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Evaluation of Siemens Atellica-IM IgG (sCOVG) SARS-CoV-2 serology assay for the detection of anti-SARS-CoV-2 IgG antibodies

Contents

Document control

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Executive summary

This document sets out the evaluation of the Siemens Atellica-IM SARS-CoV-2 IgG (sCOVG) serology assay for the detection of anti-SARS-CoV-2 IgG antibodies to the spike protein in serum samples.

The assessment was conducted by the Diagnostic Support Group (DSP) at PHE Porton between 5 November 2020 and 15 December 2020. 115 serum samples from convalescent patients, 348 negative samples and 152 confounder samples were included in the assessment.

The assay gave a specificity of 100% (95% confidence interval 98.9-100.0) in the evaluation. The manufacturer reported a specificity of 99.90% (95% CI 99.64-99.99).

The assay gave an overall sensitivity of 64.3% (95%CI 54.9-73.1), with a sensitivity of 72.5% (95%CI 61.4 to 81.9) at ≥14 days post symptom onset. The sensitivity of the assay at ≥21 days post symptom onset is 78.3% (95%CI 66.7 to 87.3). The manufacturer reported a sensitivity of 96.41% (95% CI 92.74 to 98.44) for samples ≥21 days post RT-PCR confirmation.

Introduction

Atellica-IM SARS-CoV-2 IgG (sCOVG) assay is intended for the qualitative and quantitative detection of IgG antibodies (including neutralising antibodies) to SARS-CoV-2 spike protein in human serum and plasma (lithium heparin) obtained by venepuncture or capillary puncture. 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 5 November 2020 to 15 December 20 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 IgG (sCOVG) Assay

The Atellica SARS-CoV-2 IgG (sCOVG) assay is a sandwich immunoassay for the qualitative and quantitative detection of IgG antibodies (including neutralising antibodies) to SARS Co-V-2 in human serum and plasma obtained by venepuncture or capillary puncture, manufactured by Siemens Healthcare GmbH. The assay is listed as CE marked.

Test principle

The Atellica IM sCOVG assay is a fully automated 2-step-sandwich immunoassay using acridinium-ester chemiluminescent technology. 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 Lite Reagent contains acridinium-ester-labelled anti-human IgG mouse monoclonal antibody used to detect anti-SARS-CoV-2 antibodies bound to the Solid Phase via the anti-SARS-CoV-2 IgG:SARS-CoV-2 antigen complex.

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 sCOVG assay results in Index Values and as Nonreactive or Reactive, with a measuring interval of 0.50 to 150.00:

- 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

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:

- performance characteristics have not been established for the assay used in conjunction with other manufacturers' assays for specific SARS-CoV-2 serological markers; laboratories are responsible for establishing their own performance characteristics
- the performance of the assay has not been established with cord blood, neonatal specimens, cadaver specimens, or body fluids other than serum or plasma
- results obtained with the assay may not be used interchangeably with values obtained with different manufacturers' test methods
- 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
- 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 or MERS-CoV, 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 to prevent SARS-CoV-2 transmission during blood, tissue, or organ donations

Manufacturer's performance characteristics

Sensitivity

Clinical sensitivity was determined by testing 836 samples collected from 661 unique donor subjects with a clinical diagnosis of COVID-19 based on a positive SARS-CoV-2 polymerase chain reaction (PCR) method. Table 1 describes clinical sensitivity by time of sampling following a positive PCR result:

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 to 6	368	187	181	50.82% (45.58 to 56.03)
7 to 13	194	160	34	82.47% (78.38 to 87.55)
14 to 20	79	72	7	91.14% (82.59 to 96.36)
≥ 21	195	188	7	96.41% (92.74 to 98.44)

Specificity

Clinical specificity was determined by testing 1995 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 Table 2 below:

Table 2. Specificity of the assay according to the manufacturer

Group	Number tested	Non-Reactive	Reactive	Sensitivity, % (95% CI)
Apparently Healthy	1952	1950	2	99.90% (99.63 to 99.99)
Apparently Healthy Pregnant Women	43	43	0	100.0% (91.78 to 100.0)
Total	1995	1993	2	99.90% (99.64 to 99.99)

Interferences

Interference testing was performed in accordance with CLSI Document EP07-ed3 using the Atellica IM sCOVG assay with 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
Cholesterol	500 mg/dL
Protein, total	12 g/dL

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 using the Atellica IM sCOVG assay with the Atellica IM Analyzer. No false positive results were observed with the potential cross-reactants listed in the following table:

Table 4. Cross-reactions according to the manufacturer

Condition	Number of samples tested	Number Reactive with Atellica IM sCOVG Assay
Autoimmune diseasea	14	0
Candida albicans antibody	10	0
Chlamydia pneumoniae IgG	10	0
Chlamydia trachomatis IgM	4	0
Cytomegalovirus (CMV) IgG	5	0
Cytomegalovirus (CMV) IgM	5	0
Epstein-Barr Virus (EBV) IgG	5	0
Epstein-Barr Virus (EBV) IgM	5	0
Haemophilus influenzae b	20	0
Hepatitis A infection (HAV) IgM	4	0
Hepatitis B Core Antigen (Anti-HBc) IgM	5	0
Hepatitis C infection (HCV) antibody	5	0
Human anti-mouse antibody (HAMA)	2	0
Human coronavirus antibodyb	29	0
Human herpes virus (HHV) IgM	2	0

Condition	Number of samples tested	Number Reactive with Atellica IM sCOVG Assay
Human immunodeficiency virus (HIV) antibody	9	0
Influenza antibody	29	0
Influenza A antibody	6	0
Influenza B antibody	10	0
Measles antibody	5	0
Mycoplasma pneumoniae IgG	19	0
Parvovirus B19 antibody	5	0
Respiratory pathogen antibodiesc	23	0
Respiratory syncytial virus (RSV)	23	0
Streptococcus pneumoniae anti-PCP IgG	10	0
Toxoplasma gondii antibody	10	0
Toxoplasma gondii IgG	20	0
Varicella zoster virus (VZV) antibody	4	0
Total	295	0

a) This group consists of samples from 14 subjects with autoimmune disease states, including anti-nuclear antibody (ANA; N = 5), Graves' disease (N = 5) and rheumatoid factor (RF; N = 4).
b) This panel includes 29 subjects who had antibodies to multiple human coronaviruses including coronavirus HKU (N = 24), coronavirus OC43 (N = 27), coronavirus 229E (N = 29), and coronavirus NL63 (N = 21).
c) This panel consists of samples from 19 subjects with antibodies to multiple respiratory pathogens, including Adenovirus antibodies (N = 8), Bordetella pertussis IgG (N = 19), Chlamydia pneumoniae IgG (N = 23), Chlamydia psittaci IgG (N = 3), Chlamydia psittaci IgM (N = 1), Haemophilus influenzae b (Hib) IgG (N = 11), Influenza A IgG (N = 22), Influenza A IgM (N = 1), Influenza B IgG (N = 18), Influenza B IgM (N = 1) and Mycoplasma pneumoniae IgG (N = 6).

Testing of Atellica SARS-CoV-2 IgG (sCOVG) assay by PHE

Kits from preproduction batch (Lot number: 002, PN: 11206970), were used for the evaluation outlined below. The evaluation took place at PHE Porton Down between 5 November 2020 and 15 December 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 an Atellica IM instrument.

- positive samples – 115 convalescent samples defined by a positive PCR from a swab sample for that patient
- confounder negative samples – 97 samples from the Sero-Evaluation Unit (SEU), Manchester that are rheumatoid factor (25 samples), CMV (22 samples), EBV (26 samples) or VZV (24 samples) positive
- lyme negatives – 45 samples were obtained from the Rare and Imported Pathogens Laboratory 2015 Lyme disease 2015 negative collection
- seasonal coronavirus samples – 10 samples (Confirmed seasonal coronavirus sera)
- negative historical samples – 348 samples (from SEU)

Testing results

Sensitivity

The overall sensitivity of the Atellica IM assay was measured as being 64.3% (95%CI 54.9 to 73.1).

Table 5. /Overall sensitivity of the Atellica-IM SARS-CoV-2 IgG (sCOVG) assay from the PHE assessment

No. Samples	Positive	Negative	Sensitivity (95% CI)
115	74	41	64.3% (54.9-73.1)

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

Table 6. Assay sensitivity of the Atellica-IM SARS-CoV-2 IgG (sCOVG) assay by interval when tested with PHE's sample set. Seven samples were excluded due to lack of information on sample interval

Group	Interval (days)	Positive	Negative	Total	Sensitivity (95% CI)
	<= 10	8	16	24	33.3% (15.6 to 55.3)

Group	Interval (days)	Positive	Negative	Total	Sensitivity (95% CI)
Reported onset to sample date	11 to 20	6	9	15	40.0% (16.3 -to 67.7)
	21 to 30	34	11	45	75.6% (60.5 to 87.1)
	31 to 40	12	2	14	85.7% (57.2 to 98.2)
	41 to 50	8	2	10	80.0% (44.4 to 97.5)
	From 14 days	58	22	80	72.5% (61.4 to 82.9)
	From 21 days	54	15	69	78.3% (66.7 to 78.3)

Specificity

Three sample sets were used to determine the specificity of the assay, 142 confounder samples, 10 seasonal CoV samples and 348 negative historical samples.

Table 7. Specificity of the Atellica-IM SARS-CoV-2 IgG (sCOVG) assay from the PHE assessment

Category	n	Positive	Negative	Specificity (95% CI)
Negative samples	348	0	348	100% (98.9-100.0)
Confounders + RIPL samples	152	4	148	97.4% (93.4-99.3)
Seasonal CoV	10	0	10	100% (69.2-100.0)

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 72.5% (58/80) and specificity calculated at 100.0% (348/348).

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

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	100.0% (88.4 to 100.0)	97.0% (95.0 to 98.0)

Precision

To demonstrate the repeatability of the assay, 5 sample pools 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 that the assay performed within acceptable parameters for precision with inter-assay %CV of <5 for each sample pool tested

Table 9. Precision data for the Atellica SARS-CoV-2 IgG (sCOVG) assay from the PHE assessment

	Mean/SD/%CV	Date of Testing					Inter-Assay Mean	Inter-Assay SD	Inter-Assay % CV
		Day 1 10/12/20	Day 2 11/12/20	Day 3 12/12/20	Day 4 14/12/20	Day 5 15/12/20			
Precision 1	Mean	5.48	5.19	5.37	5.74	5.65	5.483	0.222	4.043
	SD	0.174	0.348	0.207	0.104	0.141			
	% CV	3.19	6.69	3.86	1.82	2.49			
Precision 2	Mean	0.48	0.496	0.494	0.518	0.50	0.499	0.011	2.277
	SD	0.008	0.011	0.023	0.031	0.016			
	% CV	1.71	2.29	4.66	6.01	3.16			
Precision 3	Mean	2.48	2.44	2.53	2.53	2.61	2.520	0.065	2.595
	SD	0.055	0.075	0.091	0.053	0.119			
	% CV	2.25	3.08	3.60	2.09	4.57			
Precision 4	Mean	0.99	0.98	1.02	1.07	1.02	1.018	0.036	3.580
	SD	0.042	0.035	0.051	0.081	0.059			
	%CV	4.26	3.56	5.05	7.59	5.76			
Precision 5	Mean	1.17	1.19	1.23	1.28	1.25	1.225	0.044	3.559
	SD	0.047	0.05	0.015	0.044	0.041			
	%CV	4.02	4.20	1.28	3.49	3.26			
Precision 6	Mean	1.62	1.57	1.66	1.71	1.70	1.654	0.057	3.443
	SD	0.037	0.063	0.064	0.081	0.046			
	%CV	2.32	4.05	3.85	4.78	2.74			

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, seasonal and negative samples).

Figure 1. Scatterplot of results by sample category

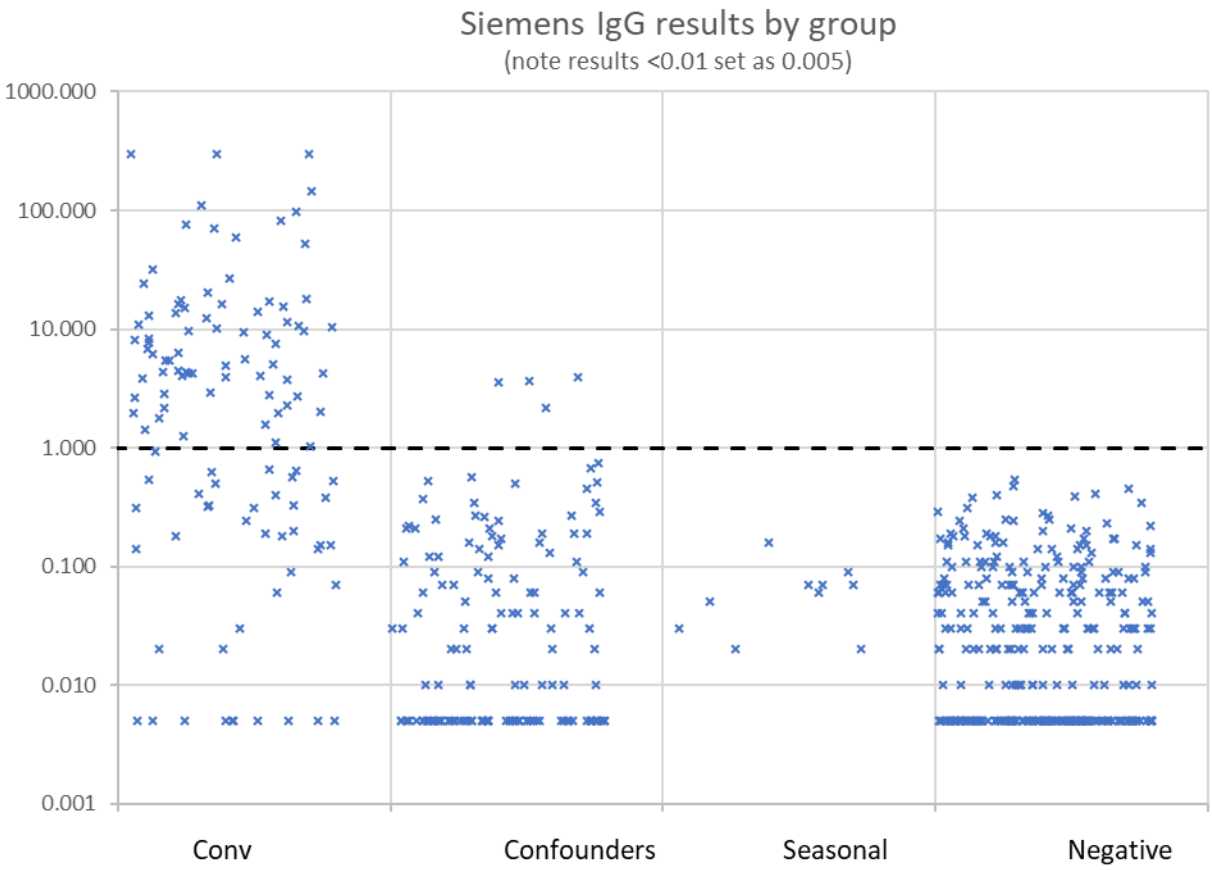


Figure 2 shows a scatterplot analysis of convalescent samples according to their time since symptom onset. The diagonal line in the plot shows the increase in antibody titre over time.

Figure 2. Scatterplot of time since symptom onset

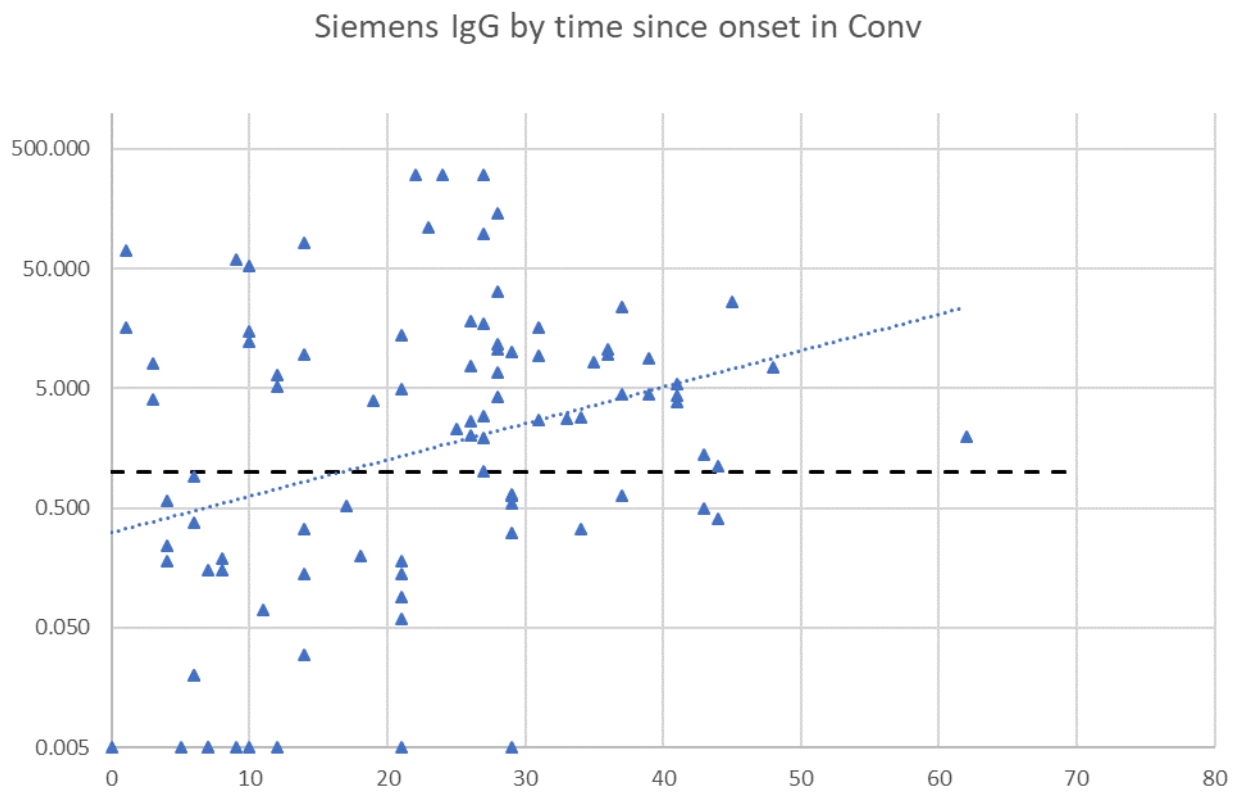


Figure 3 shows the distribution of antibodies against the manufacturer's cut-off of 1.0. To assess the cut-off for the assay, the distribution of the assay units in the negative samples are assessed (see Figure 4). It is usually desirable that a cut-off is set at least about 3 standard deviations (SD) 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 0.02 and the half-normal standard deviation was 0.399(log10) (right hand part of the distribution >= 0.06). 0.06 + 2.58 SD = 0.64 (anti-logged) and 0.06 + 3SD = 0.94 (anti-logged). So a cut-off of mean + 3 SD of 0.94 is close to the manufacturer's cut-off. The manufacturer cut-off gives a theoretical specificity of 99.89%.

Figure 3. Antibody distribution on a logarithmic scale (The **light blue lines denote the manufacturer's cut off values**)

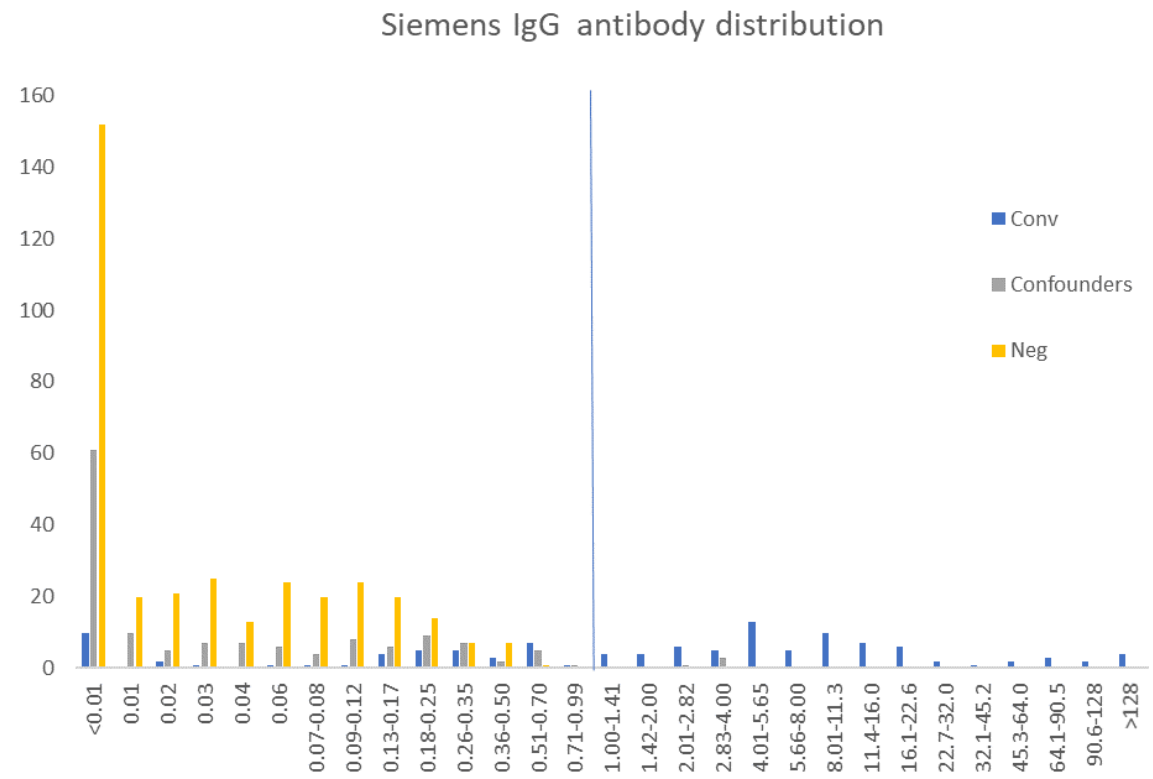
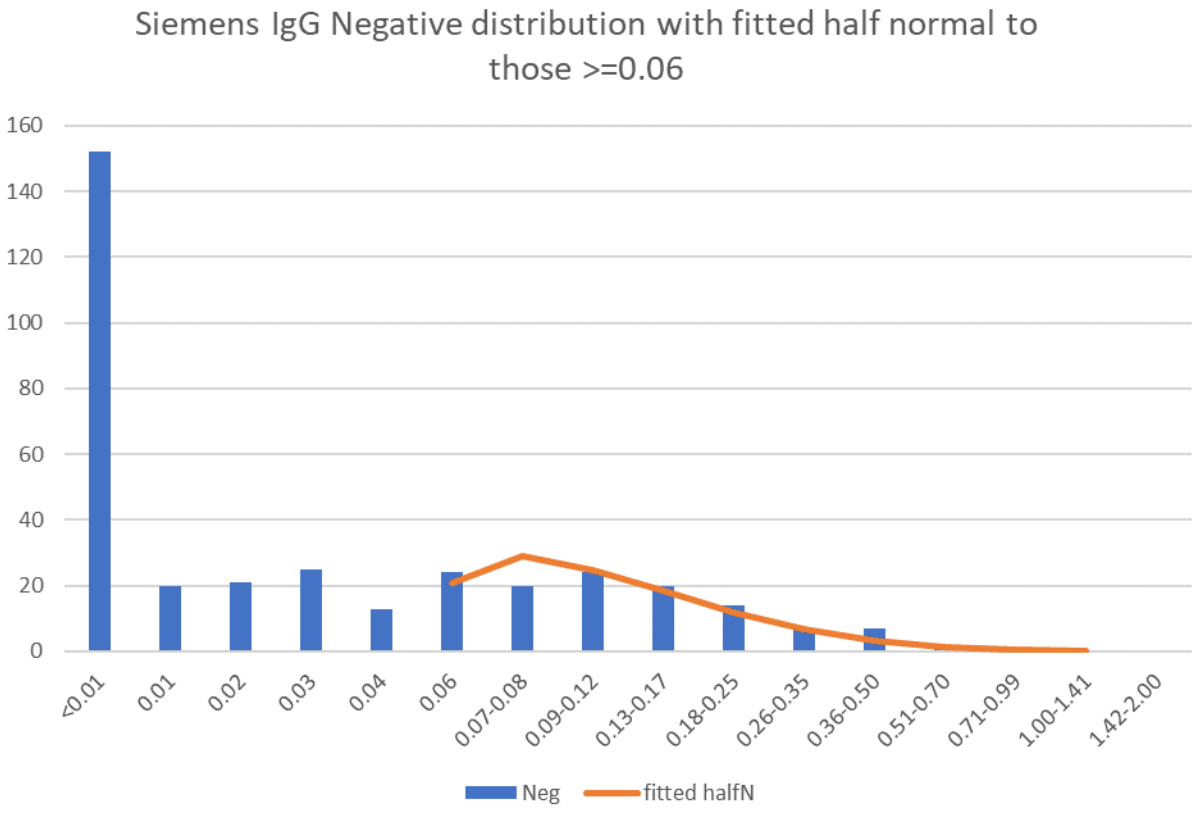


Figure 4. Negative distribution with a fitted half normal



Conclusions

In conclusion, the Siemens Atellica SARS-CoV-2 IgG (sCOVG) gave a specificity of 100% (95%CI 98.9-100.0) in this evaluation; the reported specificity of the manufacturer is 99.90% (95%CI 99.64-99.99).

In this evaluation, the sensitivity of the Siemens SARS-CoV-2 IgG (sCOVG) assay increased from 72.5% (95%CI 61.4 to 81.9) for samples collected ≥14 post symptom onset to 78.3% (95%CI 66.7 to 87.3) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity was 64.3% (95%CI 54.9 to 73.1). The manufacturer reported a sensitivity of 96.41% (95%CI 92.74 to 98.44) for samples taken ≥21 days' post RT-PCR diagnosis.

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