Evaluation of Sentinel Diagnostics COVID-19 Anti-S1 Total Ab test, a new immunoturbidimetric assay for the detection of total Anti-S1 antibodies in human serum and plasma

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Background and aim

Sentinel Diagnostics has developed the COVID-19 Anti-S1 Total Ab test, a new immunoturbidimetric assay for the semiquantitative determination of human total antibodies specific for Spike protein S1 of SARS-CoV-2 in human serum and plasma. Serology methods are extremely helpful in understanding the SARS-CoV-2 spread in the population and in monitoring the immunity coverage given by vaccination campaigns, especially when able to discriminate neutralizing from non-neutralizing antibodies. The aim of this study was to evaluate the analytical and clinical performances of Sentinel COVID-19 anti-S1 Total Ab assay and to complete the Design Validation of the product in an external clinical laboratory and under routine conditions on fully automated SENTiFIT® 270 Analyzer.

Methods

The COVID-19 Anti-S1 Total Ab test (Ref. 1158701) is a particle enhanced turbidimetric immunoassay (PETIA) and allows detection and semi-quantification of total antibodies to Spike 1 viral protein of the SARS-CoV-2 in human serum and plasma. The sample is incubated with reaction buffer (Reagent 1) and mixed with polystyrene nanoparticles coated with recombinant protein representing the S1 antigen (immunoparticles Reagent 2). Presence of specific antibodies to the Spike 1 viral protein in the sample mediates immunoparticles agglutination. Sample turbidity, measured by light absorbance, increases with specific antibodies-immunoparticles complex formation and is proportional to the titer of novel coronavirus (SARS-CoV-2) antibodies in the serum. The detected light absorbance allows qualitative detection and semi-quantification of antibodies to SARS-CoV-2 via interpolation on an established calibration curve. Statistical analysis was performed with Analyse-it. Results are presented in Arbitrary Unit (AU/mL) and Binding Antibody Unit (BAU/mL).

Results

Cross reactivity

Cross reactivity was evaluated in specimens obtained before December 2019. 90 human samples IgG positive to the following pathogens: Mumps, Adenovirus, *Mycoplasma pneumoniae*, Parainfluenza viruses 1/2/3, Respiratory Syncytial virus, Parvovirus B19, Varicella Zoster virus, Influenza A/B viruses, *Cytomegalovirus*, Herpes Simplex 1/2 viruses, Epstein-Barr Capsid antigen, Enterovirus, Rubella virus, Hantavirus, HIV-1, Hepatitis B/C viruses, *Haemophilus Influenzae* and Hepatitis B Core were tested. All 90 samples were classified as negative, showing a 100% specificity of the assay.

			COVID-19 Anti-S1 Total Ab	
Specificity	**100,0%**		Positive	Negative
Pre-COVID-19 outbreak samples	Positive	0	0	0
	Negative	90	0	90

Clinical concordance vs. swab/PCR test

264 human serum samples from individuals previously tested with a PCR, were tested with the COVID-19 Anti-S1 Total Ab kit and the results were compared to the known PCR qualitative characterization. COVID-19 Anti-S1 Total Ab clinical decision level was: 1,50 AU/mL or 11,70 BAU/mL.

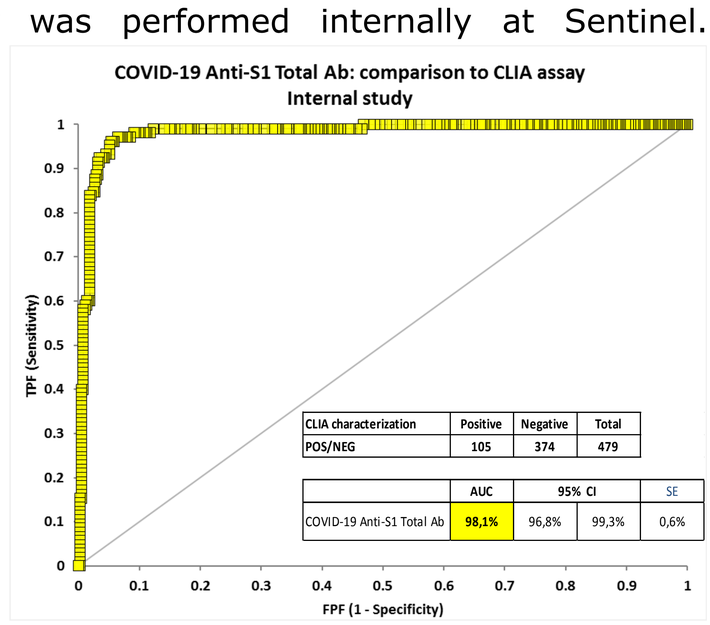
		COVID-19 Anti-S1 positive	COVID-19 Anti-S1 negative		
PCR negative	205	3	202	Specificity	**98,5%**
PCR positive	59	55	4	Sensitivity	**93,0%**

Interferences

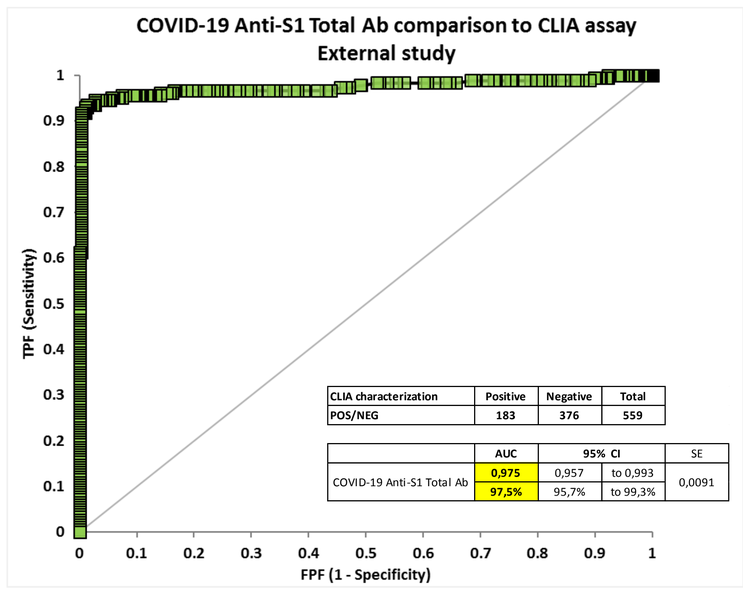
No interferences were observed, neither in the internal nor in the external study, by the presence of conjugated Bilirubin up to 60,0 mg/dL, Hemoglobin up to 1,00 g/dL, Lipids up to 500 mg/dL, ASO titer up to 1370 IU/mL and RF up to 885 IU/mL. Triglycerides testing was performed using commercially available IntraLipid material. The external evaluation study was based on routine samples with elevated concentration of the interfering substances.

Clinical concordance vs. on-market antibodies immunoassays

Two studies were performed to assess the concordance of the results of COVID-19 Anti-S1 Total Ab assay with those obtained with two different CLIA (Heterogeneous Chemiluminescence fully automated assays). The first study was performed internally at Sentinel. COVID-19 anti-S1 Total Ab clinical decision level was 1,50 AU/mL or 11,70 BAU/mL. 479 (105 positive and 374 negative) patients' samples were tested with the COVID-19 Anti-S1 Total Ab test and with an automated commercial available CLIA method for the detection of total antibodies to SARS-CoV-2.



The second study was externally performed at CDI Centro Diagnostico Italiano under routine condition. COVID-19 Anti-S1 Total Ab clinical decision level was 1,50 AU/mL or 11,70 BAU/mL. 553 (177 positive and 376 negative) patients' samples were tested with the COVID-19 Anti-S1 Total Ab kit and with the automated commercial available CLIA method in use in the Lab routine for detection of total antibodies to SARS-CoV-2.



Conclusions

COVID-19 Anti-S1 Total Ab test met the requirements of robustness in terms of analytical endogenous interferences, absence of cross-reactivity versus other viral diseases and clinical specificity and sensitivity versus PCR. Clinical performance of the PETIA reagent was comparable with the routine CLIA method, thus offering a valid alternative testing method on open clinical chemistry platforms. Additionally, the use of COVID-19 Anti-S1 Total Ab test in combination with COVID-19 Anti-NC Total Ab test, can support a differential analysis of the immunological status of the COVID-19 seropositivity (vaccination vs. infection).