirmed SARS-CoV-2 infection were tested with the Elecsys Anti-SARS-CoV-2 assay. One or more consecutive samples from these patients were collected after PCR confirmation at various time points.

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Days post PCR confirmation	N	Reactive	Non-reactive	Sensitivity, % (95% CI)
0-6	116	76	40	65.5 (56.1-74.1%)
7-13	59	52	7	88.1 (77.1-95.1%)
≥ 14	29	29	0	100 (88.1-100%)

Table 2: Sensitivity of the assay according to the manufacturer

A total of 5272 samples were tested with the Elecsys Anti-SARS-CoV-2 assay. All samples were obtained before December 2019. 10 false positive samples were detected. The resulting overall specificity in the internal study was 99.81%. The 95% lower confidence limit was 99.65%.

Interferences

Interference was tested with the endogenous substance biotin up to a concentration of 4912 nmol/L or 1200 ng/mL. No impact on results was observed. Potential endogenous interferences e.g. haemolysis, bilirubin, rheumatoid factors and pharmaceutical compounds other than biotin were not tested and an interference cannot be excluded.

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Testing of Elecsys Anti-SARS-CoV-2 assay by PHE

Three kits of the Elecsys Anti-SARS-CoV-2 (Lot 49025901, exp 31/05/20) were obtained from Roche on 4 May 2020. Two further kits were delivered on 7 May 2020 and were used for precision testing.

Procedure for testing

Research operators from DSP and RIPL performed testing of kits using the following sample sets. All testing was performed per the manufacturer's instructions on a Roche Cobas e 411 instrument.

- positive samples- 93 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 77 samples. Two of these samples had an unknown interval. The remaining 14 samples, the interval is the patient admitted to hospital to sample collection date so the interval for these samples is artificially low
- confounder negative samples- 50 samples from the Sero-Evaluation Unit (SEU), Manchester that are rheumatoid factor (12 samples), CMV (6 samples), EBV (19 samples) or VZV (13 samples) positive. All but one were negative using the EuroImmun IgG assay
- Porton negative samples- 35 samples from the RIPL 2015 Lyme disease negative sample collection
- Manchester negative samples- 387 historic samples from the Seroepidemiology Unit (SEU)

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Testing results

Sensitivity

Total number of convalescent samples (n)	Positive	Negative	Sensitivity
93	78	15	83.87% (74.8-90.7)

Table 3: Overall sensitivity of the assay from the PHE assessment

Please note that the sensitivity of the assay according to different intervals (date of symptom onset to sample collection) is not given here, as this data was missing from 14 samples. Of the remaining samples, only 2 had an interval of ≤14 days.

The number of positive samples based on interval is given in Table 4 below

Group	Positive	Negative	Total	Sensitivity (95% CI)
<= 10 from admission	10	4	14	71.4% (42.0-90.4)
11 to 20	3	1	4	75.0% (21.9-98.7)
21 to 30	28	7	35	80.0% (62.5-90.9)
31 to 40	28	2	30	93.3% (76.5-98.8)
41 to 50	8	0	8	100.0% (59.8-100)
Interval not known	1	1	2	50.0% (26.7-97.3)
From 14 days	64	9	73	87.7% (77.9-93.8)
From 21 days	67	19	77	87.0% (67.4-85.8)

Table 4:Assay sensitivity by interval when tested with PHE's sample set

The data accords with the manufacturer's supplied data and the sensitivity increases as the interval increases, with 100% positivity seen at over 40 days since symptom onset. The samples in the first row had an unclear interval, as the date from admission into

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hospital was supplied rather than the date of symptom onset but appears to align with a symptom onset around 20 days.

Specificity

Three sample sets were used to determine the specificity of the assay: 50 confounder samples, 35 RIPL Lyme disease negative samples and 387 negative historical samples.

Category	**n**	**Reactive**	**Non-reactive**	**Specificity (95% CI)**
Total	472	0	472	100% (94.2-100)
Confounder + RIPL samples	85	0	85	100% (95.8-100.0)
Negative samples	387	0	387	100% (99.1-100.0)

Table 5: Specificity of the assay from the PHE assessment

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 specificity calculated at 100%

Seroprevalence	**PPV (95%CI)**	**NPV (95%CI)**
10%	100% (91.1-100)	98.6% (97.6-99.3)

Table 6: Positive and negative predictive values assuming 10% seroprevalence

Precision

To demonstrate the repeatability of the assay, four pools of SARS-CoV-2 antibody positive samples and one pool of SARS-CoV-2 negative samples were run on five consecutive 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 <5 for each sample pool tested. Data is shown in table 7 below.

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Sample ID	Mean/SD/%CV	Date of Testing					Inter-Assay Mean	Inter-Assay SD	Inter-Assay % CV
		Day 1 11/05/20	Day 2 12/05/20	Day 3 13/05/20	Day 4 14/05/20	Day 5 15/05/20			
15067	Mean	30.89	30.57	29.83	29.91	29.32	30.10	0.79	2.61
	SD	0.54	0.54	0.92	0.35	0.46			
	% CV	1.74	1.77	3.09	1.16	1.58			
15068	Mean	16.04	15.88	15.37	15.05	14.71	15.41	0.57	3.70
	SD	0.21	0.31	0.19	0.35	0.31			
	% CV	1.3	1.93	1.23	2.35	2.09			
15069	Mean	0.08	0.08	0.09	0.08	0.09	0.08	0.00	4.59
	SD	0.00	0.00	0.00	0.00	0.00			
	% CV	3.98	3.08	4.87	2.85	4.59			
15116	Mean	39.12	36.53	36.43	37.62	37.57	37.13	1.25	3.38
	SD	0.65	0.64	0.35	0.58	0.39			
	% CV	1.66	1.76	0.97	1.54	1.10			
15117	Mean	42.2	42.03	42.03	42.01	41.61	41.98	0.86	2.06
	SD	0.99	1.56	0.24	0.62	0.62			
	% CV	2.35	3.71	0.58	1.46	1.48			

Table 7: Precision data for Roche Elecsys Anti-SARS-CoV-2 assay.

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Statistical Analysis

The scatterplot in Figure 1 shows the distribution of the samples by group (convalescent, confounder + RIPL samples and negative samples). There is very little variation with the negative samples, that pool around the COI 0.1 mark, with a few high negative values. The convalescent samples are much more widely distributed with some samples pooling with the high negatives just below the cut-off of COI 1.0.

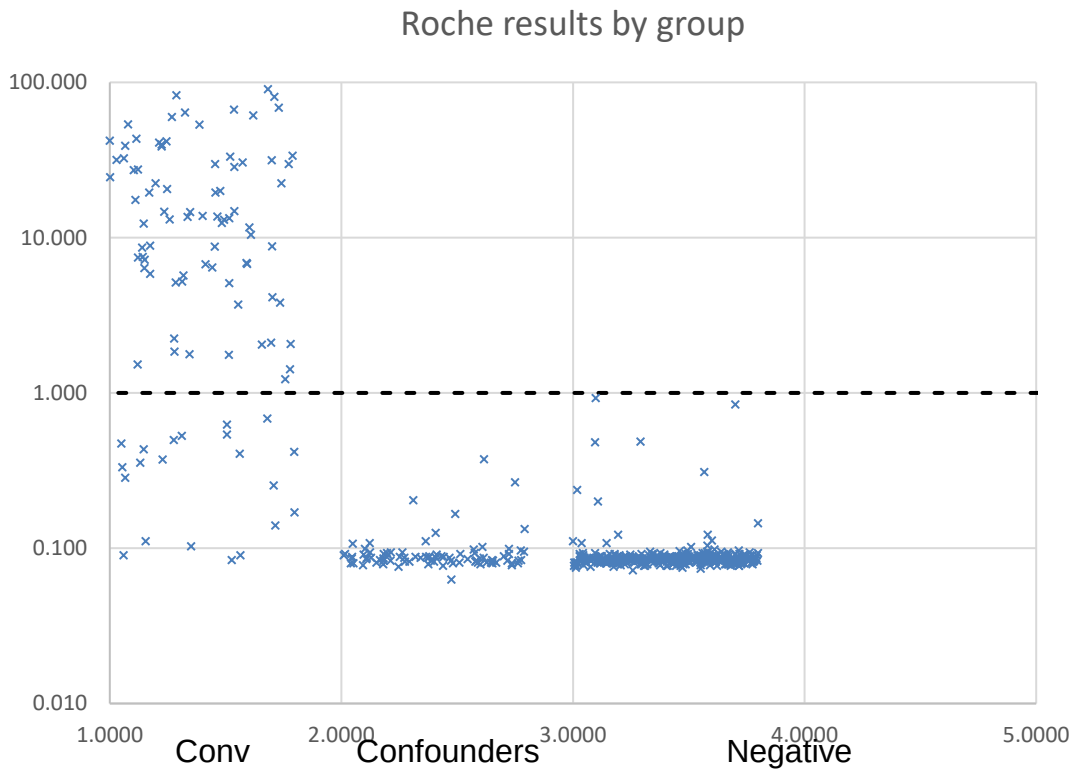

Figure 1: Scatterplot of results by sample category

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Figure 2 shows a scatterplot analysis of samples according to their time since symptom onset. For this analysis, 14 samples that did not have an accurate time since onset (the dates supplied were the admission to hospital dates rather than the time since symptom onset) were not included in the analysis.

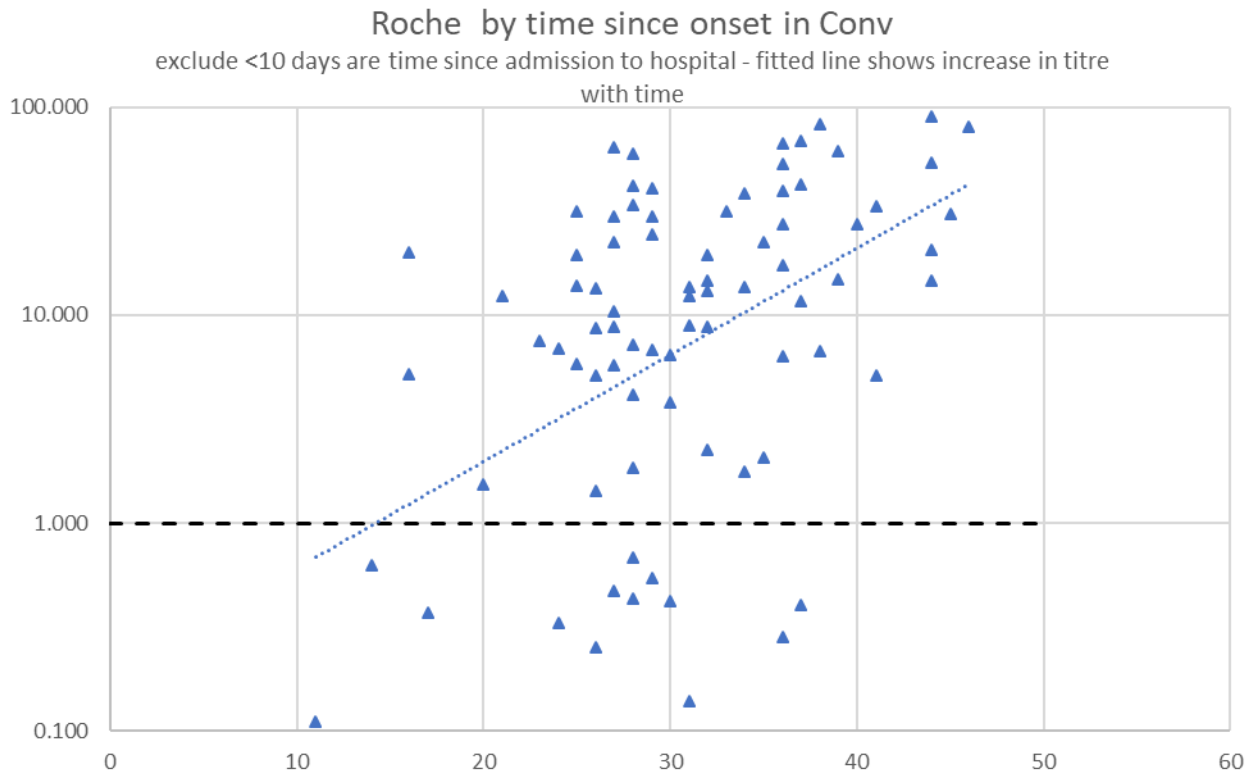

Figure 2: Scatterplot of time since symptom onset (excluding 14 samples that did not have an accurate time since symptom onset)

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Figure 3 shows the distribution of antibodies against the manufacturer's cut-off of COI 1.0. The results indicate a heavy tail to the negative distribution. 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 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 log10 results <0.8 the mean was 0.086 and SD 0.170 of those above the mean (right hand part of the distribution). Mean + 2.58 SD = 0.399 and mean + 3SD = 0.519. Therefore, a cut-off of mean + 3 SD of 0.519 is well below the manufacturer's cut-off. This gives a theoretical specificity of 100%.

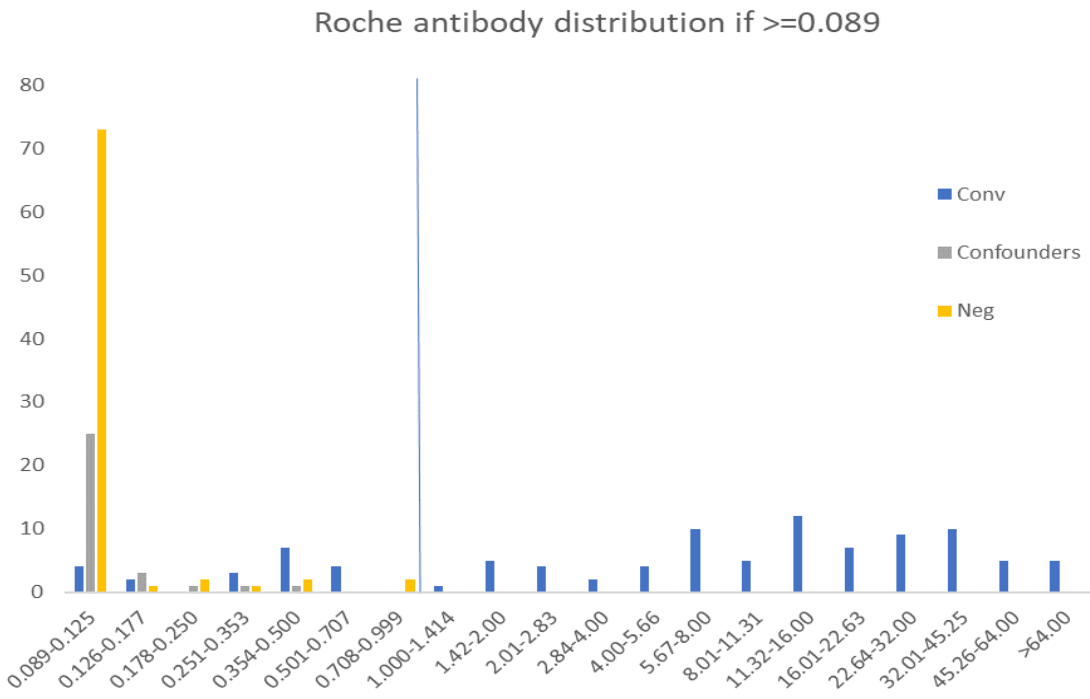

Figure 3: Antibody distribution on a logarithmic scale. The light blue line denotes the manufacturer's cut-off at a value of COI 1.0

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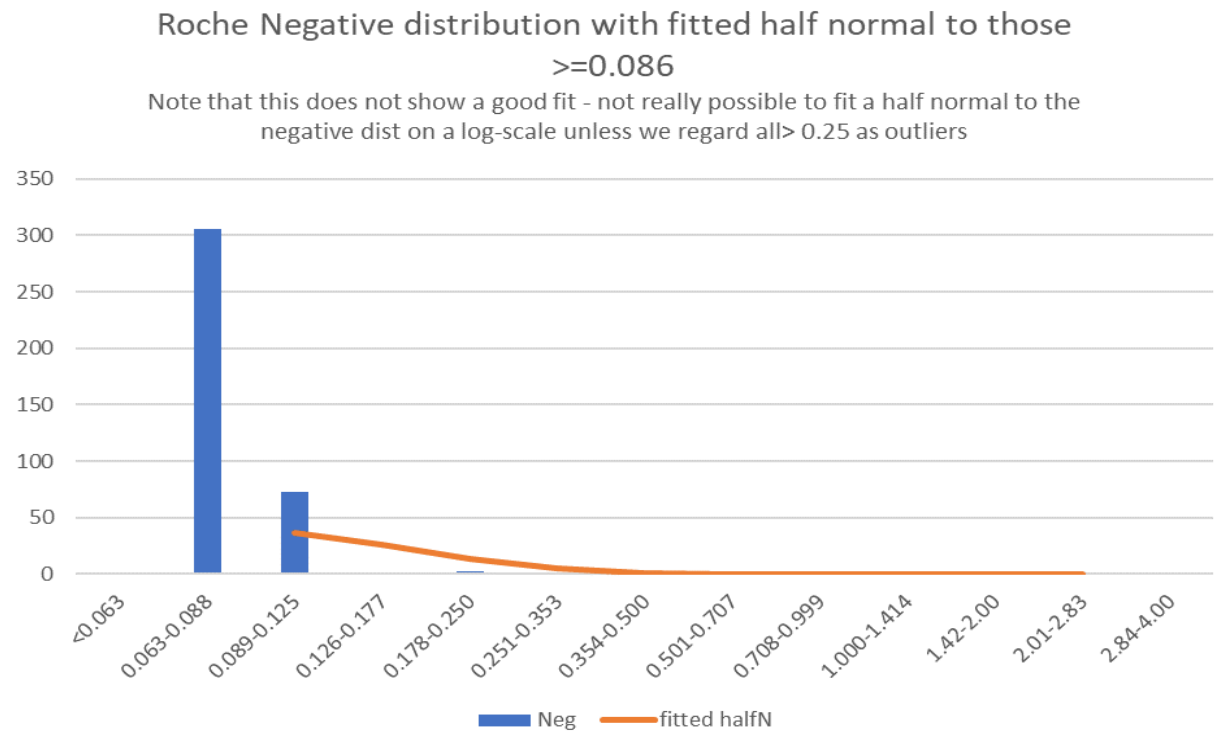

Figure 4: Negative distribution with a fitted half normal

Conclusions

In conclusion, the Elecsys Anti-SARS-CoV-2 assay is a very highly specific assay with a specificity of 100%.

The sensitivity of the assay varied over time, increasing from 75% for an interval of ≤20 days to 100% sensitivity >40 days' post symptom onset. The sensitivity of the assay from 21 days' post symptom onset is 87.7%. The overall sensitivity of the assay is 83.87%.

This is in line with the sensitivity data supplied by the manufacturer. The cut-off used by the manufacturer was found to be on the high side and could be reduced with very little loss in specificity.

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