Public Health England

Protecting and improving the nation's health

# Evaluation of the Abbott SARS-CoV-2 IgG for the detection of anti-SARS-CoV-2 antibodies

# About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. We do this through world-leading science, research, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health and Social Care, and a distinct delivery organisation with operational autonomy. We provide government, local government, the NHS, Parliament, industry and the public with evidence-based professional, scientific and delivery expertise and support.

Public Health England
Wellington House
133-155 Waterloo Road
London SE1 8UG
Tel: 020 7654 8000
www.gov.uk/phe
Twitter: @PHE_uk
Facebook: www.facebook.com/PublicHealthEngland

Prepared by: Jackie Duggan, Rare and Imported Pathogens Laboratory, PHE Porton Down
For queries relating to this document, please contact: Tim Brooks, Clinical Services Director, Rare and Imported Pathogens Laboratory, PHE Porton Down (tim.brooks@phe.gov.uk)



First published 18 May 2020; this version published 8 June 2020
PHE supports the UN Sustainable Development Goals

# Contents

About Public Health England 2

Document control 4

Executive summary 5

Introduction 6

Abbott SARS-CoV-2 IgG assay 7

- Test principle 7
- Interpretation of the result 7
- Manufacturer's listed limitations 8
- Manufacturer's performance characteristics 9

Testing of Abbott SARS-CoV-2 assay by PHE 12

- Procedure for testing 12
- Testing results 12
- Statistical analysis 14
- Conclusions 18

# Document control

| Current version publication date | Author | Amendments |
|---|---|---|
| 18 May 2020 | Jackie Duggan, Kevin Brown, Tim Brooks, Stephanie Migchelsen | |
| 19 May 2020 | Jackie Duggan, Kevin Brown, Tim Brooks, Stephanie Migchelsen | Data on Precision testing added |
| 23 May 2020 | Jackie Duggan, Kevin Brown, Nick Andrews, Tim Brooks, Stephanie Migchelsen | Correction to Table 1; minor wording amendment to clarify Sensitivity and Specificity results; 95% confidence intervals added |
| 8 June 2020 | Jackie Duggan, Kevin Brown, Nick Andrews, Tim Brooks, Stephanie Migchelsen | Error in the Executive Summary corrected; two confounders were incorrectly removed from previous analyses and have now been included |

# Executive summary

This document sets out the preliminary assessment of the Abbott SARS-CoV-2 IgG kit for the detection of anti-SARS-CoV-2 in human serum samples using the Abbott Architect *i*2000SR system.

The assessment was conducted by the Clinical Service Unit (CSU) at PHE Colindale between 4 and 7 May 2020. Ninety-six samples from convalescent patients and 760 negative samples were included in the evaluation.

All negative samples tested negative by the assay, giving a specificity of 100.00% (95% confidence interval 99.1-100.0) in the evaluation. The manufacturer reported a specificity of 99.6% (95%CI 99.1-99.9).

In this evaluation, the assay had an overall sensitivity of 92.7% (95%CI 85.6-97.0), with a sensitivity of 93.9% (95%CI 86.3-98.0) for samples ≥14 days post symptom onset. The sensitivity of the assay at ≥21 days post symptom onset is 93.5% (95%CI 85.5-97.9). The manufacturer reports a sensitivity of 100% (95%CI 95.9-100) for samples ≥14 post symptom onset.

# Introduction

The SARS-CoV-2 IgG kit is a chemiluminescent microparticle assay (CMIA) used for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma on the Architect *i* system. This report details a preliminary assessment of the assay conducted at PHE Colindale between 4-7 May 2020 to inform the use of the assay by NHS laboratories for the detection of anti-SARS-CoV-2 antibodies in patient samples.

# Abbott SARS-CoV-2 IgG assay

The SARS-CoV-2 IgG kit is an CMIA assay manufactured by Abbott Laboratories. The assay is listed as CE marked.

As per the manufacturer's information, the SARS-CoV-2 IgG assay is designed to detect immunoglobulin class G (IgG) antibodies to the nucleocapsid protein of SARS-CoV-2 in serum and plasma from patients with signs and symptoms of infection who are suspected of coronavirus disease (COVID-19) or in serum and plasma of subjects that may have been infected by SARS-CoV-2.

## Test principle

The assay is an automated, two step immunoassay for the detection of IgG antibodies to SARS-CoV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA). The antigen used in the assay is SARS-CoV-2 nucleocapsid.

1. the patient sample, SARS-CoV-2 antigen-coated paramagnetic microparticles and assay diluent are combined and incubated. IgG antibodies present in the patient sample bind to the antigen coated microparticles. The mixture is washed. Anti-human IgG acridinium-labelled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.
2. the resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics.
3. this relationship is reflected in the calculated Index (S/C). The presence or absence of IgG antibodies to SARS-CoV-2 in the sample is determined by comparing the chemiluminescent RLU in the reaction to the calibrator RLU.

## Interpretation of the result

The ARCHITECT *i* System calculates the calibrator mean chemiluminescent signal from 3 calibrator replicates and stores the result. Results are reported by dividing the sample result by the stored calibrator result. The default result unit for the SARS-CoV-2 IgG assay is Index (S/C). The cut off is 1.4 Index (S/C).

| Index (S/C) | Interpretation |
|---|---|
| <1.4 | Negative for anti-SARS-CoV-2 antibodies |
| ≥1.4 | Positive for anti-SARS-CoV-2 antibodies |

Table 1: Manufacturer's interpretation of the results

## Manufacturer's listed limitations

The limitations of the assay are:

- results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
- results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E, have not been evaluated with this assay. In a population of patients with non-COVID-19 respiratory illnesses, no cross-reactivity has been observed.
- not to be used to screen units of blood for SARS-CoV-2 infection.
- immunocompromised patients who have COVID-19 may have a delayed antibody response and produce levels of antibody which may not be detected as positive by the assay.
- specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as SARS-CoV-2 IgG that employ mouse monoclonal antibodies.
- heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed.
- rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.

# Manufacturer's performance characteristics

## Clinical performance

A study was performed to determine the clinical performance of the SARS-CoV-2 IgG assay.

To estimate the positive percent agreement (PPA), 122 serum and plasma specimens were collected at different times from 31 subjects who tested positive for SARS-CoV-2 by a polymerase chain reaction (PCR) method and who also presented with COVID-19 symptoms. Each specimen was tested using the SARS-CoV-2 IgG assay. The PPA and the 95% confidence interval (CI) were calculated.

To estimate the negative percent agreement (NPA), 1070 serum and plasma specimens from subjects assumed to be negative for SARS-CoV-2 were tested. Of the 1070 specimens, 997 specimens were collected prior to September 2019 (pre-COVID-19 outbreak). An additional 73 specimens were collected in 2020 from subjects who were exhibiting signs of respiratory illness but tested negative for SARS-CoV-2 by a PCR method. All 1070 specimens were tested using the SARS-CoV-2 IgG assay. The NPA and the 95% CI were calculated.

The results of both groups are presented in the following two tables.
**Positive Agreement by Days Post-Symptom Onset**

| **Days Post Symptom Onset** | **n** | **Positive** | **Negative** | **PPA (95% CI)** |
|---|---|---|---|---|
| <3 | 4 | 0 | 4 | 0.00% (0.0-60.2%) |
| 3-7 | 8 | 2 | 6 | 25.00% (3.2-65.1%) |
| 8-13 | 22 | 19 | 3 | 86.46% (65.1-97.1%) |
| ≥ 14 | 88* | 88 | 0 | 100% (95. 9-100%) |

Table 2: Manufacturer's reported assay sensitivity based on days post-symptom onset.

* Five specimens from 1 immunocompromised patient were excluded from the study. See assay limitations above. When the results from these specimens were included, the PPA at ≥ 14 days post-symptom onset was 96.8% (95%CI 90.9-99.3).

**Negative Agreement by Category**

| **Category** | **n** | **Positive** | **Negative** | **NPA (95% CI)** |
|---|---|---|---|---|
| Pre-COVID-19 Outbreak | 997 | 4 | 993 | 99.6% (99.0-99.9) |
| Other respiratory illness | 73 | 0 | 73 | 100% (95.1-100) |
| Total | 1070 | 4 | 1066 | 99.6% (99.1-99.90) |

Table 3: Manufacturer's reported assay specificity

## Interferences and analytical specificity

The SARS-CoV-2 IgG assay was evaluated for potential cross-reactivity from individuals with other medical conditions. A total of 182 specimens from 36 different categories were tested. One hundred eighty-one (181) specimens were negative and 1 specimen was positive by the SARS-CoV-2 IgG assay. The data are summarised in the following table. Bold indicates other respiratory illness categories.

| **Category** | **n** | **Positive** | **Negative** |
|---|---|---|---|
| Adenovirus | 5 | 0 | 5 |
| Antinuclear Antibody (ANA) | 5 | 0 | 5 |
| Autoimmune Hepatitis | 5 | 0 | 5 |
| Cytomegalovirus (CMV) IgG | 5 | 1 | 4 |
| CMV Immunoglobulin Class M (IgM) | 5 | 0 | 5 |
| Double-Stranded Deoxyribonucleic Acid (dsDNA) Antibody | 5 | 0 | 5 |
| Epstein-Barr Virus (EBV) IgG | 5 | 0 | 5 |
| EBV IgM | 5 | 0 | 5 |
| *Escherichia coli (E. coli)* Antibody | 5 | 0 | 5 |
| HAMA | 5 | 0 | 5 |
| Hemodialysis Patients | 5 | 0 | 5 |
| Hepatitis A Virus (HAV) | 5 | 0 | 5 |
| Hepatitis B Core (HBc) IgM | 4 | 0 | 4 |
| Hepatitis B Virus (HBV) | 5 | 0 | 5 |
| Hepatitis C Virus (HCV) | 5 | 0 | 5 |
| Hepatitis D Virus (HDV) | 5 | 0 | 5 |
| Herpes Simplex Virus (HSV) | 5 | 0 | 5 |
| Heterophilic Antibody Positive | 5 | 0 | 5 |
| Human Immunodeficiency Virus (HIV) | 5 | 0 | 5 |

Table 4: Manufacturer’s reported analytical specificity

# Testing of Abbott SARS-CoV-2 assay by PHE

Ten kits of 100 tests per kit of the SARS-CoV-2 IgG assay (reagent batch number 16253FN00, exp date 16/07/2020), control kit (lot number 16268FN00) and calibration kit (lot number 16265FN00) were obtained from Abbott on 29/04/20.

## Procedure for testing

Research operators from VRD performed testing of kits using the following sample sets. All testing was performed per the manufacturer's instructions on an Abbott Architect i2000SR automated instrument.

- positive samples- 96 samples defined by a positive PCR from a swab sample for that patient. All patients were healthy prior to their acquisition of COVID-19 disease
- confounder negative samples- 351 samples that are rheumatoid factor, CMV, EBV or VZV positive
- seasonal coronavirus positive samples- 11 samples
- negative samples- 395 historic samples (samples stored before mid-2019). These samples have been chosen based on their collection before mid-2019 to ensure they are SARS-CoV-2 antibody negative, but will contain samples containing antibodies to other seasonal coronaviruses to provide an additional screen for the assay

## Testing results

### Clinical sensitivity

The sensitivity was calculated using convalescent patient serum. Of the 96 sera used, 14 had an onset date ≤14 days prior to sample collection and 82 had an onset date ≥14 days prior to sample collection.

| Group | Interval (days) | Positive | Negative | Total | Sensitivity (95% CI) |
|---|---|---|---|---|---|
| Hospital admission to sample date | <= 10 | 12 | 2 | 14 | 87.5% (57.2-98.2) |
| Reported onset to sample date | 11 to 20 | 5 | 0 | 5 | 100% (47.8-100) |
| | 21 to 30 | 29 | 2 | 31 | 93.5% (78.6-99.2) |
| | 31 to 40 | 35 | 2 | 37 | 94.6% (81.8-99.3) |
| | 41 to 50 | 8 | 1 | 9 | 88.9% (51.8-99.7) |
| | From 14 days | 77 | 5 | 82 | 93.9% (86.3-98.0) |
| | From 21 days | 71 | 5 | 76 | 93.4% (85.3-97.8) |

Table 5: Assay sensitivity by interval when tested with PHE's sample set

It should be noted here that none of the patients with previously positive PCR who tested negative by this assay had been hospitalised for COVID-19 disease and most likely had a mild disease outcome.

## Specificity

Specificity was calculated based on testing samples collected in 2019, and before the onset of COVID infection in the UK and gave a specificity of 100% (95% CI 99.1-100). In addition, two groups of samples were tested for interfering samples: 11 samples positive for seasonal coronavirus, and a second group who tested positive for rheumatoid factor, EBV, CMV or VZV. None of the seasonal coronavirus samples tested positive.

| Category | n | Negative | Positive | Specificity (95% CI) |
|---|---|---|---|---|
| Negative sample collection | 395 | 395 | 0 | 100% (99.1-100.0) |
| Seasonal coronavirus positive | 11 | 11 | 0 | 100% (71.5-100.0) |
| Confounders | 354 | 352 | 2 | 99.4% (98.0-99.9) |

Table 6: Result of testing negative and confounder samples in PHE's sample sets

## Precision

Five pools of sera (4 positive, 1 negative) were tested at different times over 2 days by different operators to assess the intra-laboratory variation of the assay. Table 8 gives the mean, SD and %CV of the testing for each pooled sample. The data shows that the assay performed within acceptable parameters for precision with inter-assay %CV of <1 for each sample pool tested.

| Pool ID | Mean | SD | %CV |
|---|---|---|---|
| Pool 1 | 7.306 | 0.086 | 1.8 |
| Pool 2 | 7.318 | 0.123 | 1.7 |
| Pool 3 | 4.036 | 0.021 | 0.5 |
| Pool 4 | 6.994 | 0.140 | 2.0 |
| Pool 5 (negative) | 0.092 | 0.0044 | 4.8 |

Table 7: Precision of Abbott SARS-CoV-2 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 93.9% (77/82) and specificity calculated at 395/395 (100%) based on the negative sample collection.

| Seroprevalence | PPV (95%CI) | NPV (95%CI) |
|---|---|---|
| 10% | 100% (91.8-100) | 99.3% (98.5-99.8) |

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

## Statistical analysis

The plots below show the statistical analysis on the data obtained.

Figure 1 shows the plot of the samples in the positive sample pool plotted against the time after symptom onset. An asymptomatic case, which tested positive by PCR, can be seen in the lower right quadrant of the graph. All other samples that tested negative have a longer symptom onset date with one sample that has a symptom onset date of >40 days.

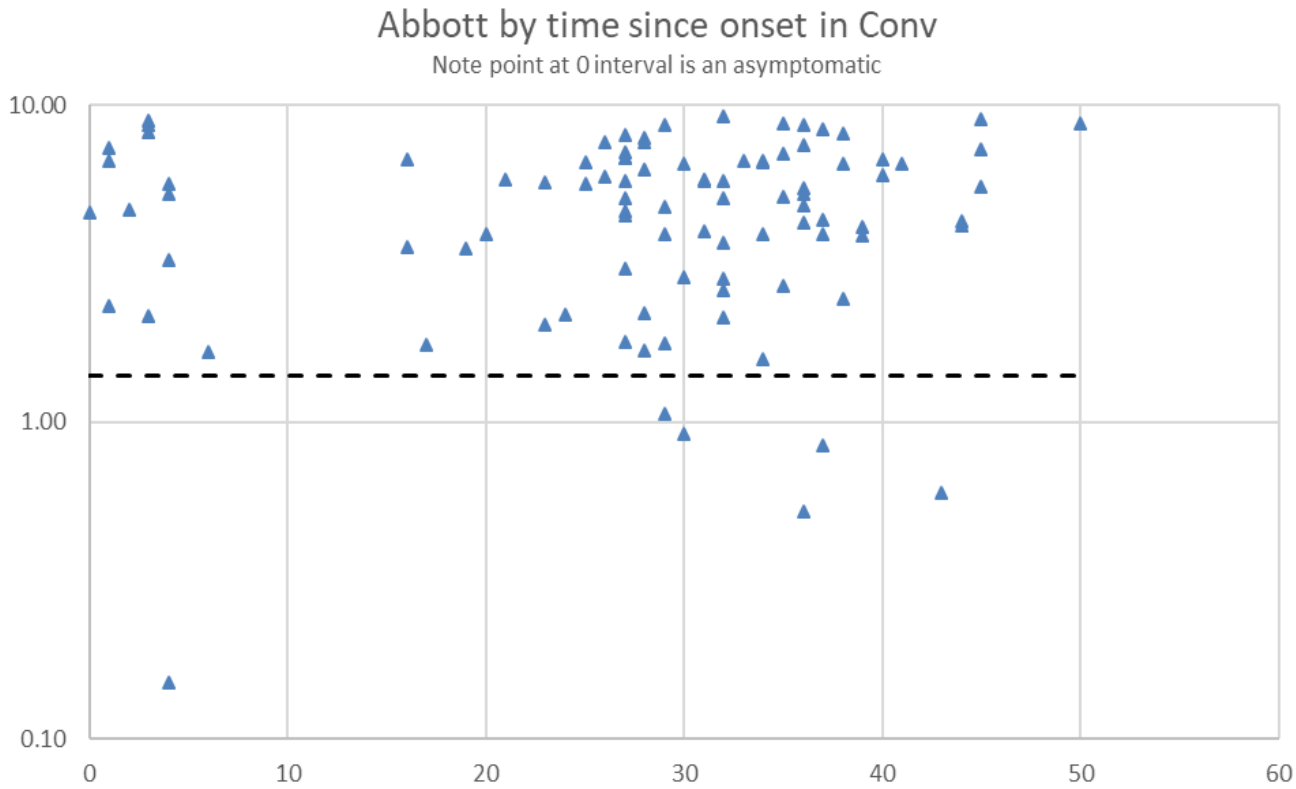


Figure 1. Graph showing the positive sample set plotted against time since symptom onset using the manufacturer's cut off of 1.4

Figure 2 shows the distribution of all the sample results according to sample group. Most of the negative samples fall well below the assay cut off of 1.4. Figure 3 shows the data presented as a scatterplot.

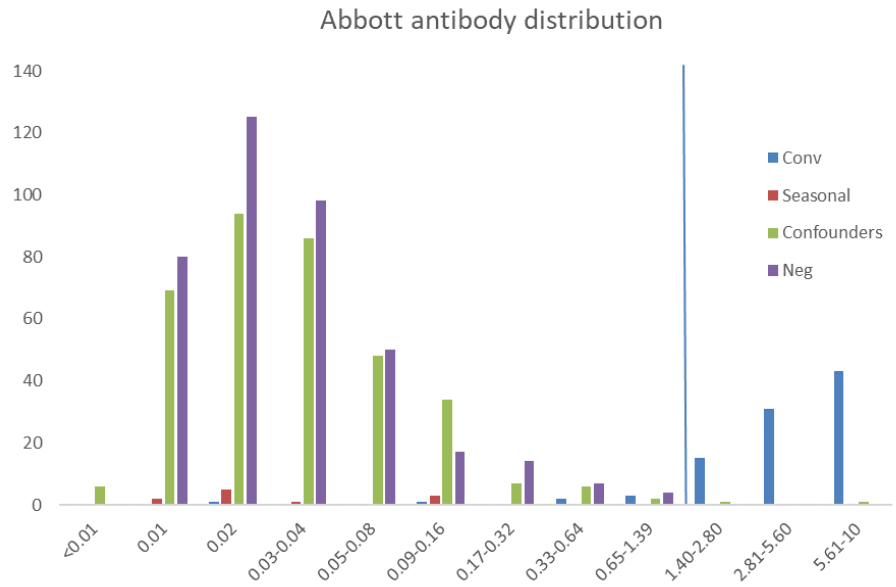


Figure 2: Distribution of antibodies according to sample group. A logarithmic scale was used for the assay value on the X axis. The Y axis represents the number of samples at each particular assay value.

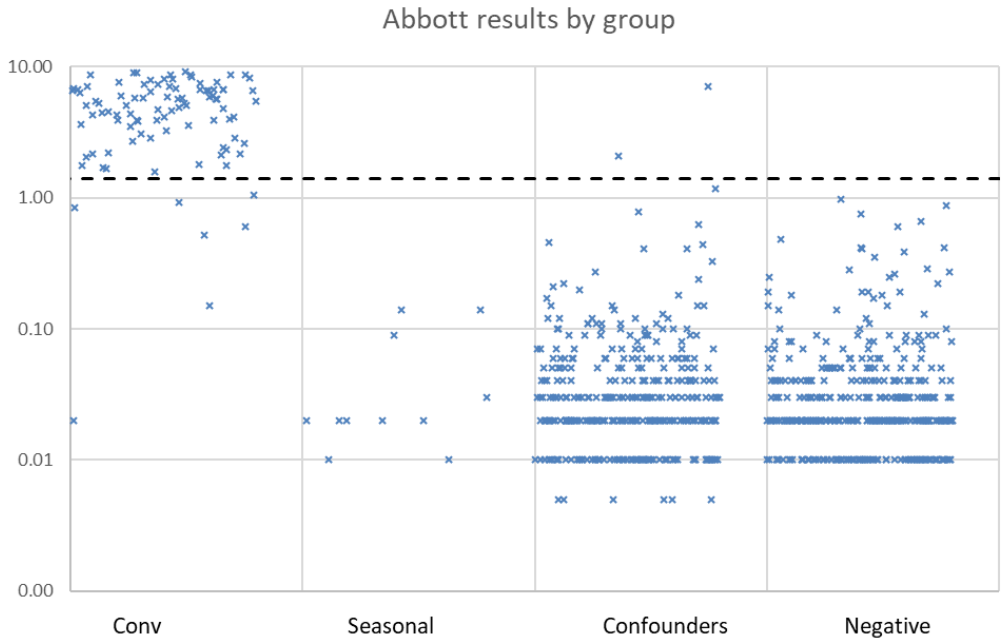


Figure 3: Scatterplot of all samples according to sample group.

Figure 4 shows the assessment of the negative samples based on the cut off of 1.4 based on a fitted half normal of >= 0.05. To assess the cut-off for the assay the distribution of the assay units in the negative samples are assessed. 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 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. From the graph, the right hand score + 3SD is 1.3042, so the manufacturer's cut off of 1.4 is found to be appropriate for this assay.

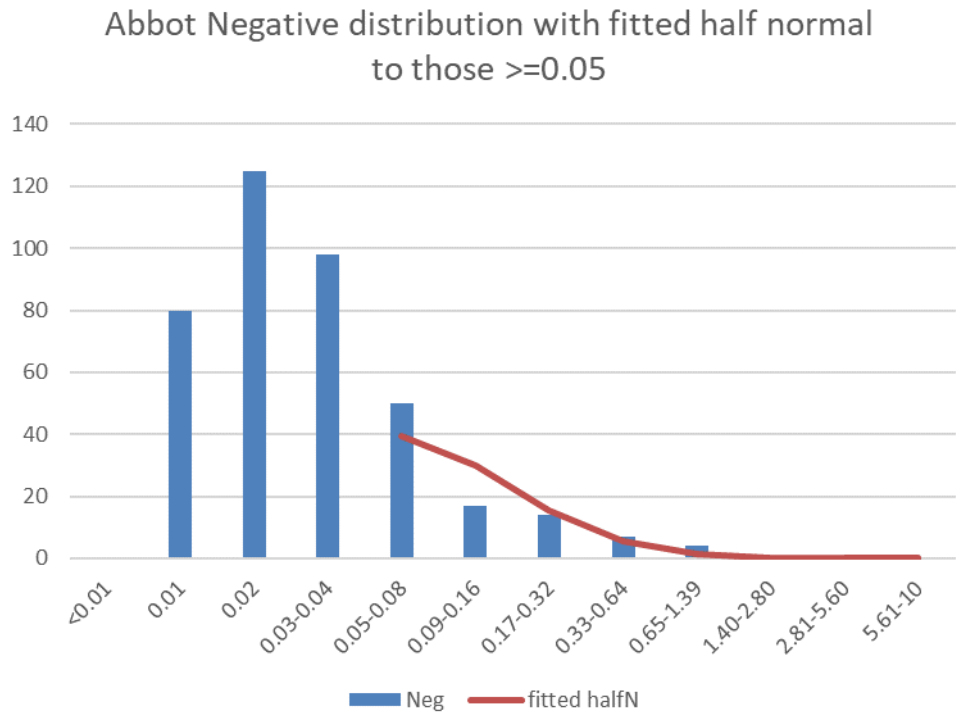


Figure 4: Assessment of the manufacturer's cut off looking at the negative sample distribution with a fitted half normal of >=0.05

## Conclusions

In conclusion, the Abbott SARS-CoV-2 gave a specificity of 100% (95% CI 97.79-100) in this evaluation; the manufacturer-reported specificity is 99.6% (99.1-99.90).

In this evaluation, the sensitivity of the Abbott SARS-CoV-2 IgG assay was 93.9% (95%CI 86.3-98.0) for samples collected ≥14 days post symptom onset and sensitivity was 93.5% (95%CI 85.5-97.9) for samples collected ≥21 days post symptom onset. For all samples, the sensitivity in this evaluation was 92.7% (95%CI 85.6-97.0). The manufacturers reported a sensitivity of 86.3% for samples <14 days and a sensitivity of 100% for samples ≥14 days post symptom onset.