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Evaluation of Siemens Atellica-IM Total (COV2T) SARS-CoV-2 serology assay for the detection of anti-SARS-CoV-2 total antibodies

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Executive summary

This document sets out the evaluation of the Siemens Atellica-IM SARS-CoV-2 Total (COV2T) 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 3-12 June 2020. 100 serum samples from convalescent patients and 499 negative serum samples were included in the assessment.

The assay gave a specificity of 100% (95% confidence interval 99.1-100) in this evaluation. The manufacturer's reported a specificity of 99.82% (95%CI 91.59-99.98).

The assay gave an overall sensitivity of 86.0% (95%CI 77.6-92.1), with a sensitivity of 89.4% (95%CI 80.8-95.0) at ≥14 days post symptom onset. The sensitivity of the assay at ≥21 days post symptom onset was 92.4% (95%CI 84.2-97.2). The manufacturer reported a sensitivity of 100% (95%CI 91.59-100) for samples ≥14 days post RT-PCR confirmation.

Introduction

Atellica-IM SARS-CoV-2 Total (COV2T) assay is intended for the detection of total antibodies to SARS-CoV-2 in human serum and plasma. The assay is an antigen sandwich immunoassay using acridinium ester chemiluminescent technology. The assay is intended for use on the Atellica IM immunoassay analysers. This report details an evaluation of the assay conducted at PHE Porton Down between 3-12 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.

Atellica-IM SARS-CoV-2 Total (COV2T) Assay

The Atellica SARS-CoV-2 Total (COV2T) assay is a sandwich immunoassay for the detection of total antibody, including IgM and IgG to SARS Co-V-2 in human serum and plasma manufactured by Siemens Healthcare GmbH. The assay is listed as CE marked.

Test principle

The Atellica IM COV2T assay is a fully automated 1-step antigen sandwich immunoassay using acridinium ester chemiluminescent technology, in which antigens are bridged by antibodies present in the patient sample. The solid phase contains a preformed complex of streptavidin-coated microparticles and biotinylated SARS-CoV-2 recombinant antigens. This reagent is used to capture anti-SARS-CoV-2 antibodies in the patient sample. The light reagent contains acridinium-ester-labelled SARS-CoV-2 recombinant antigens used to detect anti-SARS-CoV-2 antibodies bound to the solid phase.

A direct relationship exists between the amount of SARS-CoV-2 antibodies present in the patient sample and the amount of relative light units (RLUs) detected by the system.

A result of reactive or nonreactive is determined according to the Index Value established with the calibrators.

Interpretation of the result

The system reports Atellica IM COV2T assay results in Index Values and as nonreactive or reactive:

- nonreactive < 1.0 Index – these samples are considered negative for SARS-CoV-2 antibodies
- reactive ≥ 1.0 Index – these samples are considered positive for SARS-CoV-2 antibodies

The cut-off value for the Atellica IM COV2T assay was verified based on clinical agreement of results.

Results of this assay should always be interpreted in conjunction with the patient’s medical history, clinical presentation, and other findings.

Manufacturer's listed limitations

The limitations of the assay are:

- results are not intended to be used as the sole basis for patient management decisions
- test results should be interpreted in conjunction with clinical observations, patient history, epidemiological information, and other laboratory findings
- the performance of the assay has not been established with cord blood, neonatal specimens, cadaver specimens, or body fluids other than serum or plasma
- it is currently unknown how long SARS-CoV-2 antibodies persist following infection and if the presence of antibodies confers protective immunity
- a reactive test result does not exclude past or present infection by other coronaviruses, such as SARS-CoV-1, MERS-CoV, HKU1, 229E, NL63, or OC43, or due to cross-reactivity from pre-existing antibodies or other possible causes
- a nonreactive test result does not exclude the possibility of exposure to or infection with SARS-CoV-2 – patient specimens may be nonreactive if collected during the early (preseroconversion) phase of illness or due to a decline in titre over time; in addition, the immune response may be depressed in elderly, immunocompromised, or immunosuppressed patients
- this test should not be used for donor screening

Manufacturer's performance characteristics

Sensitivity

Clinical sensitivity was determined by testing 250 samples from individuals with a clinical diagnosis of COVID-19 based on a positive polymerase chain reaction (PCR) method. The results are shown in the table below:

Table 1: Sensitivity of the assay according to the manufacturer

Days post RT-PCR diagnosis	Number tested	Reactive	Non-reactive	Clinical Sensitivity, % (95% CI)
0 – 6	89	54	35	60.67% (49.75-70.87)
7 – 13	119	116	3	97.48% (92.81-99.48)
≥ 14	42	42	0	100.0% (91.59-100.0)

Specificity

Clinical specificity was determined by testing 1091 samples collected prior to the COVID-19 outbreak (before November 2019) from apparently healthy individuals and

apparently healthy pregnant women in the United States. The results are shown in the table below.

Table 2: Specificity of the assay according to the manufacturer

Group	Number tested	Reactive	Non-reactive	Sensitivity, % (95% CI)
Apparently Healthy	993	991	2	99.80% (99.27-99.98)
Apparently Healthy Pregnant Women	98	98	0	100.0% (96.31-100.0)
Total	**1091**	**1089**	**2**	99.82% (99.34-99.98)

Interferences

Interference testing was performed in accordance with CLSI Document EP07-ed3[1] using the Atellica IM Analyzer. Testing demonstrated ≤ 10% change for each substance. The following results were obtained.

Table 3: Interferences and their tested concentrations according to the manufacturer

Substance	Substance Test Concentrations
Haemoglobin	1000mg/dL
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Triglycerides (Intralipid)	2000mg/dL
Biotin	3500ng/mL

Cross-reactions

Cross-reactivity was determined in accordance with CLSI Document EP07-ed3. The assay was evaluated for potential cross-reactivity in specimens with other viral and microbial antibodies and other disease states.

[1] Clinical and Laboratory Standards Institute. Interference Testing in Clinical Chemistry; Approved Guideline—Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2018. CLSI Document EP07-ed3.

Table 4: Cross-reactions according to the manufacturer

Condition	**Number of samples tested**	**Number Reactive with Atellica IM COV2T Assay**
Anti-nuclear autoantibodies (ANA)	5	0
Chlamydia IgG	5	0
Cytomegalovirus (CMV) IgG	5	0
Epstein-Barr Virus (EBV) IgG	5	0
Epstein-Barr Virus (EBV) IgM	5	0
Graves' Disease	5	0
Hepatitis A infection (HAV) IgM	5	0
Hepatitis B Core Antigen (Anti-HBc) IgM	5	0
Hepatitis C infection (HCV) antibody	5	0
Human anti-mouse antibody (HAMA)	4	0
Human herpes virus (HHV)	3	0
Human immunodeficiency virus (HIV) antibody	10	0
Influenza antibody	10	0
Measles antibody	5	0
Parvovirus B19 antibody	5	0
Rheumatoid factor	4	0
Varicella zoster (VZV) antibody	5	0
Total	**91**	**0**

Testing of Atellica SARS-CoV-2 Total (COV2T) assay by PHE

Atellica SARS-CoV-2 Total (COV2T) assay kits, batch number 11206711, expiry 2021-05-12, were used in this evaluation. The evaluation took place at PHE Porton Down between 3-12 June 2020.

Procedure for testing

Research operators from DSP and RIPL performed testing of kits using serum sample sets. All testing was performed per the manufacturer's instructions on an Atellica IM instrument. The serum 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 86 samples; for the remaining 14 samples, the interval is measured from the time the patient was admitted to hospital to sample collection date so the interval for these samples is artificially low
- 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 114 samples from PHE Immunoassay Group (IAG) sample collection
- Manchester negative samples – 285 historic samples from the SEU

The sample cohort used for this study had some difference in its composition to the sample cohorts used to evaluate the other serology antibody tests; 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

The overall sensitivity of the Atellica IM assay was measured as being 86.0% (95%CI 77.6-92.1).

Table 5: Overall sensitivity of the Atellica-IM SARS-CoV-2 Total (COV2T) assay from the PHE assessment

No. Samples	Positive	Negative	Sensitivity (95% CI)
100	86	14	86.0% (77.6-92.1)

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

Table 6: Assay sensitivity of the Atellica-IM SAS-CoV-2 Total (COV2T) assay by interval when tested with PHE's sample set

Group	Interval (days)	Positive	Negative	Total	Sensitivity (95% CI)
Hospital admission to sample date	<= 10	10	4	14	71.4% (41.9-91.6)
Reported onset to sample date	11 to 20	3	4	7	42.9% (9.9-81.6)
	21 to 30	35	2	37	94.6% (81.8-99.3)
	31 to 40	28	4	32	87.5% (71.0-96.5)
	41 to 50	10	0	10	100.0% (69.2-100)
	From 14 days	76	9	85	89.4% (80.8-95.0)
	From 21 days	73	6	79	92.4% (84.2-97.2)

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 (IAG+SEU).

Table 7: Specificity of the Atellica-IM SARS-CoV-2 Total (COV2T) assay from the PHE assessment

Category	n	Positive	Negative	Specificity (95% CI)
Negative samples	399	0	399	100.0% (99.1-100.0)
Confounder + RIPL samples	100	0	100	100.0% (96.4-100.0)

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 89.4% (76/85) and specificity calculated at 100.0% (399/399).

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

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	100.0% (91.5-100.0)	98.8% (97.9-99.5)

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 9 below shows the results for 3 sample pools. The first 2 sample pools returned results that were outside the maximum threshold for the assay. From the 3 sample pools that returned a result within the measurable parameter of the assay, it shows that the assay performed within acceptable parameters for precision with inter-assay %CV of <5.

Table 9: Precision data for the Atellica SARS-CoV-2 Total (COV2T) assay from the PHE assessment

	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 08/06/20	Day 4 09/06/20	Day 5 10/06/20			
Pool 3	Mean	8.21	8.18	7.91	8.20	7.82	8.06	0.27	3.32
	SD	0.30	0.21	0.27	0.20	0.10			
	% CV	3.69	2.55	3.39	2.44	1.22			
Pool 4	Mean	4.45	4.28	4.24	4.17	4.14	4.25	0.13	3.10
	SD	0.06	0.05	0.12	0.07	0.08			
	% CV	1.31	1.14	2.85	1.65	2.00			
Pool 5	Mean	2.44	2.39	2.19	2.34	2.31	2.34	0.12	4.98
	SD	0.08	0.10	0.12	0.06	0.06			
	% CV	3.16	4.17	5.30	2.50	2.44			

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) with a cut-off of 1.0.

Figure 1: Scatterplot of results by sample category

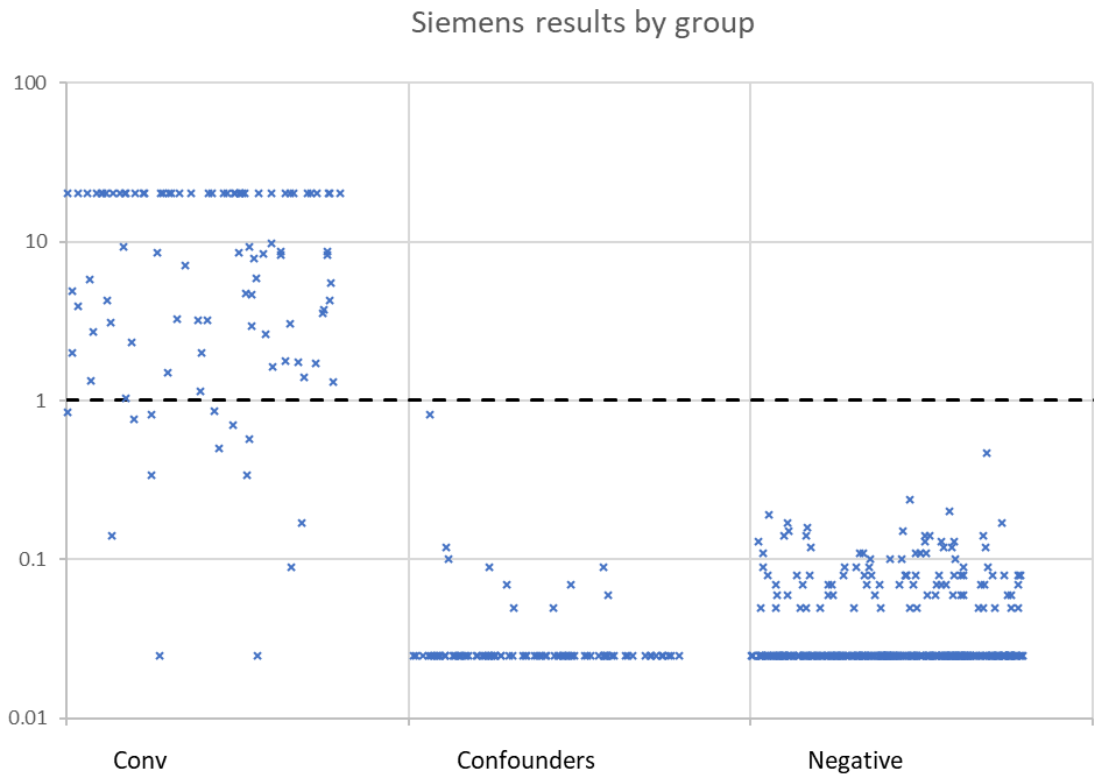


Figure 2 shows a scatterplot analysis of samples according to their time since symptom onset. For this analysis, 14 samples that did not have an accurate time since onset (the dates supplied were the admission to hospital dates rather than the time since symptom onset) were not included in the analysis.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

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

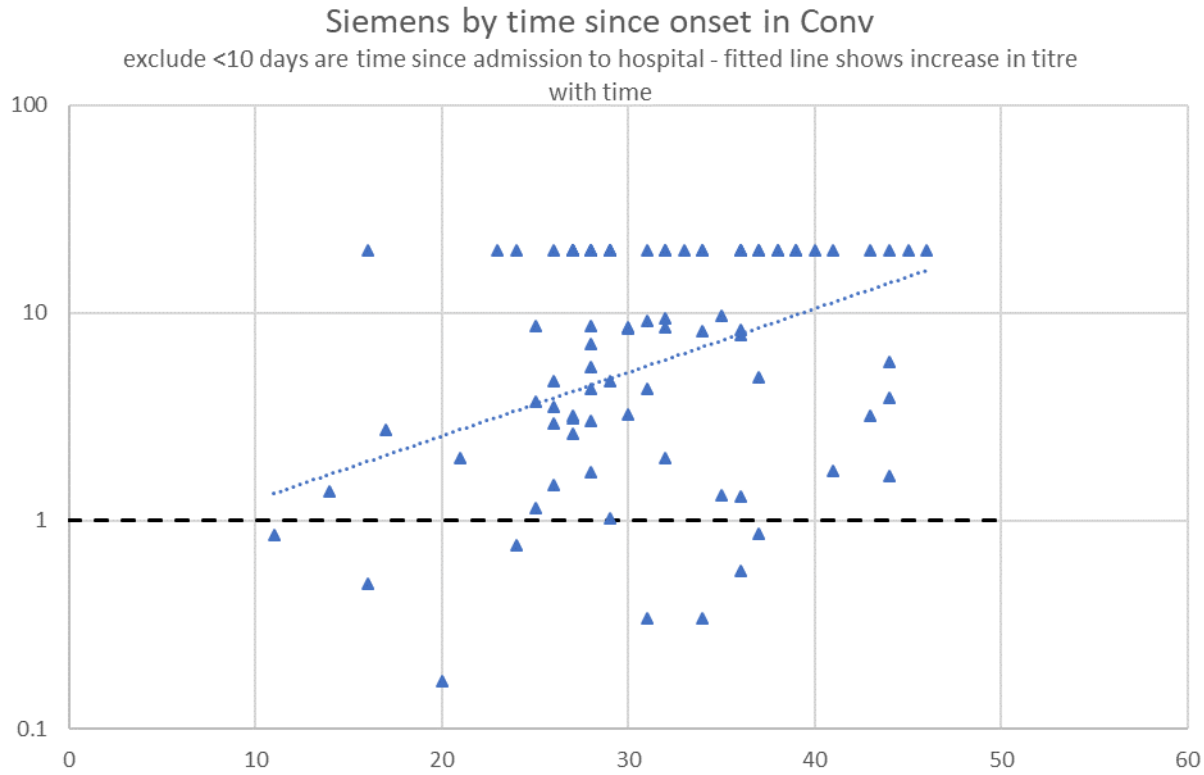
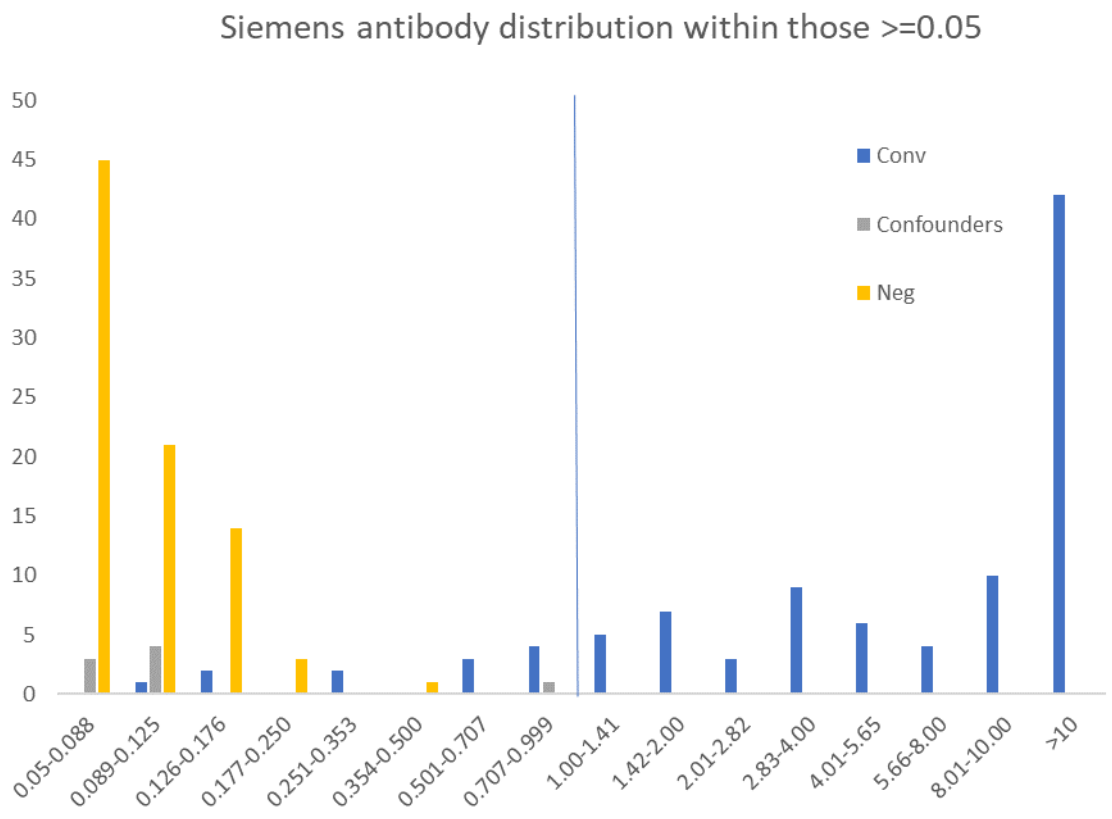


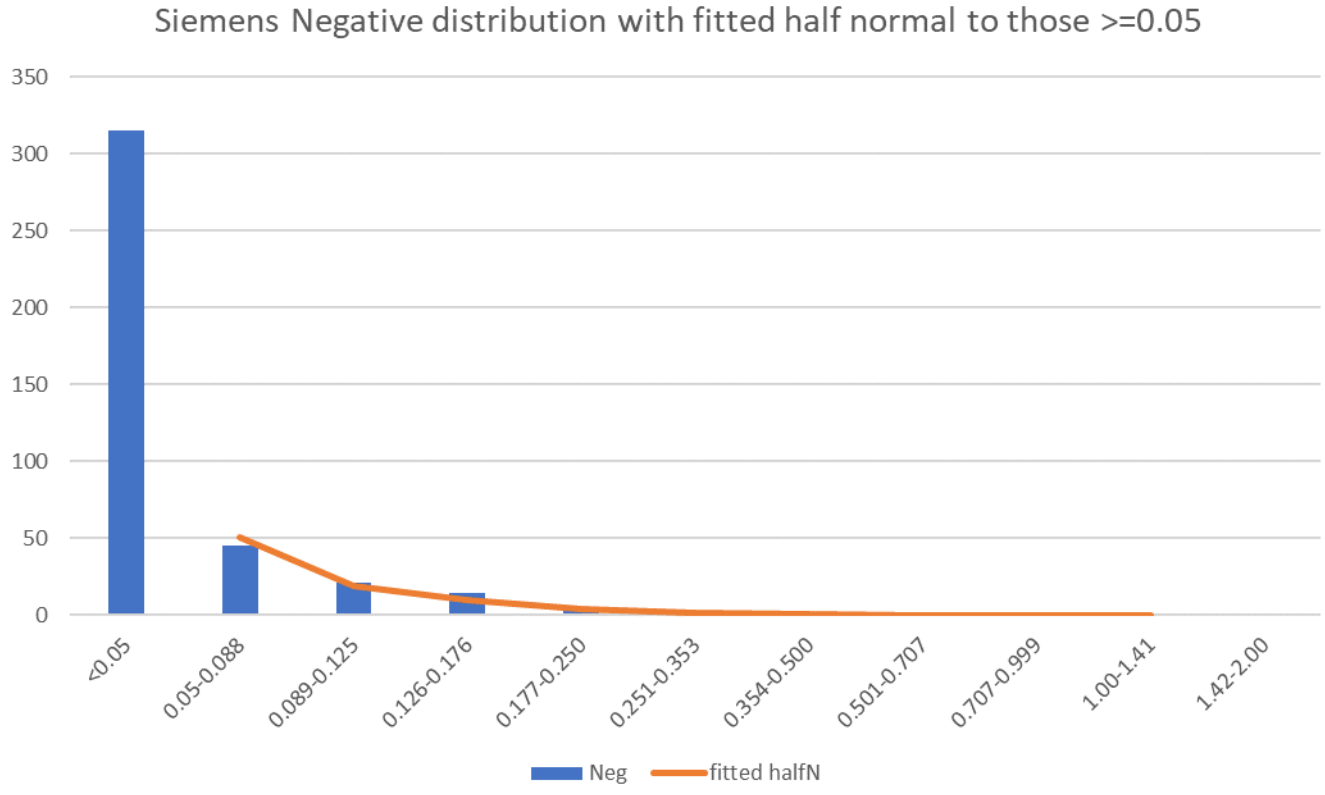
Figure 3 shows the distribution of antibodies against the manufacturer's cut-off of 1.0. To assess the cut-off for the assay, the distribution of the assay units in the negative samples are assessed (see Figure 4). It is usually desirable that a cut-off is set at least about 3 standard deviations (SD) 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 <1 the mean was <0.05 and the half-normal standard deviation was 0.292(log10) (right hand part of the distribution >= 0.05). 0.05 + 2.58 SD = 0.28 (anti-logged) and 0.05 + 3SD = 0.38 (anti-logged). So, a cut-off of mean + 3 SD of 0.38 is well below the manufacturer's cut-off. The manufacturer cut-off gives a theoretical specificity of 100% ignoring outlier false positives.

Figure 3: Antibody distribution on a logarithmic scale.



The light blue line denotes the manufacturer's cut off value.

Figure 4: Negative distribution with a fitted half normal



Conclusions

In conclusion, the Siemens Atellica SARS-CoV-2 Total (COV2T) gave a specificity of 100.0% (95%CI 99.1-100) in this evaluation; the reported specificity of the manufacturer is 99.82% (95%CI 99.34-99.98).

In this evaluation, the sensitivity of the Siemens SARS-CoV-2 Total (COV2T) assay was 89.4% (95%CI 80.8-95.0) for samples collected ≥14 post symptom onset and 92.4% (95%CI 84.2-97.2) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity was 86.0% (95%CI 77.6-92.1). The manufacturer reported a sensitivity of 97.48% (95%CI 92.81-99.48) for samples 7-13 days and a sensitivity of 100% (95%CI 99.59-100.0) for samples taken ≥14 days' post RT-PCR diagnosis.