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Evaluation of the Ortho Clinical Diagnostics Vitros Immunodiagnostic Products Anti-SARS-CoV-2 IgG serology assay for the detection of anti-SARS-CoV-2 antibodies

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Contents

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Executive summary

This document sets out the evaluation of the Ortho Clinical Diagnostics VITROS Immunodiagnostic Products anti-SARS-CoV-2 IgG 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 between11/5/20/20 and 15/05/20. 93 serum samples from convalescent patients and 490 negative samples were included in the assessment.

The assay gave a specificity of 99.7% (95% confidence interval 98.6-100). The manufacturer reported a specificity of 100% (95%CI 99.1-100).

The assay gave an overall sensitivity of 77.4% (95%CI 67.6-85.4), with a sensitivity ≥14 days of 79.7% (95%CI 69.2-88.0). The sensitivity of the assay at ≥21 days' post symptom onset is 81.3% (95%CI 70.7-89.4). The manufacturer reported a sensitivity of 90.0% (95%CI 76.3-97.2) for samples taken >15 days' post symptom onset.

Introduction

The VITROS Immunodiagnostic Products anti-SARS-CoV-2 IgG assay, manufactured by Ortho Clinical Diagnostics, is intended for the detection of IgG antibodies to SARS-CoV-2 in human serum and plasma. The assay is a chemiluminescent immunoassay (CLIA) 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 11/05/20 and 13/05/20 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.

VITROS Anti-Sars-CoV-2 IgG Assay

The ‘VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Reagent Pack’ when used in combination with the ‘VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Calibrator’ is a chemiluminescent immunoassay test intended for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum. The assay is manufactured by Ortho Clinical Diagnostics Inc. The VITROS Anti-SARS-CoV-2 IgG test is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The VITROS Anti-SARS-CoV-2 IgG test should not be used to diagnose acute SARS-CoV-2 infection. The assay has FDA Emergency Use Authorisation and is listed as CE marked.

As per the manufacturer’s information, the assay uses the structural spike protein of SARS-CoV-2 as its antigen.

Test principle

The VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG test is performed using the VITROS Anti-SARS-CoV-2 IgG Reagent Pack and the VITROS Anti-SARS-CoV-2 IgG Calibrator on the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems. An immunometric technique is used; this involves a two-stage reaction. In the first stage antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 spike protein coated on wells. Unbound sample is removed by washing. In the second stage horseradish peroxidase (HRP)-labelled murine monoclonal anti-human IgG antibodies are added in the conjugate reagent. The conjugate binds specifically to the antibody portion of the antigen-antibody complex. If complexes are not present, the unbound conjugate is removed by the subsequent wash step.
The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent is added to the wells. The HRP in the bound conjugate catalyses the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indicative of the amount of SARS-CoV-2 IgG antibody present.
The sample volume used per assay run is 20µL, the total sample volume required to run the assay is 55µL.

Interpretation of the result

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

Result Calculation

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Signal at Cutoff (Cutoff value)}}$$

Interpretation of Results

The following table summarizes the interpretation of results obtained with the VITROS Anti-SARS-CoV-2 IgG test on the VITROS Immunodiagnostic and VITROS Integrated Systems.

VITROS Anti-SARS-CoV-2 IgG Test Result (S/C)	Interpretation
<1.0	Specimen is non-reactive for Anti-SARS-CoV-2 IgG
≥1.0	Specimen is reactive for Anti-SARS-CoV-2 IgG

Table 1: Interpretation of results according to the manufacturer's instructions

Manufacturer's listed limitations

The limitations of the assay are:

- heterophilic antibodies in serum samples may cause interference in immunoassays. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results that are inconsistent with clinical observations indicate the need for additional testing
- a non-reactive result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody detected by the test
- the results obtained with this test should only be interpreted in conjunction with clinical findings, and the results from other laboratory tests and evaluations

- this test should not be used for screening of donated blood for the purpose of preventing COVID-19 transmission

Manufacturer's performance characteristics

Sensitivity

58 samples collected from patients confirmed to be SARS-CoV-2 positive by PCR were tested. Of the 58 PCR positive samples, 51 were reactive in the VITROS Anti-SARS-CoV-2 IgG assay and 7 were non-reactive. Reactivity was correlated with elapsed days after onset of symptoms. For the 40 samples collected > 15 days after symptoms were reported, 36 were Reactive for a Positive Percent Agreement to PCR of 90.0%.

The results are summarised in the table below.

Days between Symptom Onset and Serum Collection	**Number Reactive**	**Number Non-Reactive**	**Total number tested**	**PPA (95% CI)**
12-15	15	3	18	83.3% (58.6-96.4)
>15	36	4	40	90.0% (76.3-97.2)

Table 2: Sensitivity of the assay based on days between symptom onset and serum collection (interval)

Specificity

Four hundred and seven presumed SARS-CoV-2 negative samples from healthy blood donors were tested resulting in 100% clinical specificity (95% CI: 99.1–100.0%).

Interferences

The VITROS Anti-SARS-CoV-2 IgG test was evaluated for interference consistent with CLSI document EP7[1]. Of the compounds tested, none was found to interfere with the clinical interpretation of the test in negative and weakly reactive samples at the concentrations indicated.

Compound	**Concentration**	
Bilirubin, conjugated	40.0 mg/dL	475 µmol/L
Bilirubin, unconjugated	40.0 mg/dL	684 µmol/L
Biotin	3510 mg/mL	14.3 µmol/L
Haemoglobin	1000 mg/dL	0.156 mmol/L
Intralipid	2000 mg/dL	N/A

Table 3: Manufacturer's reported interferences with concentrations used.
N/A = not applicable (alternative units are not provided)

Cross-reactions

The VITROS Anti-SARS-CoV-2 IgG test was evaluated for potential cross-reactivity in anti-SARS-CoV-2 negative samples from medical conditions unrelated to SARS-CoV-2 infection. The results are summarised in the table below.

[1] CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Sample category	**Number of samples**	**Non-Reactive**	**Reactive**
Adenovirus antibody	2	2	0
Influenza A IgG	5	5	0
Influenza A IgM	3	3	0
Influenza B IgG	5	5	0
Influenza B IgM	1	1	0
Coxsackie Virus Antibody	5	5	0
Echovirus Antibody	5	5	0
Polio Virus	4	4	0
Anti-Respiratory Syncytial Virus	3	3	0
HCV Antibody	5	5	0
Anti Nuclear Antibody	5	5	0

Table 4: Manufacturer's reported cross-reactions of the VITROS Anti-SARS-CoV-2 IgG assay

Testing of VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Assay by PHE

9 kits of VITROS Anti-SARS-CoV-2 IgG assay were received from Ortho-Clinical Diagnostics. The evaluation took place on a Vitros ECiQ instrument at PHE Porton Down between 11/05/20 and 15/05/20.

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 the Vitros ECiQ.

- 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 79 samples. For the remaining 14 samples, the interval was measured from when the patient was admitted to hospital to sample collection date, so the interval for these samples is artificially low
- confounder negative samples- 49 samples from the Sero-Evaluation Unit (SEU), Manchester that are rheumatoid factor (12 samples), CMV (6 samples), EBV (19 samples) or VZV (12 samples) positive
- Porton negative samples. 50 samples from the RIPL 2015 Lyme disease negative sample collection
- Manchester negative samples- 391 historic samples from the SEU

Testing results

Sensitivity

No. Samples	**Positive**	**Negative**	**Sensitivity (95% CI)**
93	72	21	77.4% (67.6-85.4)

Table 5: Overall sensitivity of the VITROS Anti-SARS-CoV-2 IgG assay from the PHE assessment

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

Group	Interval (days)	Positive	Negative	Total	Sensitivity (95% CI)
Hospital admission to sample date	<= 10	9	5	14	64.3% (35.1-87.2)
Reported onset to sample date	11 to 20	2	2	4	50.0% (6.8-93.2)
	21 to 30	28	7	35	80.00% (63.1-91.6)
	31 to 40	25	5	30	83.3% (65.3-94.4)
	41 to 50	8	2	10	80.0% (44.4-97.5)
	From 14 days	63	16	79	79.7% (69.2-88.0)
	From 21 days	61	14	75	81.3% (70.7-89.4)

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

Specificity

Three sample sets were used to determine the specificity of the assay, 49 confounder samples, 50 RIPL Lyme disease negative samples and 391 negative historical samples).

Category	n	Positive	Negative	Specificity (95% CI)
Negative samples	391	1	390	99.7% (98.6-100)
Confounder + RIPL samples	99	1	98	99.0% (94.5-100)

Table 7: Specificity of the VITROS Anti-SARS-CoV-2 IgG assay from the PHE assessment

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.

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	16.08	16.04	15.98	15.74	15.84	15.936	0.221	1.389
	SD	0.084	0.270	0.164	0.23	0.181			
	% CV	0.52	1.684	1.028	1.462	1.147			
15068	Mean	2.986	2.896	2.906	3.014	2.846	2.929	0.092	3.16
	SD	0.074	0.126	0.033	0.056	0.045			
	% CV	2.49	4.344	1.157	1.856	1.602			
15069	Mean	0.01	0.01	0.01	0.01	0.01	0.01	0.0	0.0
	SD	0.00	0.00	0.00	0.0	0.0			
	% CV	0.00	0.00	0.00	0.0	0.0			
15116	Mean	11.0	10.52	10.5	11.02	10.94	10.796	0.279	2.582
	SD	0.3	0.083	0.07	0.083	0.114			
	% CV	2.72	0.795	0.673	0.759	1.042			
15117	Mean	14.5	13.9	14.02	14.22	14.06	14.14	0.251	1.779
	SD	0.07	0.173	0.148	0.192	0.134			
	% CV	0.487	1.24	1.05	1.353	0.954			

Table 8: Precision data for VITROS Anti-SARS-CoV-2 IgG Assay.

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 79.7% (63/79) and specificity calculated at 99.7% (390/391).

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	97.2% (86.1-99.9)	97.8% (96.7-98.7)

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

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). There is a tighter grouping of samples in the negative sample sets with the positive samples showing a wider distribution of assay results.

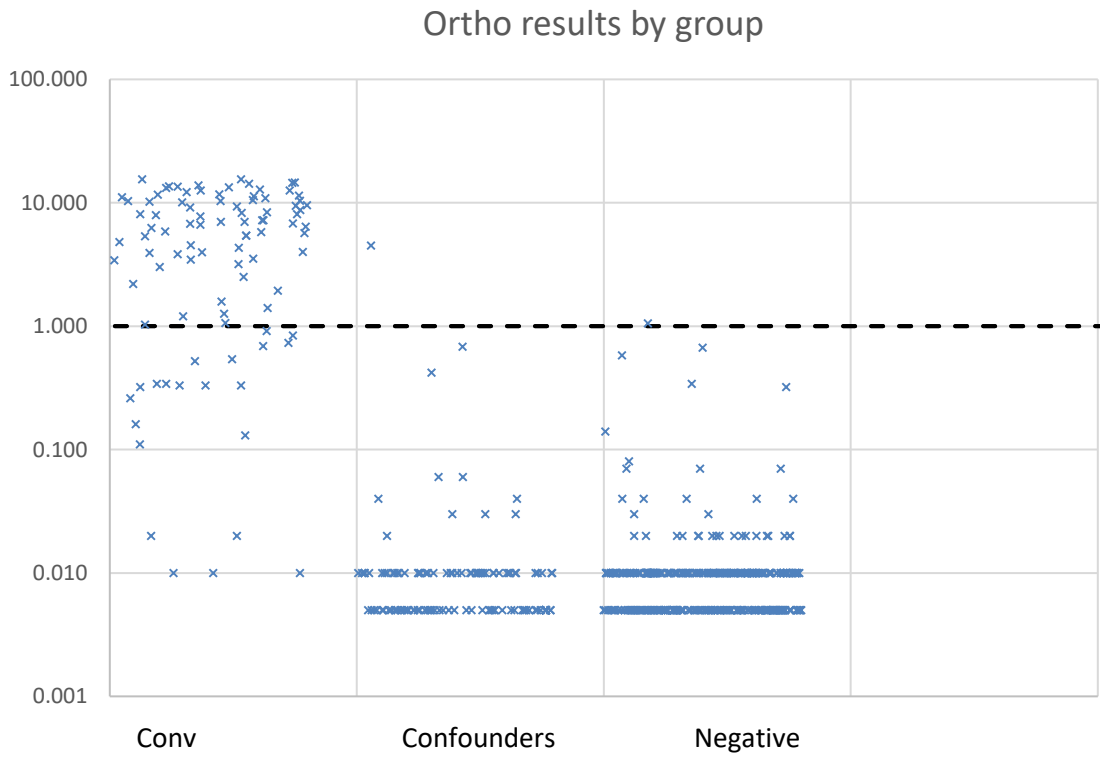

Figure 1: Scatterplot of results by sample category

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.

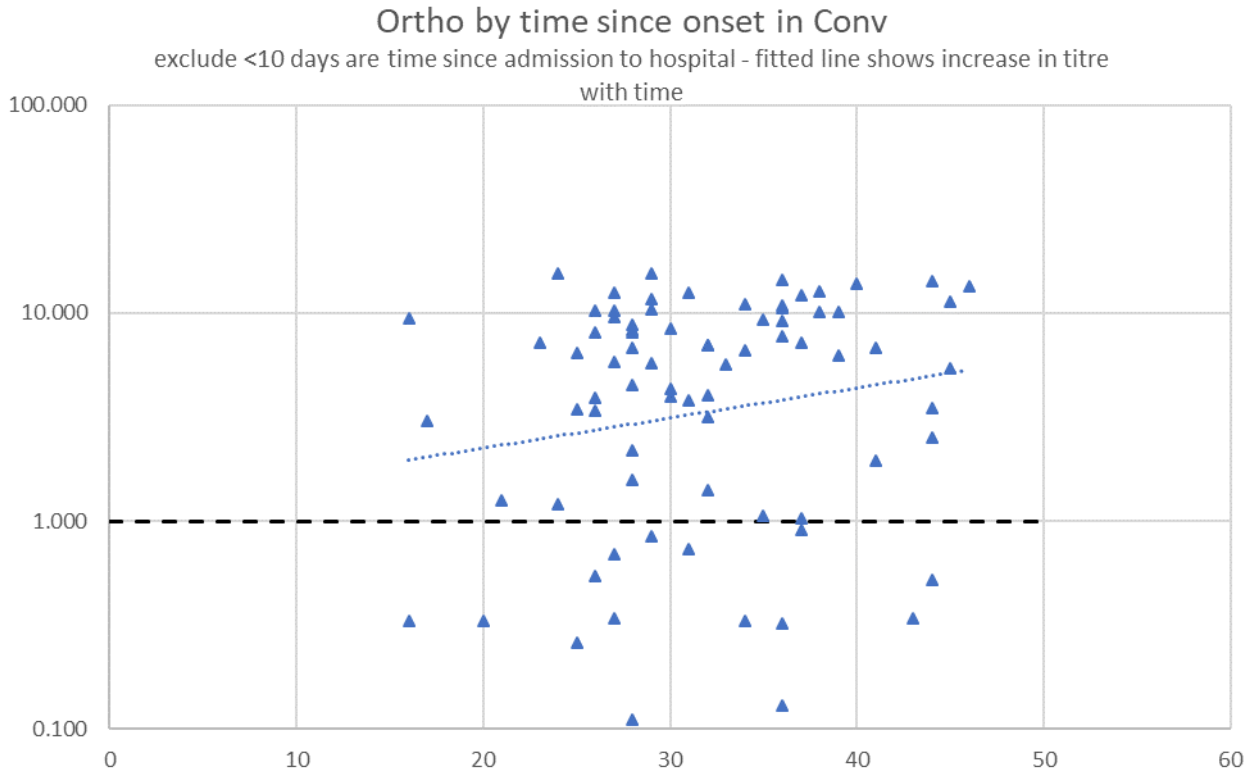

Figure 2: Scatterplot of time since symptom onset (excluding 14 samples that did not have an accurate time since symptom onset)

Figure 3 shows the distribution of antibodies against the manufacturer's cut-off. 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 <0.01 (-2 log10) and the half-normal standard deviation was 0.54 (log10) (right hand part of the distribution >= a value of 0.02). 0.02 + 2.58 SD = 0.49 (anti-logged) and 0.58 + 3SD = 0.83 (anti-logged). So a cut-off of mean + 3 SD of 0.83 is below the manufacturer's cut-off. The manufacturer cut-off gives a theoretical specificity of 99.9% ignoring outlier false positives.

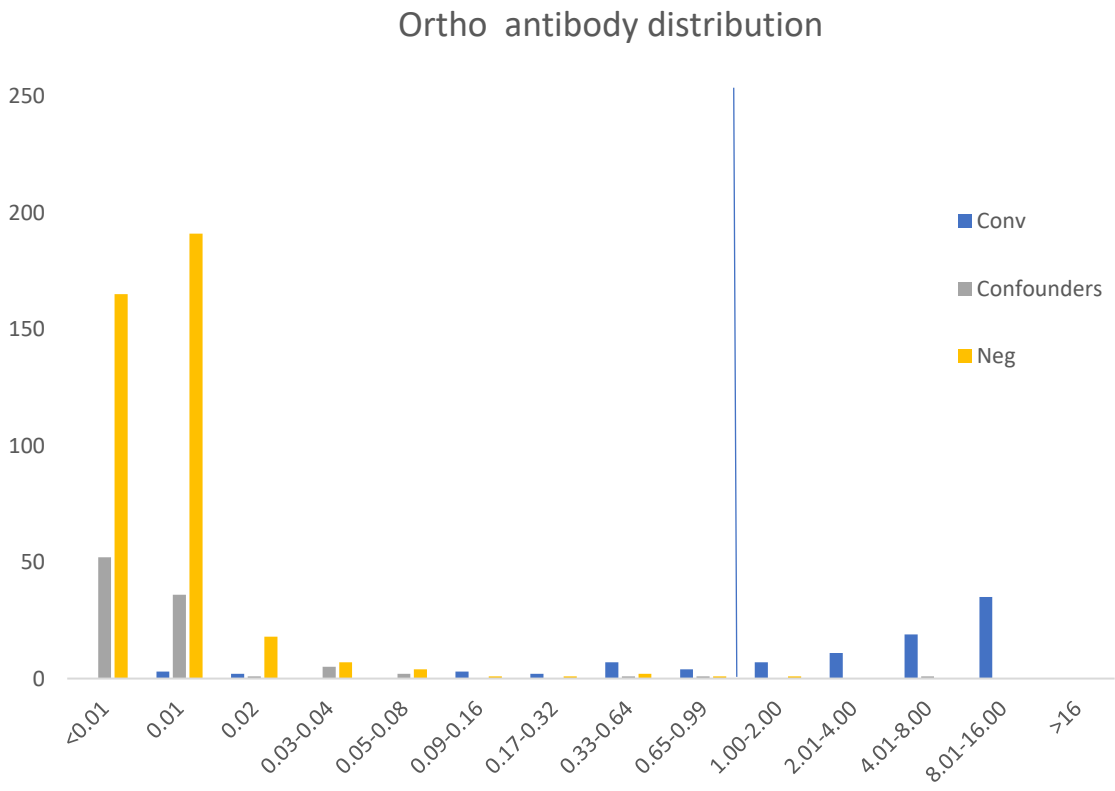

Figure 3: Antibody distribution on a logarithmic scale. The light blue line denotes the manufacturer's cut-off of 1.0

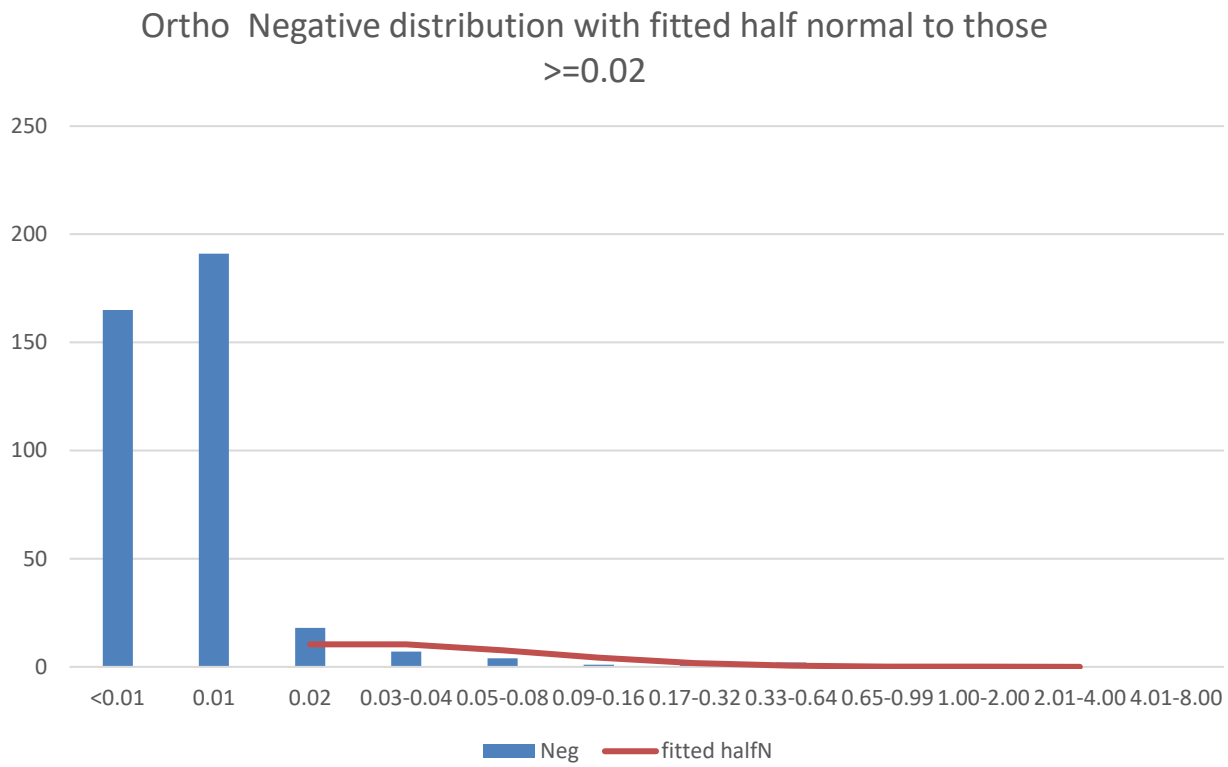

Figure 4: Negative distribution with a fitted half normal

Conclusions

In conclusion, the VITROS Anti-SARS-CoV-2 IgG assay gave a specificity of 99.7% (95%CI 98.6-100) in this evaluation; the reported specificity of the manufacturer is 100% (99.1-100).

In this evaluation, the sensitivity of the VITROS Anti-SARS-CoV-2 IgG assay increased from 79.7% (95%CI 69.2-88.0) for samples collected ≥14 post symptom onset to 81.3% (95%CI 70.7-89.4) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity was 77.4% (95%CI 67.6-85.4). The manufacturer reported a sensitivity of 83.3% (95%CI 58.6-96.4) for samples ≤15 days and a sensitivity of 90.0% (95%CI 76.3-97.2) for samples taken >15 days' post symptom onset.