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Evaluation of Roche Elecsys Anti-SARS-CoV-2 S serology assay for the detection of anti-SARS-CoV-2 S antibodies

Contents

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Executive summary

This document sets out the evaluation of the Roche Elecsys Anti-SARS-CoV-2 S serology assay for the detection of anti-SARS-CoV-2 spike protein antibodies in serum samples.

The assessment was conducted by the Diagnostic Support Group (DSP) at Public Health England (PHE) Porton between October and December 2020. Serum samples from convalescent patients and negative samples were included in the assessment.

All negative samples tested negative by the assay, giving a specificity of 100% (95% confidence interval 99.1-100). The manufacturer reported a specificity of 99.98% (95%CI 99.91-100.0).

The assay gave an overall sensitivity of 95.5% (95%CI 93.2-97.1), with a sensitivity greater than or equal to 14 days of 97.7% (95%CI 95.9-98.8). The sensitivity of the assay greater than or equal to 21 days post symptom onset was 98.5% (95%CI 96.9-99.4). The manufacturer reported a sensitivity of 98.8% (95%CI 98.1-99.3) for samples greater than or equal to 14 post-PCR confirmation.

Introduction

Elecsys Anti-SARS-CoV-2 S serology assay is intended for the detection of antibodies (including IgG) to SARS-CoV-2 spike (S) protein receptor binding domain (RBD) 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 October and December 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.

Roche Elecsys Anti-Sars-CoV-2 S Assay

The Elecsys Anti-SARS-CoV-2 S 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 RBD domain of the S 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 5 main steps in the assay which are:

1. 1st incubation: 12 µL of sample, biotinylated SARS-CoV-2 S-RBD specific recombinant antigen and SARS-CoV-2 S-RBD specific recombinant antigen labelled with a ruthenium complex* form a sandwich complex
2. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin
3. 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.
4. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
5. Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the Cobas link.

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

The sample volume used in the assay is 12µL; the total minimum sample volume required to run the assay is 100µL.

Interpretation of the Result

The analyzer automatically calculates the analyte concentration of each sample in U/mL. The results can be interpreted as follows:

Table 1: Manufacturer's interpretation of the results

Result	Interpretation
<0.8U/mL	Negative for anti-SARS-CoV-2-S antibodies
≥0.8U/mL	Positive for anti-SARS-CoV-2-S antibodies

Manufacturer's listed limitations

The limitations of the assay are:

1. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
2. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
3. 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. It has also been reported that certain patients with confirmed infection do not develop SARS-CoV-2 antibodies. Furthermore, waning of antibody titers has been reported in some individuals within a range of months after infection, a feature which has also been reported for other coronaviruses.

Sensitivity and Specificity

A total of 1610 samples from 402 symptomatic patients (including 297 samples from 243 hospitalised patients) with a PCR confirmed SARS-CoV-2 infection was tested with the Elecsys Anti-SARS-CoV-2 S assay. One or more sequential samples from these patients were collected after PCR confirmation at various time points.

1423 of the tested samples had a sampling date of 14 days or later after diagnosis with PCR. 1406 of these 1423 samples were determined as greater than or equal to 0.8 U/mL in the Elecsys Anti-SARS-CoV-2 S assay and hence considered positive, resulting in a sensitivity of 98.8 % (95 % CI: 98.1-99.3 %) in this sample cohort.

Table 2: Sensitivity of the assay according to the manufacturer

U/mL	Days after diagnosis with positive PCR					
	0-6	**7-13**	**14-20**	**21-27**	**28-34**	**>35**
<0.4	4	16	7	3	0	0
0.4-<0.8	0	6	7	0	0	0
0.8-<1.5	2	3	4	1	0	0
1.5-<2.5	0	2	6	2	0	0
2.5-<5	3	10	9	12	10	40
5-<10	1	7	7	15	25	49
10-<20	0	11	19	32	25	62
20-<50	1	13	19	40	38	183
50-<100	3	9	11	34	48	232
100-<150	1	4	11	11	21	135
150-<200	2	4	2	5	11	95
200-<250	3	8	0	1	5	47
>250	15	59	28	20	14	77
≥0.8	31	130	116	173	197	920
Total	35	152	13	176	197	920
Sensitivity %	88.6	85.5	89.2	98.3	100	100
Cumulative sensitivity, % (95 % Confidence Interval (CI)), %)	86.1(80.3-90.7)		98.8 (98.1-99.3)			

A total of 5991 samples were tested with the Elecsys Anti-SARS-CoV-2 assay. All samples were obtained before October 2019. 1 false positive sample was detected. The resulting overall specificity in the internal study was 99.98%. The 95% lower confidence limit was 99.91%.

Table 3: Specificity of the assay according to the manufacturer

Cohort	N	Reactive	Specificity %	95% CI %
Diagnostic routine (Europe)	2528	0	100	99.85-100
Blood donors (USA)	2713	1	99.96	99.75-100
Blood donors (Africa)	750	0	100	99.51-100
Overall	5991	1	99.98	99.91-100

Interferences

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Table 4: Interferents tested according to the manufacturer

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Haemoglobin	≤ 1000 mg/dL or ≤ 10 g/L
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7.0 g/dL or ≤ 70 g/L
IgA	≤ 1.6 g/dL or ≤ 16 g/L
IgM	≤ 1.0 g/dL or ≤ 10 g/L

Cross-Reactivity

1100 samples containing potentially cross-reacting analytes were tested with the Elecsys Anti-SARS-CoV-2 S assay. All samples were obtained before October 2019. No cross-reactivity was found. The resulting overall specificity was 100%.

Table 4: Cross-reactivity of the Elecsys Anti-SARS-CoV-2 S assay

Indication	N	Reactive	Specificity %
SARS-Co-V-2 related			
MERS Co-V (anti-S1 IgG+)	7	0	100
Common coronavirus panel[1]	94	0	100
Infectious respiratory diseases			
Bordetella pertussis	34	0	100
Chlamydia pneumoniae	33	0	100
Common cold panel[2]	21	0	100
Enterovirus	17	0	100
Haemophilus influenzae B	40	0	100
Influenza A	25	0	100
Influenza B	25	0	100
Influenza vaccinees	25	0	100
Mycoplasma pneumoniae	3	0	100
Parainfluenza	31	0	100
Respiratory syncytial virus	23	0	100
Other infectious diseases			
Borrelia	6	0	100
Candida albicans	13	0	100
Chlamydia trachomatis	10	0	100
CMV acute (IgM+, IgG+)	86	0	100
E. coli (anti-E. coli reactive)	10	0	100
EBV acute (IgM+, VCA IgG+)	106	0	100
Gonorrhea	5	0	100
HAV acute (IgM+)	10	0	100
HAV late (IgG+)	15	0	100
HAV vaccinees	15	0	100

HBV acute	12	0	100
HBV chronic	12	0	100
HBV vaccinees	15	0	100
HCV	50	0	100
HEV	12	0	100
HIV	10	0	100
HSV acute (IgM+)	24	0	100
HTLV	6	0	100
Listeria	6	0	100
Measles	10	0	100
Mumps	14	0	100
Parvovirus B19	30	0	100
Plasmodium falciparum	8	0	100
Rubella acute (IgM+, IgG+)	12	0	100
Toxoplasma gondii (IgM+, IgG+)	8	0	100
Treponema pallidum	62	0	100
VZV (Varicella zoster virus)	30	0	100
Autoimmune diseases			
AMA (anti-mitochondrial antibodies)	30	0	100
ANA (anti-nuclear antibodies)	2	0	100
Haemophiliacs	15	0	100
Rheumatoid arthritis	10	0	100
SLE (systemic lupus erythematosus)	10	0	100
Hepatic diseases			
Alcohol-induced hepatitis/cirrhosis	13	0	100
Drug-induced hepatitis/cirrhosis	10	0	100
Fatty liver	10	0	100
Liver cancer	10	0	100
Non-viral liver disease	15	0	100

[1] 100 pre-pandemic samples were screened for reactivity to Coronavirus HKU1, NL63, 229E, or OC43. 94 out of 100 samples showed serologic reactivity to antigens of at least 1, typically several of these viruses. These 94 samples were assessed for reactivity in the Elecsys Anti-SARS-CoV-2 S assay

[2] 21 potentially cross-reactive samples from individuals with common cold symptoms, collected

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

8 kits of the Elecsys Anti-SARS-CoV-2 S (Lot 51054400, expiry 10/2020) were obtained from Roche in October 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 on a Roche Cobas e 801 instrument.

1. Positive samples – 485 convalescent samples defined by a positive PCR from a swab sample for that patient.
2. Confounder negative samples – 114 samples including 60 samples from the Sero-Epidemiology Unit (SEU), Manchester that were rheumatoid factor (13 samples), CMV (7 samples), EBV (20 samples) or VZV (20 samples) positive.
3. Seasonal coronavirus positive samples – 10 samples
4. Porton negative samples- 44 samples from the RIPL 2015 Lyme disease negative sample collection
5. Manchester negative samples- 398 historic samples from the Sero-Epidemiology Unit (SEU)

Testing results

Sensitivity

Table 5: Overall sensitivity of the assay from the PHE assessment

Total number of convalescent samples (n)	Positive	Negative	Sensitivity (95% CI)
485	463	22	95.5% (93.2-97.1)

The number of positive samples based on interval is given in Table 6 below.

Table 6: Assay sensitivity by interval when tested with PHE's sample set

Group	Interval (days)	Positive	Negative	Total	Sensitivity (95% CI)
Reported onset to sample date	0 to 6	1	2	3	33.3% (0.8-90.6)
	7 to 13	3	9	12	25.0% (5.5-57.2)
	14 to 20	7	4	11	63.6% (30.8-89.1)
	21 to 27	20	7	27	74.1% (53.7-88.9)
	28 to 34	41	0	41	100.0% (91.4-100.0)
	35 to 41	136	0	136	100.0% (97.3-100.0)
	42+	255	0	255	100.0% (98.6-100.0)
	From 14 days	459	11	470	97.7% (95.9-98.8)
	From 21 days	452	7	459	98.5% (96.9-99.4)
	All	463	22	485	95.5% (93.2-97.1)

Specificity

Three sample sets were used to determine the specificity of the assay: 60 confounder samples, 10 seasonal coronavirus samples and 44 RIPL Lyme disease negative samples and 398 negative historical samples.

Table 7: Specificity of the assay from the PHE assessment

Category	n	Reactive	Non-reactive	Specificity (95% CI)
Negative samples	398	0	398	100% (99.1-100.0)
Confounder + seasonal coronavirus + RIPL samples	114	1	113	99.1% (95.2-100.)

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 greater than or equal to 14 days following onset of symptoms, with sensitivity of 97.7% (459/470) and specificity of 100% (398/398).

Table 8: Positive and negative predictive values assuming 10% seroprevalence

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	100% (92.2-100)	99.7% (99.5-99.9)

Precision

To demonstrate the repeatability of the assay, 4 SARS-CoV-2 antibody positive samples and one pool of SARS-CoV-2 negative samples were run on 5 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 less than 3.7 for each sample pool tested. Data is shown in Table 9 below.

Table 9: Precision data for Roche Elecsys Anti-SARS-CoV-2 S assay

Sample ID	Mean/SD/%CV	Date of testing					Inter-Assay Mean	Inter-Assay SD	Inter-Assay % CV
		Day 1	Day 2	Day 3	Day 4	Day 5			
Sample 1	Mean	417.800	418.800	422.800	439.800	444.800	428.800	12.590	2.936
	SD	3.033	3.271	3.493	5.541	6.017			
	% CV	0.726	0.781	0.826	1.260	1.353			
Sample 2	Mean	40.960	41.660	41.060	42.760	43.560	42.000	1.128	2.686
	SD	0.219	0.513	0.873	0.598	0.513			
	% CV	0.535	1.231	2.127	1.399	1.177			
Sample 3	Mean	184.800	186.600	183.600	193.800	199.800	189.720	6.886	3.630
	SD	2.168	1.817	3.782	2.588	1.095			
	% CV	1.173	0.974	2.060	1.336	0.548			
Sample 4	Mean	127.000	127.800	126.400	131.400	134.600	129.440	3.477	2.686
	SD	1.225	0.837	1.342	1.140	0.894			
	% CV	0.964	0.655	1.061	0.868	0.665			
Sample 5	Mean	0.400	0.400	0.400	0.400	0.400	0.400	0.000	0.000
	SD	0.000	0.000	0.000	0.000	0.000			
	% CV	0.000	0.000	0.000	0.000	0.000			

Statistical Analysis

The scatterplot in Figure 1 shows the distribution of the samples by group (convalescent, confounder + RIPL samples and negative samples), with the dashed black line indicating the positive cut-off (less t0.8 U/ml).

Figure 1: Scatterplot of results by sample category

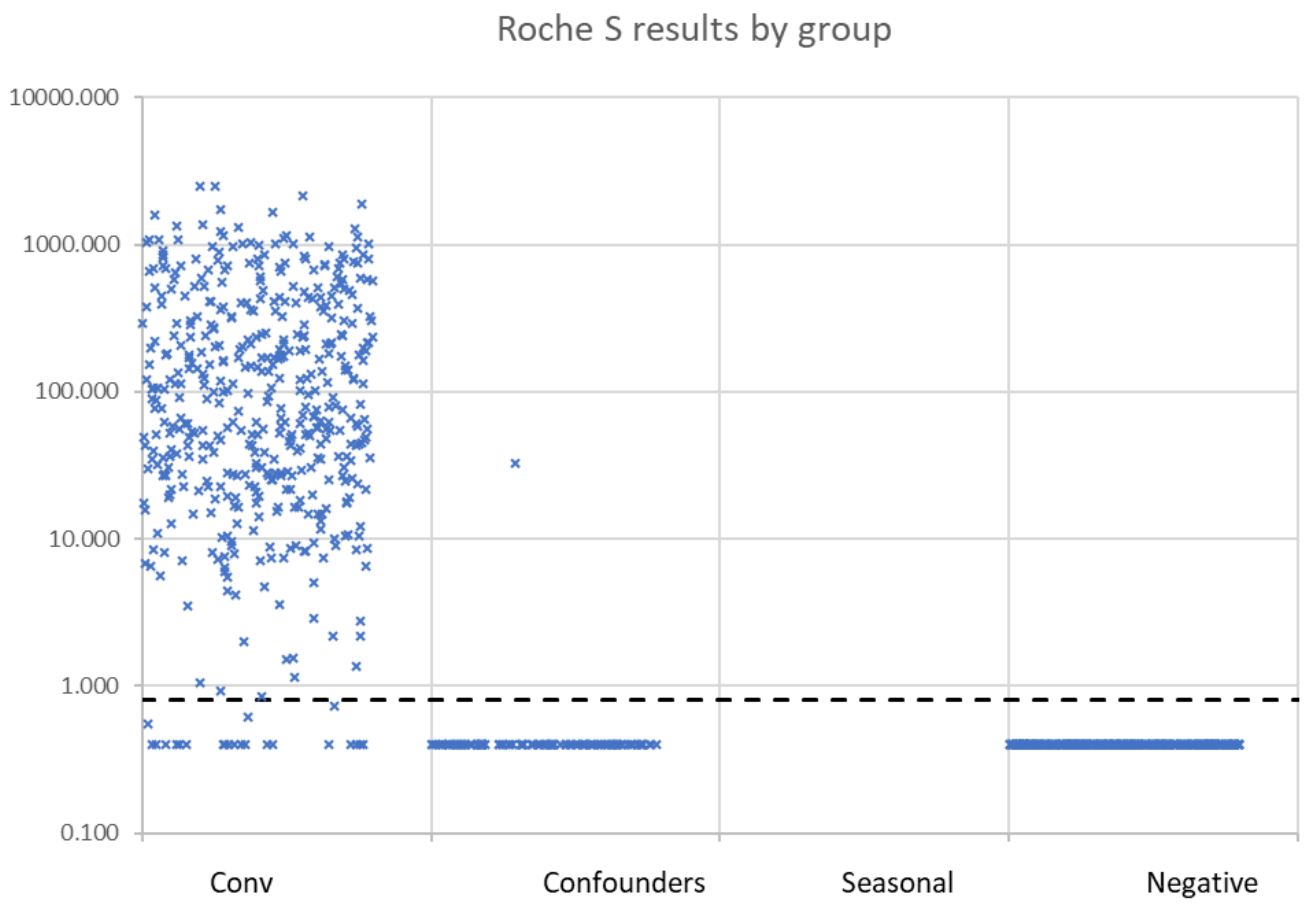


Figure 2 shows a scatterplot analysis of samples according to their time since symptom onset.

Figure 2: Scatterplot of time since symptom onset

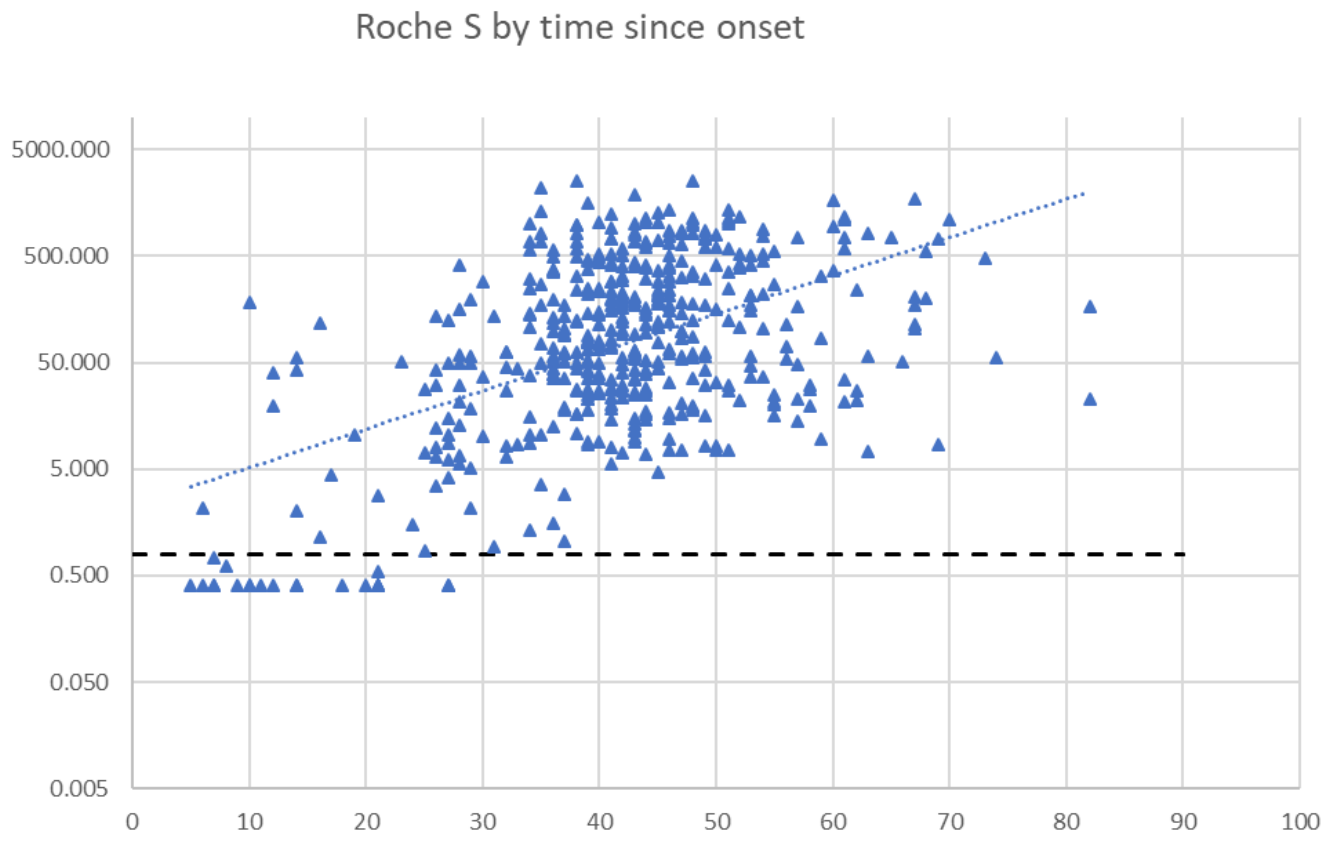
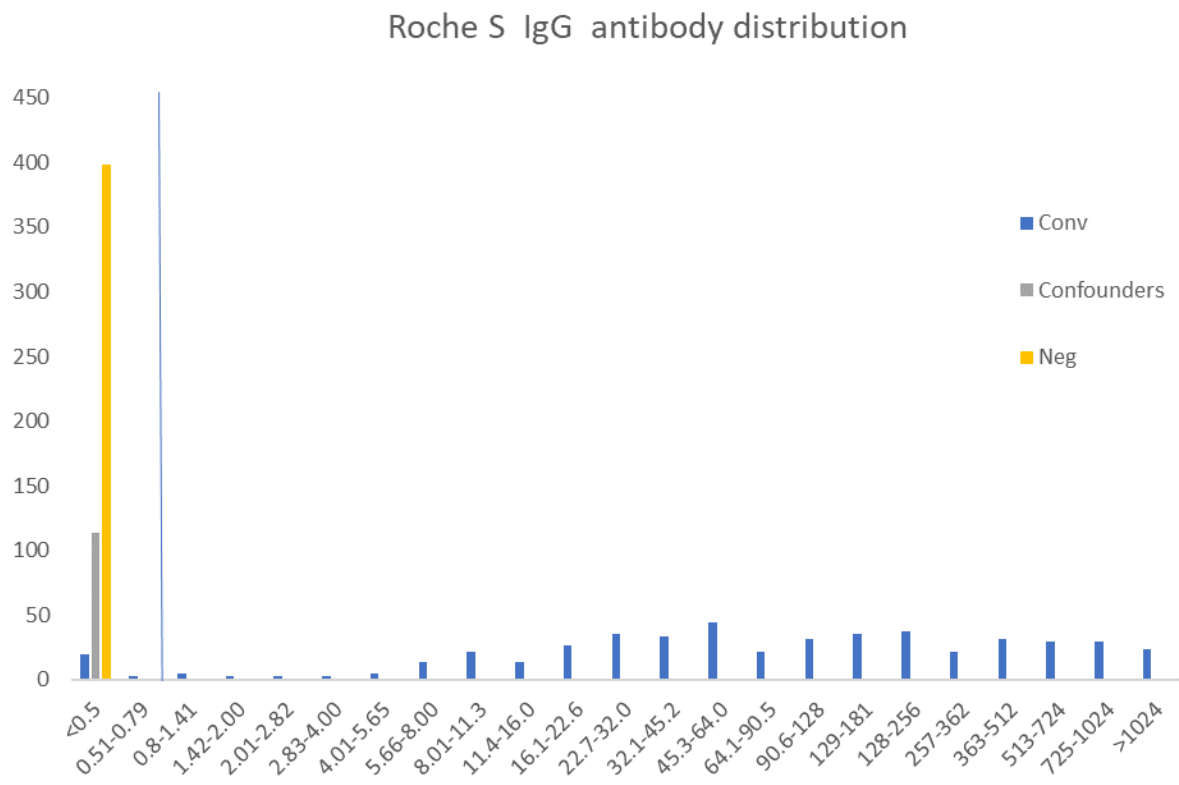


Figure 3 shows the distribution of antibodies against the manufacturer's cut-off of 0.8 U/mL. The results indicate a heavy tail to the negative distribution.

Figure 3: Antibody distribution on a logarithmic scale for all samples. The light blue line denotes the manufacturer's cut-off at a value of 0.8 U/mL



Theoretical specificity based on the quantitative negative distribution could not be assessed as all results were 0.4 U/ml.

Conclusions

In conclusion, the Elecsys Anti-SARS-CoV-2 S assay gave a specificity of 100% (95%CI 99.1-100) in this evaluation; the manufacturer reported a specificity of 99.98% (95%CI 99.91-100).

In this evaluation, the overall sensitivity of the Elecsys Anti-SARS-CoV-2 assay was 95.5% (93.2-97.1). The sensitivity was 97.7% (95%CI 95.9-98.8) for samples taken greater than or equal to 14 since symptom onset and 98.5% (95%CI 96.9-99.4) for samples taken greater than or equal to 21 days since symptom onset. The manufacturer reported a sensitivity of 98.8% (95%CI 98.1-99.3) for samples greater than or equal to 14 days post-PCR confirmation.

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