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Evaluation of the Euroimmun Anti-SARS-CoV-2 ELISA (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 Euroimmun anti-SARS-CoV-2 ELISA (IgG) serology assay for the detection of anti-SARS-CoV-2 in serum samples. Please note that data in this evaluation was extracted from seroprevalence studies undertaken at PHE Porton. The sample panel presented is the same as used in other evaluation studies. This report is being published to present the data in a manner comparable to previously published evaluations of commercial serological assays.

The assessment was conducted by the Diagnostic Support Group (DSP) at PHE Porton between 5/04/20 and 21/05/20; precision testing was completed 4-9/06/20. Ninety-3 serum samples from convalescent patients and 499 negative samples were included in the assessment.

The assay gave a specificity of 99.0% (95% confidence interval 97.5-99.7), which accords with the manufacturer's reported specificity of 99.6%.

The assay gave an overall sensitivity of 72.0% (95%CI 61.8-80.9), with a sensitivity ≥14 days of 73.4% (95%CI 62.3-82.7). The sensitivity of the assay at ≥21 days post symptom onset was 74.7% (95%CI 63.3-84.0). The manufacturer reported a sensitivity ≥10 days post symptom onset of 94.4%.

Introduction

Euroimmun anti-SARS-CoV-2 ELISA (IgG) assay is intended for the detection of IgG antibodies to SARS-CoV-2 in human serum and plasma. The assay is an enzyme immunoassay (ELISA) 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 5 April-21 May 2020; precision testing was completed 4-9 June 2020. This evaluation supersedes a previous, unpublished, evaluation from March 2020, which was undertaken to inform PHE's use of the Euroimmun assay in seroprevalence surveys.

The sample panel described herein is the same as used in other published evaluation studies. This current evaluation was prepared to be comparable to previously published evaluations of commercial serological assays. These evaluations were undertaken 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 Euroimmun assay includes a borderline zone which reflects the biological variation of any ELISA between different runs. The borderline 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 varies according to whether the borderline 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 that the instructions detail a repeat and/or second test process which the manufacturer recommends for a borderline result; this was not possible due to limited sample volume and absence of a second sample.

Euroimmun Anti-SARS-CoV-2 ELISA (IgG) assay

The Anti-SARS-CoV-2 ELISA (IgG) assay is an ELISA assay manufactured by Euroimmun Medizinische Labordiagnostika AG. The assay is listed as CE marked. The U.S. Food and Drug Administration (FDA) has provided Emergency Use Authorization (EUA) for EUROIMMUN's Anti-SARS-CoV-2 ELISA (IgG) serology test.

As per the manufacturer's information, the assay uses the recombinant structural spike 1 (S1) protein of SARS-CoV-2 expressed in the human cell line HEK 293.

Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with recombinant S1 SARS-CoV-2 protein. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation step using an enzyme-labelled anti-human IgG antibody (enzyme conjugate) catalyses a colour reaction. The sample volume used in the assay is 10µL, the total sample volume required for running the assay is 100µL.

Interpretation of the result

Results can be evaluated semi-quantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator. The ratio is calculated according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{ratio}$$

The manufacturer recommends interpreting the result as follows:

Ratio < 0.8 = negative
Ratio ≥0.8 to <1.1 = borderline
Ration ≥1.1 = positive

For duplicate determinations, the mean of the 2 values should be taken. If the 2 values deviate substantially from each other, the manufacturer recommends retesting the samples.

Manufacturer's listed limitations of the assay

For a medical diagnosis, the serological test result should always be interpreted together with the clinical symptoms of the patient and other results, for example, from direct pathogen detection. A negative serological test does not exclude the presence of the disease.

The pipetting volumes, incubation times, temperatures, and preparation steps given in the instructions for use must be adhered to.

Correct performance of sample collection and storage is crucial for the test results.

The test system is validated for human serum and plasma samples only.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependant. It is therefore recommended to use a thermostatically adjusted ELISA incubator in all incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction. The same variations also apply to the incubation times. However, the calibrators are subject to the same influences, with the results that variations will be largely compensated in the calculation of the results.

Insufficient washing (for example, less than 3 washes, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.

Residual liquid (>10µL) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.

The partial or complete adjustment of the test system to the use of different instruments for automated sample processing or other liquid handling devices may result in differences between the results obtained with automated processing and those obtained by manual procedure. It is the responsibility of the user to validate the instruments used so that they yield test results in the reliable range.

Manufacturer's performance characteristics

Sensitivity

The sensitivity was determined by investigating 166 samples from 152 European patients, using the Anti-SARS-CoV-2 ELISA (IgG). In these patients, infections with SARS-CoV-2 had been confirmed by RT-PCR based on a sample taken at the early phase of infection. In samples taken prior to day 10 (time point after onset of symptoms or positive direct detection), the Anti-SARS-CoV-2 ELISA (IgG) showed a sensitivity of

43.7%. The sensitivity of the Anti-SARS-CoV-2 ELISA (IgG) in samples collected after day 10 was 94.4%. Borderline results (n = 7) were not considered in the calculation.

Table 1: Sensitivity of the Anti-SARS-CoV-2 ELISA (IgG) assay according to the manufacturer

Group	**Anti-SARS-CoV-2 ELISA (IgG)**			
	Positive	Negative	Borderline	Sensitivity
<10 days after symptom onset	38	49	2	43.7%
>10 days after symptom onset	68	4	1	94.4%

Specificity

The specificity of the Anti-SARS-CoV-2 ELISA (IgG) was determined by analysing 222 patient samples that were positive for antibodies against other human pathogenic coronaviruses, other pathogens or for rheumatoid factors. Additionally, 1122 samples from blood donors, children and pregnant women obtained before the occurrence of SARS-CoV-2 (before January 2020) were analysed. The results in the borderline range (n = 7) were not considered in the calculation. The specificity of the Anti-SARS-CoV-2 ELISA (IgG) amounted to 99.6%.

Table 2: Specificity of the Anti-SARS-CoV-2 ELISA (IgG) assay according to the manufacturer

Panel	**n**	**Specificity**
Blood donors	849	99.5%
Pregnant women	199	99.5%
Children	74	100%
Older people	97	100%
Infections with other human pathogenic coronaviruses	23	100%
Influenza (freshly vaccinated including courses)	40	100%
Acute EBV infections and heterophilic antibodies	22	100%
Rheumatoid factors	40	100%
Total	**1344**	**99.6%**

Interferences

Haemolytic, lipaemic and icteric samples showed no influence on the result up to concentrations of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA.

Cross-reactions

Due to low homologies between the S1 protein within the coronavirus family, cross-reaction to most of the human pathogenic representatives of this virus family are virtually excluded. However, due to the close relationship SARS-CoV-1 and SARS-CoV-2, cross-reactions between these 2 viruses are likely. Sera from patients with SARS-CoV-1, MERS-CoV, HCoV-229, HCoV-HKU1, HCoV-NL63 and HCoV-OC43 infections were investigated to examine this further. Pronounced cross-reactions occurred with SARS-CoV-1. Cross-reactions to other human pathogenic coronaviruses were not observed.

Testing of Euroimmun SARS-CoV-2 ELISA (IgG) assay by PHE

Seven batches of Euroimmun Anti-SARS-CoV-2 ELISA (IgG) – EI 2606-9601 G were received from Euroimmun. The evaluation took place at PHE Porton Down between 5 April and 21 May 2020; precision testing was completed 4-9 June 2020.

In this evaluation, borderline samples were included in the negative data set for consistency with other evaluations – note that the instructions detail a repeat and/or second test process which the manufacturer recommends for a borderline 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 the following sample sets. All testing was performed per the manufacturer's instructions on a Stratec Biomedical Gemini automated ELISA platform.

Positive samples

Ninety-three 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 79 samples. Two of these samples had an interval of ≤ 14 days. For the remaining 14 samples, the interval is measure from when the patient was admitted to hospital to sample collection date so the interval for these samples is artificially low.

Confounder negative samples

Fifty 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

Fifty samples from the RIPL 2015 Lyme disease negative sample collection.

Manchester negative samples

Three hundred and ninety-nine historic samples from the SEU.

Testing results

Sensitivity

The sensitivity of the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) assay based on the PHE assessment of the assay is shown in Table 3 below, using a threshold of 1.1.

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

Total number of convalescent samples (n)	Positive	Borderline	Negative	Sensitivity (95% CI)
93	67	8	18	72.0% (61.8-80.9)

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

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

Group	Interval (days)	Positive	Borderline	Negative	Total	Sensitivity (95% CI)
Hospital admission to sample date	<= 10	9	0	5	14	64.3 (35.1-87.2)
Reported onset to sample date	11 to 20	2	0	2	4	50.0% (6.8-93.2)
	21 to 30	26	5	4	35	74.3% (56.7-87.5)
	31 to 40	22	3	5	30	73.3% (54.1-87.7)
	41 to 50	8	0	2	10	80.0% (44.4-97.5)
	From 14 days	58	8	13	79	73.4% (62.3-82.7)
	From 21 days	56	8	11	75	74.7% (63.3-84.0)

The samples in the row "<= 10 from admission" have an interval based on admission to hospital, so the intervals are artificially low for these samples.

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. The specificity of the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) assay is shown in Table 5 below.

Table 5: Specificity of the Anti-SARS-CoV-2 ELISA (IgG) assay from the PHE assessment

Category	n	Positive	Borderline + Negative	Specificity (95% CI)
Negative samples	399	4	395	99.0% (97.5-99.7)
Confounder + RIPL samples	100	2	98	98.0% (93.0-99.8)

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 73.4% (58/79) and specificity calculated at 99.0% (395/399).

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

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	89.1% (76.2-96.8)	97.1% (95.9-98.1)

Precision

To demonstrate the repeatability of the assay, 4 pools consisting of 5 replicates of SARS-CoV-2 antibody positive samples and one pool of SARS-CoV-2 borderline 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 <6 for each sample pool tested.

Table 7: Precision data for Euroimmun Anti-SARS-CoV-2 (IgG) Assay

Sample ID	Mean/SD/%CV	Date of Testing					Inter-Assay Mean	Inter-Assay SD	Inter-Assay % CV
		Day 1 04/06/20	Day 2 05/06/20	Day 3 06/06/20	Day 4 08/06/20	Day 5 09/06/20			
Pool 1	Mean	9.00	8.43	8.59	8.43	8.64	8.62	0.26	3.02
	SD	0.26	0.13	0.14	0.08	0.13			
	% CV	2.93	1.59	1.60	0.99	1.50			
Pool 2	Mean	6.28	5.95	5.99	6.04	6.12	6.08	0.21	3.39
	SD	0.11	0.19	0.13	0.16	0.28			
	% CV	1.69	3.26	2.18	2.64	4.50			
Pool 3	Mean	3.72	3.52	3.55	3.67	3.67	3.63	0.14	3.79
	SD	0.11	0.12	0.04	0.19	0.11			
	% CV	2.84	3.52	1.13	5.17	2.93			
Pool 4	Mean	2.07	1.90	1.99	1.99	1.99	1.99	0.08	4.11
	SD	0.04	0.03	0.06	0.09	0.08			
	% CV	2.16	1.72	3.12	4.42	4.04			
Pool 5	Mean	1.07	0.98	1.02	0.99	1.03	1.02	0.05	5.39
	SD	0.07	0.01	0.04	0.02	0.07			
	% CV	6.28	0.55	3.76	2.46	6.60			

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 shows 2 cut off values at 0.8 AU/mL and 1.1 AU/mL which, according to the manufacturer equates to borderline results. For the purpose of this evaluation, a cut-off of 1.1 AU/mL was used.

Figure 1: Scatterplot of results by sample category with the manufacturer's thresholds of 0.8 and 1.1

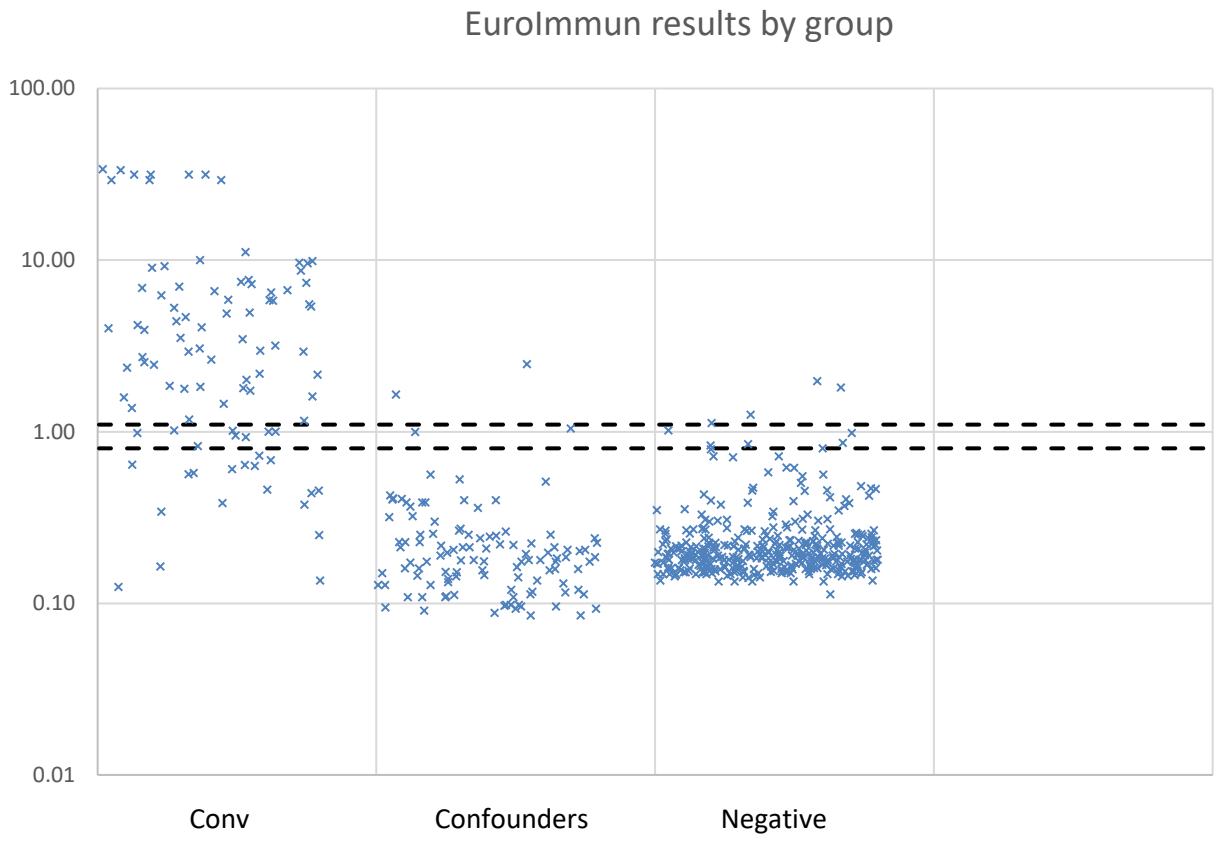
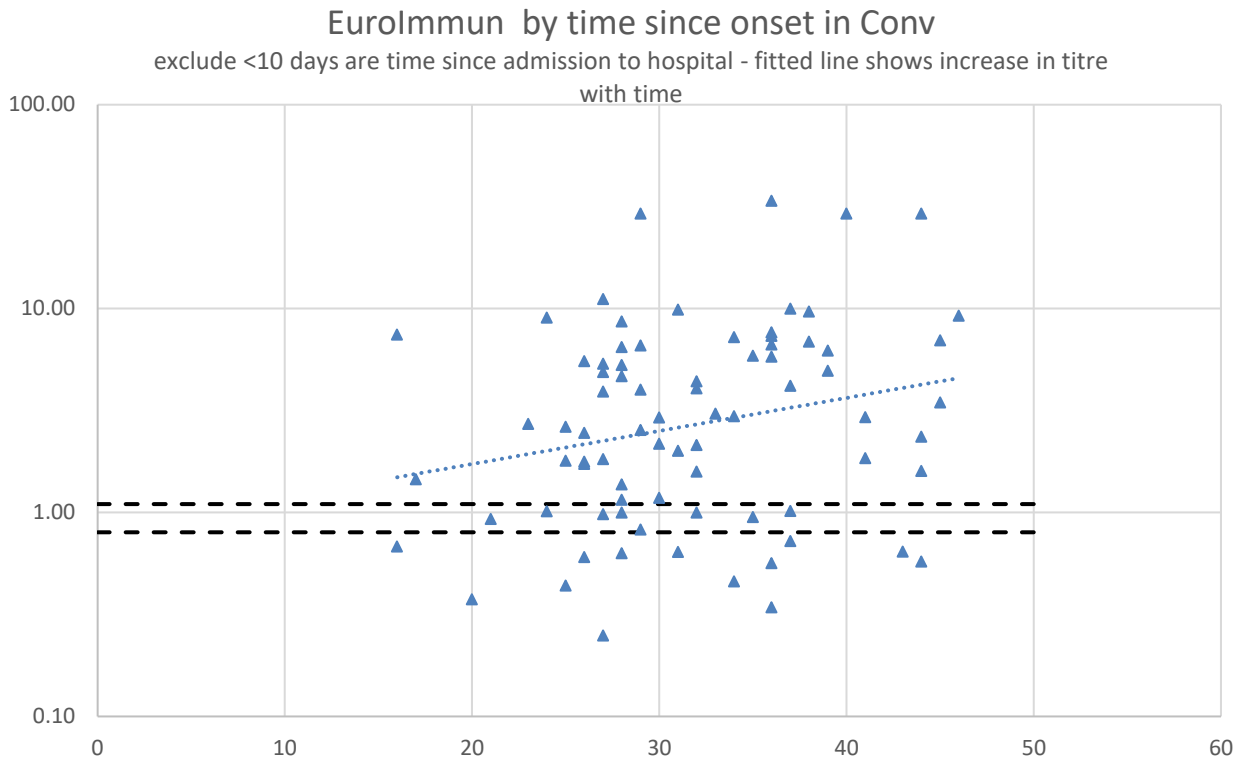


Figure 2 shows a scatterplot analysis of samples according to their time since symptom onset. 14 samples that did not have an accurate interval recorded were excluded. These samples had an interval time recorded from the patients' admission to hospital rather than the date of onset of symptoms and so the interval for these patient samples is artificially low. The dashed line shows the rise in antibody titre over time from onset of symptoms.

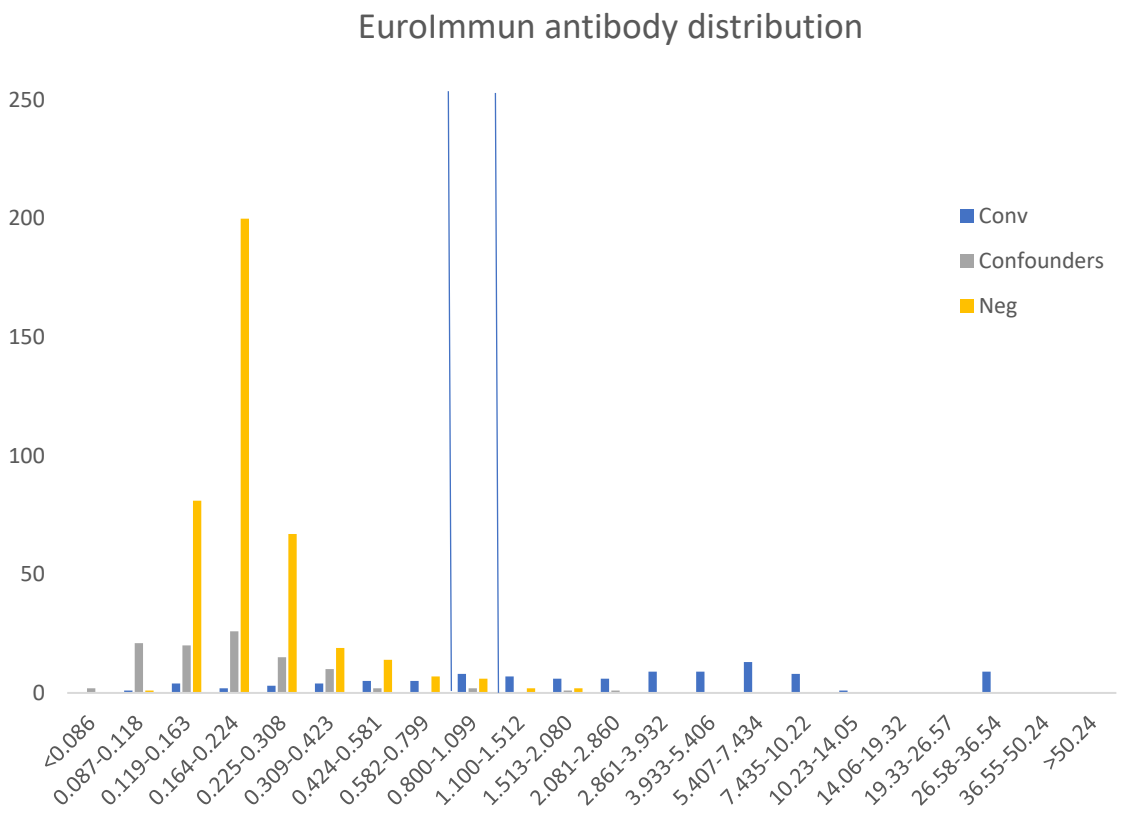
Figure 2: Scatterplot of time since symptom onset (excluding 14 samples that did not have an accurate time since symptom onset)



Manufacturer's thresholds of 0.8 and 1.1 are shown with dashed lines.

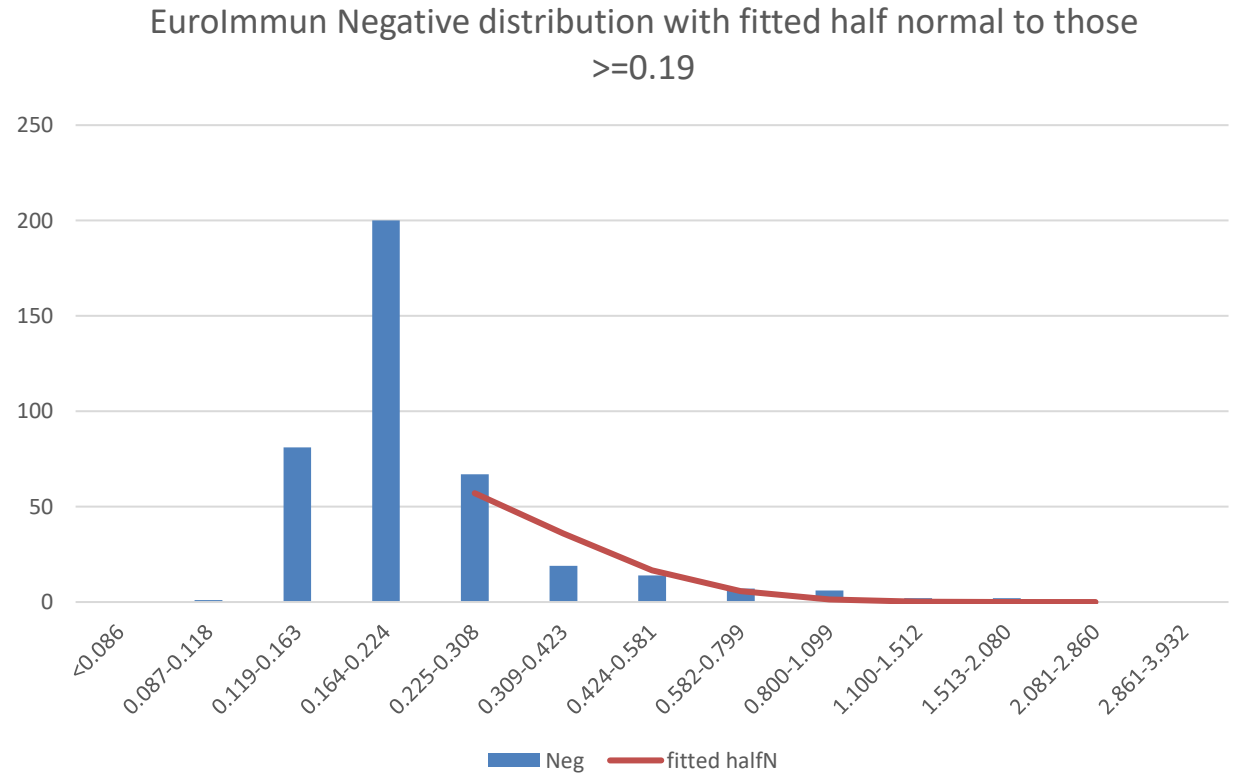
Figure 3 shows the distribution of antibodies against the manufacturer's cut-off. In order to assess the cut-off for the assay, the distribution of the assay units in the negative samples are assessed (see Figure 4). It is usually desirable that a cut-off is set about 3 standard deviations (SD) above 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 the 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 results <2 the mean was 0.21 (-0.66 log10) and the half-normal standard deviation was 0.247 (log10) (right hand part of the distribution above a value of 0.19). 0.19 + 2.58 SD = 0.82 (anti-logged) and 0.19 + 3SD = 1.04 (anti-logged). So a cut-off of mean + 3 SD of 1.04 is close to the manufacturer's cut-off. The manufacturer cut-off gives a theoretical specificity of 99.9% ignoring outlier false positives.

Figure 3: Antibody distribution on a logarithmic scale



The light blue lines denote the manufacturer's cut-off between the ratio of 0.8 and 1.1. Sample results that fall between these lines are considered to be borderline.

Figure 4: Negative distribution with a fitted half normal



Conclusions

In conclusion, the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) assay gave a specificity of 99.0% (95%CI 97.5-99.7); the manufacturer reported a specificity of 99.6%.

In this evaluation, the sensitivity of the Euroimmun Anti-SARS-CoV-2 ELISA (IgG) assay was 73.4% (95%CI 62.3-82.7) for samples collected ≥14 days post symptom onset and 74.7% (95%CI 63.3-84.0) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity in this evaluation was 72.0% (95%CI 61.8-80.9). The manufacturers reported a sensitivity of 43.7% for samples <10 days post symptom onset and a sensitivity of 94.4% for samples >10 post symptom onset.