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Evaluation of the Ortho Clinical Diagnostics Vitros Immunodiagnostic Products Anti-SARS-CoV-2 Total 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 Ortho Clinical Diagnostics VITROS Immunodiagnostic Products anti-SARS-CoV-2 Total 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 2-11 June 2020. 100 serum samples from convalescent patients and 491 negative samples were included in the assessment.

The assay gave a specificity of 99.5% (95% confidence interval 98.2-99.9). The manufacturer reported a specificity of 100% (95%CI 99.1-100).

The assay gave an overall sensitivity of 85.0% (95%CI 76.5-91.4), with a sensitivity ≥14 days after symptom onset of 91.8% (95%CI 83.8-96.6). The sensitivity of the assay at ≥21 days' post symptom onset was 93.5% (95%CI 85.5-97.9). The manufacturer reported a sensitivity of 100% (95%CI 92.7-100) for samples taken >8 days' post symptom onset.

Introduction

The VITROS Immunodiagnostic Products anti-SARS-CoV-2 Total assay, manufactured by Ortho Clinical Diagnostics, is intended for the detection of total antibody (including IgM, IgG and IgA) to SARS-CoV-2 in human serum and plasma. The assay is a chemiluminescent immunoassay (CLIA) and is processed on Ortho's 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 2-11 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.

VITROS Anti-SARS-CoV-2 Total Assay

The 'VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack' when used in combination with the 'VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Calibrator' is a chemiluminescent immunoassay test intended for the qualitative detection of total antibodies (including IgM, IgG and IgA) to SARS-CoV-2 in human serum and plasma. The assay is manufactured by Ortho Clinical Diagnostics Inc. The VITROS Anti-SARS-CoV-2 Total 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 Total test should not be used to diagnose acute SARS-CoV-2 infection. The assay 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 Total test is performed using the VITROS Anti-SARS-CoV-2 Total Reagent Pack and the VITROS Anti-SARS-CoV-2 Total 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 recombinant SARS-CoV-2 spike protein S1 antigen is added in the conjugate reagent. The conjugate binds specifically to any anti-SARS-CoV-2 captured on the well in the first stage. 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 antibody present. Signal to cut-off values will increase as the amount of SARS-CoV-2 antibody present in the sample increases.
The sample volume used per assay run is 80µL; the total sample volume required to run the assay is 115µL.

Interpretation of the result

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

Result Calculation

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

Interpretation of Results

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

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

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

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 recognised 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 – results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-CoV-2

may become detectable later following infection; at this time, it is unknown for how long IgM or IgG antibodies may persist following infection
- the presence of specific antibodies is a sign of previous or current infection

Manufacturer's performance characteristics

Sensitivity

Samples collected from 86 individual patients confirmed to be SARS-CoV-2 positive by PCR were tested. Of the 86 PCR positive samples, 80 were Reactive in the VITROS Anti-SARS-CoV-2 Total assay and 6 were Non-reactive. Five of the 6 Non-reactive samples were sent for supplemental testing and found to be negative for neutralizing antibody and were therefore not appropriate for use in the sensitivity calculation. For 69 of the 86 samples, the date of sample collection and date of onset of symptoms were provided. Reactivity was correlated with elapsed days after onset of symptoms. Antibody sensitivity and the 95% confidence intervals were calculated.
The results are summarised in the table below.

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

Days since symptoms reported*	Number Reactive	Number Non-Reactive	Total number tested	Sensitivity (95% CI)
≤8	16	4**	20	100% (79.4-100)
>8	49	0	49	100% (92.7-100)

* An additional 17 samples were tested but information about date of symptom onset was not available. Of those 17 samples, 15 were Reactive and 2 were Non-Reactive.
** These samples were negative for neutralizing antibody and removed from the Sensitivity calculation.

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%).

Interferences

The VITROS Anti-SARS-CoV-2 Total test was evaluated for interference. Commonly encountered substances were tested on one lot of reagent. Of the compounds tested, none was found to interfere with the clinical interpretation of the test.

Table 3: Manufacturer's reported interferences with concentrations used

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

N/A = not applicable (alternative units are not provided)

Cross-reactions

The VITROS Anti-SARS-CoV-2 Total 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.

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

Sample category	Number of samples	Non-Reactive	Reactive
Adenovirus antibody	2	2	0
Influenza A Antibody	5	5	0
Influenza B Antibody	5	5	0
Coxsackie Virus Antibody	5	5	0
Echovirus Antibody	5	5	0
HCV Antibody	5	5	0
Anti Nuclear Antibody	5	5	0

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

Eight kits of VITROS Anti-SARS-CoV-2 Total assay were received from Ortho-Clinical Diagnostics. The evaluation took place on a Vitros ECiQ instrument at PHE Porton Down between 2-11 June 2020.

Procedure for testing

Research operators from DSP performed testing of kits. All testing was performed per the manufacturer's instructions on the Vitros ECiQ. The sample sets used were:

- positive samples – 100 convalescent serum 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 all samples
- Manchester negative samples – 86 historic negative samples from the Sero-Epidemiology Unit (SEU)
- Porton negative samples – 313 historic negative samples from PHE Immunoassay Group (IAG); 50 samples from the RIPL 2015 Lyme disease negative sample collection
- confounder negative samples – 50 samples from the SEU that are rheumatoid factor (12 samples), CMV (6 samples), EBV (19 samples) or VZV (13 samples) positive

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

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

No. Samples	Positive	Negative	Sensitivity (95% CI)
100	85	15	85.0% (76.5-91.4)

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

Table 6: Sensitivity of the VITROS Anti-SARS-CoV-2 Total assay by interval

Group	Interval (days)	Positive	Negative	Total	Sensitivity (95% CI)
Reported onset to sample date	<= 10	5	6	11	45.5% (16.7-76.6)
	11 to 20	8	4	12	66.7% (34.9-90.1)
	21 to 30	32	5	37	86.5% (71.2-95.5)
	31 to 40	31	0	31	100% (88.8-100)
	41 to 50	9	0	9	100% (66.4-100)
	From 14 days	78	7	85	91.8% (83.8-96.6)
	From 21 days	72	5	77	93.5% (85.5-97.9)

Specificity

Three negative sample sets were used to examine the specificity of the assay. Eight samples (1 rheumatoid factor confounder sample, 1 negative sample from the IAG and 6 negative samples from the SEU) did not yield results and so were excluded from the analysis.

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

Category	n	Positive	Negative	Specificity (95% CI)
Negative samples	392	2	390	99.5% (98.2-99.9)
Confounder + RIPL samples	99	1	98	99.0% (94.5-100)

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 ≥14 days following onset of symptoms, with sensitivity calculated at 91.8% (78/85) and specificity calculated at 99.5% (390/392).

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

Seroprevalence	PPV (95%CI)	NPV (95%CI)
10%	95.2% (84.8-99.4)	99.1% (98.2-99.6)

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 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 VITROS Anti-SARS-CoV-2 Total Assay

	Mean/SD/%CV	Date of Testing					Inter-Assay Mean	Inter-Assay SD	Inter-Assay % CV
		Day 1 05/06/20	Day 2 08/06/20	Day 3 09/06/20	Day 4 10/06/20	Day 5 11/06/20			
Pool 1	Mean	464.00	443.80	441.40	440.60	445.00	446.96	9.87	2.21
	SD	5.39	5.89	2.61	5.03	4.36			
	% CV	1.16	1.33	0.59	1.14	0.98			
Pool 2	Mean	317.60	313.40	299.00	306.60	302.00	307.72	7.75	2.52
	SD	2.61	2.61	2.74	4.83	4.30			
	% CV	0.82	0.83	0.75	1.57	1.42			
Pool 3	Mean	106.60	103.40	102.60	103.00	102.20	103.56	2.26	2.18
	SD	1.82	1.52	2.07	1.58	1.64			
	% CV	1.70	1.47	2.02	1.54	1.61			
Pool 4	Mean	24.58	24.50	23.64	23.92	23.32	23.99	0.67	2.81
	SD	0.36	0.34	0.81	0.42	0.40			
	% CV	1.45	1.38	3.43	1.76	1.73			
Pool 5	Mean	10.84	10.84	10.74	10.52	10.50	10.69	0.21	2.01
	SD	0.17	0.21	0.09	0.20	0.12			
	% CV	1.54	1.91	0.83	1.95	1.17			

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

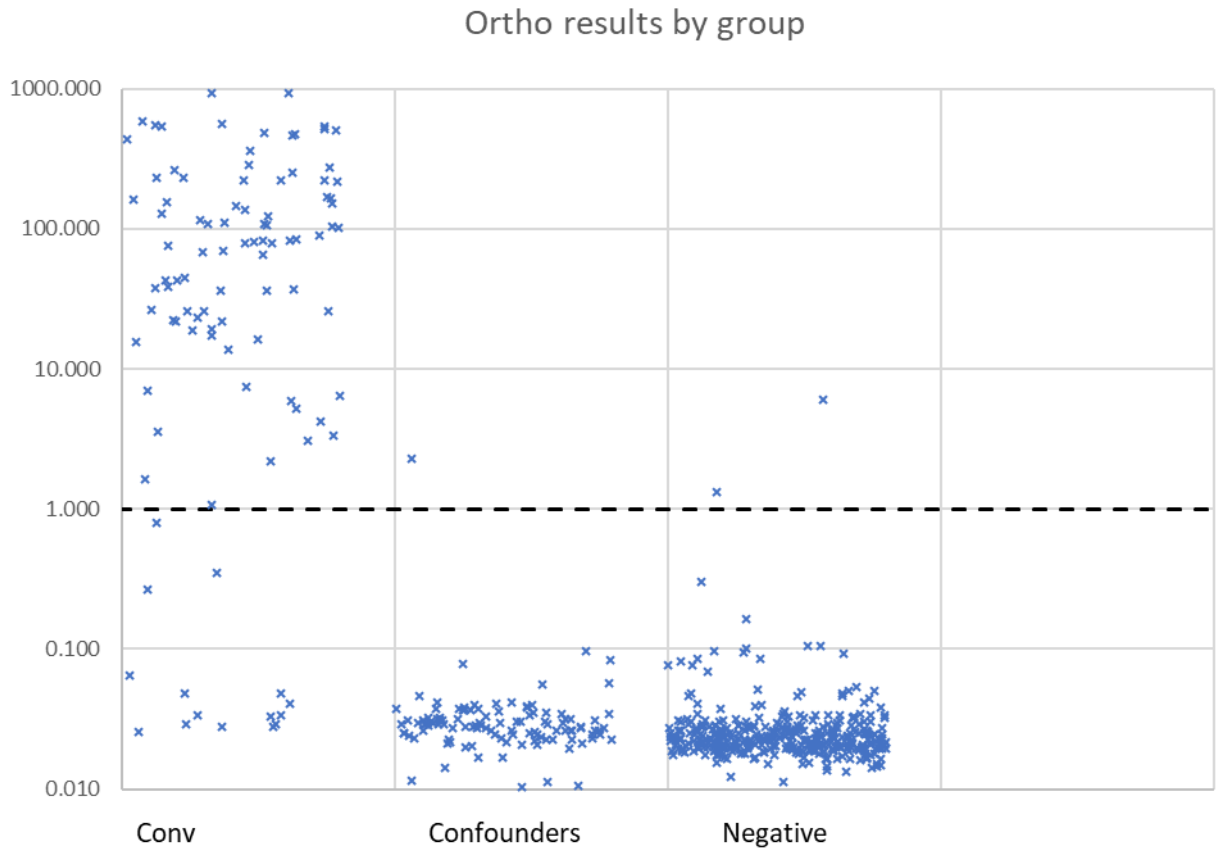


Figure 2 shows a scatterplot analysis of samples according to their time since symptom onset. The dashed line shows the rise in antibody titre over time from onset of symptoms.

Figure 2: Scatterplot of time since symptom onset

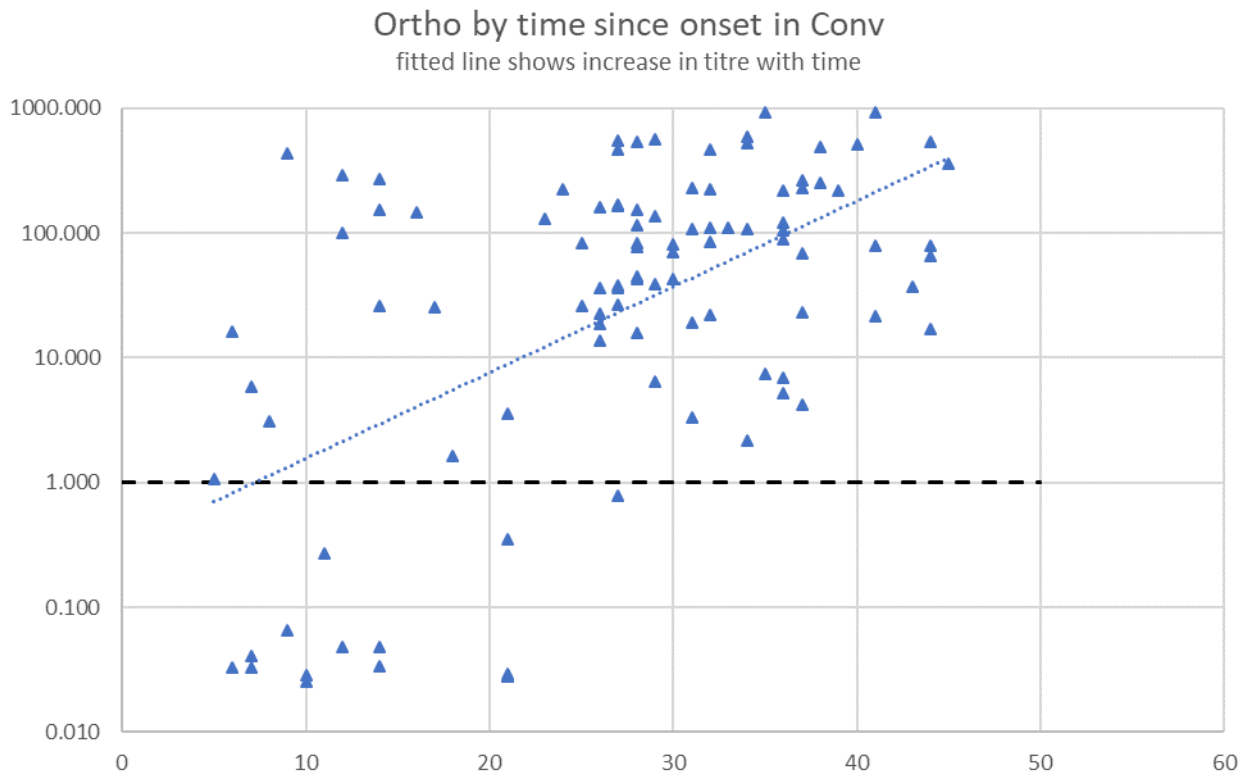
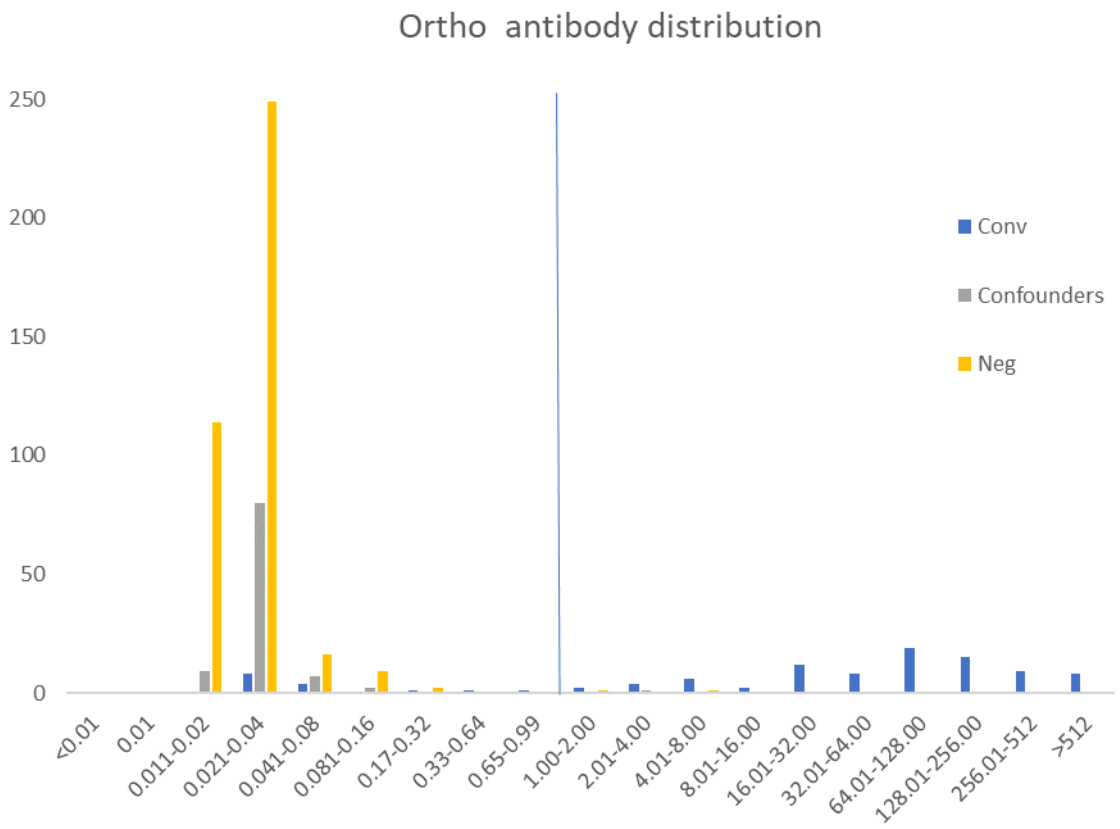


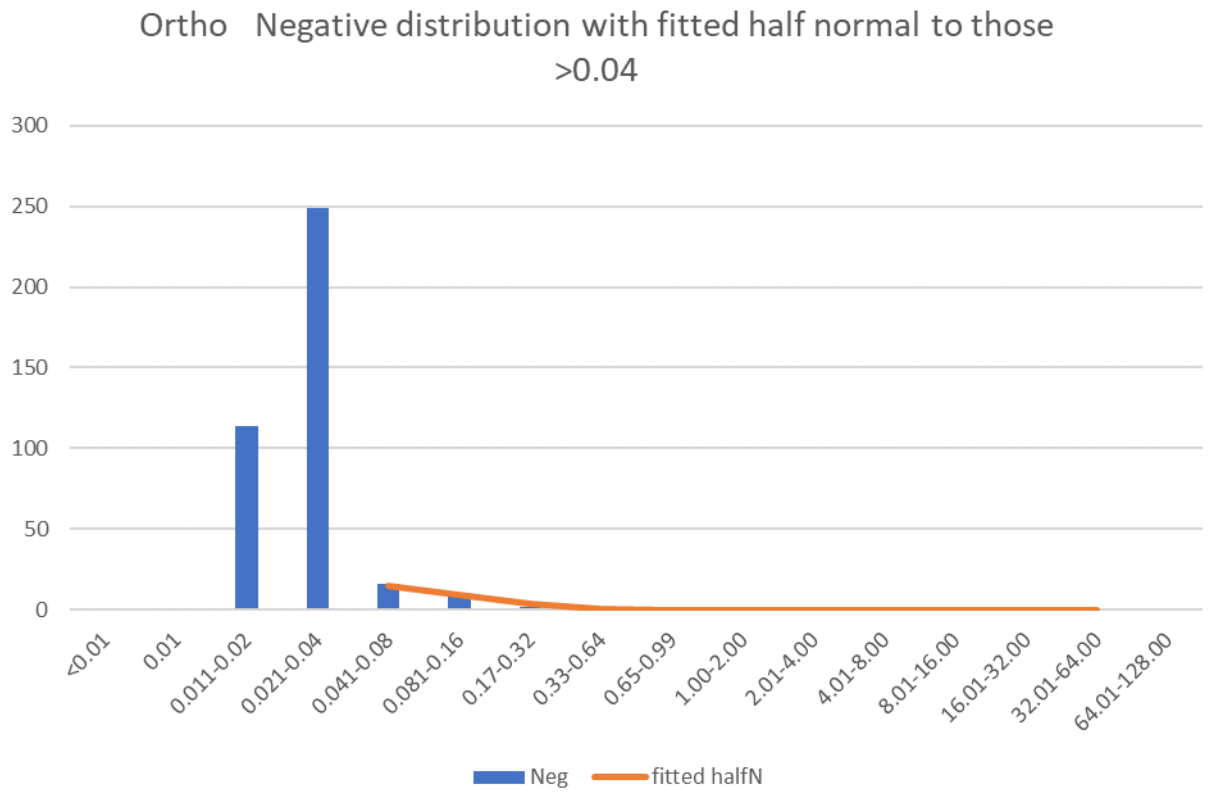
Figure 3 shows the distribution of antibodies against the manufacturer's cut-off. To assess the cut-off for the assay, the distribution of the assay units in the negative samples are assessed (see Figure 4). It is usually desirable that a cut-off is set about 3 standard deviations (SD) above the mean 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 <2 the mean was 0.025 (-1.6 log10) and the half-normal standard deviation was 0.42 (log10) (right hand part of the distribution > a value of 0.04 where this distribution gives a good fit). 0.04 + 2.58 SD = 0.49 (anti-logged) and 0.04 + 3SD = 0.74 (anti-logged). So, a cut-off of 0.04 + 3 SD of 0.74 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 Total assay gave a specificity of 99.5% (95%CI 98.2-99.9) 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 Total assay increased was 91.8% (95%CI 83.8-96.6) for samples collected ≥14 post symptom onset and 93.5% (95%CI 85.5-97.9) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity was 85.0% (95%CI 76.5-91.4). The manufacturer reported a sensitivity of 100% (95%CI 79.4-100) for samples ≤8 days and a sensitivity of 100% (95%CI 92.7-100) for samples taken >8 days' post symptom onset.