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Short communication

Standardization of two SARS-CoV-2 serology assays to the WHO 20/136 human standard reference material

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ABSTRACT

WHO 20/136 is standard reference material for SARS–COV-2 serology assays. Standardization of serology assays that target the same antigen and class of immunoglobulin will enable comparison of results between studies that use various lab-developed and commercial assays around the world. Standardization of assays will help better define immune correlates of protection and possibly immune correlates of vaccine efficacy. Two automated SARS–COV-2 anti-S1 RBD immunoglobulin serology assays on the Atellica IM Analyzer were calibrated to WHO 20/136 Standard Reference Material which was assigned 1000 binding antibody units (BAU/mL). The anti-S1 RBD IgG assay (sCOVG) cut-off Index of 1.00 corresponded to WHO 45.1 BAU/mL, and the anti-S1 RBD Ig Total assay (COV2T) cut-off Index of 1.00 corresponded to WHO 6.70 BAU/mL.

1. Introduction

The need for standardized SARS-CoV-2 quantitative immunoglobulin and neutralization assays has been highlighted by the World Health Organization (WHO) and the United States (U.S.) Centers for Disease Control and Prevention (CDC) (Gundlapalli et al., 2021; Kristiansen et al., 2021). Standardization and harmonization of SARS-CoV-2 serology assays will aid comparison of results across various assays (lab-developed and commercial) and manufacturers around the world and speed research progress towards defining humoral correlates of immunity and vaccine efficacy. The First WHO International Standard (IS) for anti-SARS-CoV-2 Immunoglobulin, human (code 20/136) was provided by the National Institute for Biological Standards and Control (NIBSC) on behalf of the WHO and in collaboration with the Coalition for Epidemic Preparedness Innovations (CEPI), for calibration and harmonization of SARS-CoV-2 neutralizing and other serology assays. The WHO IS is a sample pool of plasma from eleven individuals recovered from SARS-CoV-2 infection and contains human polyclonal antibodies to N protein, S1, S2, S1/S2 (full-length S), and S1 RBD proteins of SARS-CoV-2. The WHO IS has a single assigned value of 1000 binding antibody units (BAU/mL) per vial for serology assays of the same immunoglobulin class and specificity. Recent studies have calibrated their assays to the WHO IS (Castillo-Olivares et al., 2021; Feng et al., 2021). One study compared results of several binding antibody assays on convalescent samples from severe, moderate, and mild COVID-19 cases

and found a positive correlation between severity and RBD/S antibodies (Castillo-Olivares et al., 2021). Here, we report the WHO IS calibration of two additional anti-S1 RBD Ig assays not previously reported in the literature.

2. Materials and methods

Calibration to WHO IS reference material was performed for the Atellica® IM SARS-CoV-2 IgG (sCOVG) assay and the Atellica® IM SARS-CoV-2 Total (COV2T) assay (Siemens Healthineers, Tarrytown, NY, U.S.). Both assays are semi-quantitative, used for determining anti-S1 RBD IgG or Total (IgG and IgM) antibody levels, respectively.

The sCOVG assay is a two-step automated sandwich chemiluminescent immunoassay. In the first step, the patient specimen is incubated with the Solid Phase Reagent (containing recombinant antigens that capture antibodies in the patient sample). The antibody-antigen complex is washed. The second step involves adding Lite Reagent (mouse monoclonal anti-human IgG antibody labeled with acridinium ester). The anti-human IgG antibody in the Lite Reagent binds antibodies in the specimen that were captured by the Solid Phase Reagent. Upon binding, a signal (relative light units, RLU) is generated that is directly related with the amount of SARS-CoV-2 IgG antibody present. The measuring range is 0.50–150 Index.

The COV2T assay is a one-step (i.e., no wash step in between addition of Solid Phase Reagent and the Lite Reagent) automated sandwich

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(antibody bridging) chemiluminescent immunoassay that uses antigens to bridge patient sample antibodies. The patient sample is incubated with the Solid Phase Reagent (containing bound recombinant antigens) that captures antibodies in the specimen, followed by incubation with Lite Reagent (recombinant S1 RBD antigen labeled with acridinium ester). Upon binding of antigen in the Lite Reagent to antibody bound to the Solid Phase antigens, a signal (RLU) is generated that is directly related with the amount of SARS-CoV-2 antibodies present in the sample. The measuring range is 0.50–75 Index.

Both assays were performed according to the manufacturer’s individual instructions for use. An index value of ≥ 1.00 indicates a positive (reactive) result for both assays, established with calibrators. The Atellica IM COV2T Calibrator is processed human plasma (defibrinated and filtered) nonreactive for antibodies to SARS-CoV-2 (low calibrator) and processed (defibrinated and filtered) human plasma spiked with antibodies to SARS-CoV-2 (high calibrator). The Atellica IM COV2G Calibrator is processed (defibrinated and filtered) human plasma nonreactive for SARS-CoV-2 antibodies (low calibrator) and horse serum spiked with human monoclonal IgG antibodies to SARS-CoV-2 (high calibrator). The calibrators are used to give a quantitative patient result.

Both assays were authorized under the U.S. Food and Drug Administration Emergency Use Authorization (EUA) at the time of the study (Atellica IM sCOVG: March 2021; Atellica IM COV2T: May 2020) (FDA, 2021).

Patient sera samples (128) RT-PCR positive for SARS-CoV-2 were purchased from Cerba Research (Ghent, Belgium), Discovery Life Sciences (Huntsville, AL), and Boca Biolistics (Pompano Beach FL, USA) which had relevant institutional ethics agreements and donor consent for the collection of specimens.

2.1. sCOVG calibration

The sCOVG assay is directly calibrated to WHO IS (not yet available to customers). Customers can report positive or negative results and/or the BAU/mL associated with that result. The sCOVG assay is calibrated directly to the WHO IS by assigning BAU/mL values to internal standards when calibrating with the WHO IS. Ten dilutions were prepared with the WHO IS in negative human serum to calibrate the assay. The sCOVG assay using WHO IS calibration and Index calibration was compared by running 128 clinical samples. The relationship between the index value and BAU/mL was then calculated.

2.2. COV2T calibration

The COV2T assay is calibrated with internal standards not traceable to the WHO IS but rather an Index value as defined by Siemens Healthineers. A series of dilutions of the WHO IS was performed in negative human serum to establish the relationship between COV2T Index and BAU/mL. The eight samples in Fig. 1 B are the individual diluted WHO IS samples. The Index to BAU/mL relationship is a function of the slope and intercept of the comparison.

3. Results

Results for calibration of the two anti-S1 RBD Ig assays to the WHO IS are presented in Fig. 1 and summarized in the Table 1. The cut-off Index of 1.00 for the sCOVG assay was observed to correspond to a WHO BAU/mL value of 45.1 and the cut-off Index of 1.00 for the COV2T assay corresponded to a WHO BAU/mL value of 6.70.

4. Discussion

In this study, calibration of two common anti-S1 RBD Ig assays (sCOVG and COV2T) assays to WHO BAU/mL was performed. The WHO IS has one assigned value for serological assays, 1000 BAU/mL, which can be used to compare assays of the same immunoglobulin class and

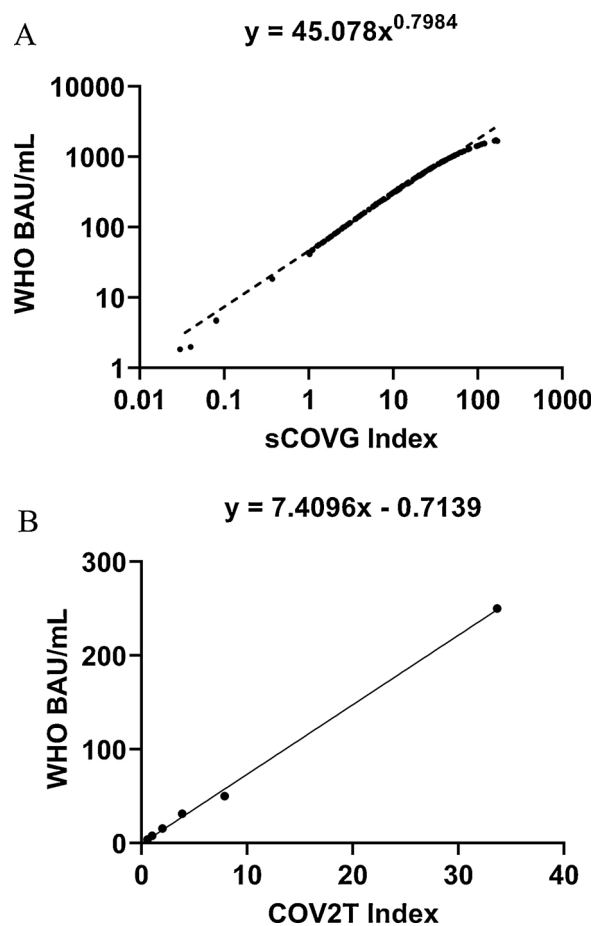


Fig. 1. A. Atellica IM sCOVG assay traceability to WHO 20/136 (BAU/mL). The Index and BAU/mL values were calculated independently using 2-point calibration from the Index and BAU/mL master curves from 128 individual positive remnant samples obtained from COVID-19 positive individuals. B. Atellica IM COV2T assay traceability to WHO 20/136 (BAU/mL). The Index and BAU/mL values were calculated independently using 2-point calibration from the Index and BAU/mL master curves from eight individual positive samples obtained from COVID-19 positive individuals.

Table 1

Atellica IM / ADVIA Centaur sCOVG assay and Atellica IM / ADVIA Centaur COV2T assay traceability to WHO 20/136 (BAU/mL).

Assay	sCOVG	COV2T
Reporting units	Index	Index
Cut off	1.00 Index	1.00 Index
R	0.972	0.999
WHO BAU/mL at 1.00 Index cut off	45.1	6.70

The sCOVG results were established using the Atellica IM sCOVG assay, which has the same reagent formulations as the ADVIA Centaur® sCOVG assay (EUA June 17, 2021) (FDA, 2021).

The COV2T results were established using the Atellica IM COV2T assay, which has the same reagent formulations as the ADVIA Centaur® COV2T assay (EUA May 29, 2020) (FDA, 2021).

antigen specificity. It will not harmonize assays that use different viral proteins or harmonize assays of total Ig to assays that measure IgG or IgM. Although the correlates of protection against SARS-CoV-2 are not completely defined, several studies have supported the involvement of antibody-mediated immunity as one potential correlate of protection. Other proposed correlates of protection such as cell-mediated immunity (involving cytotoxic cells) are currently being investigated (DiPiazza et al., 2021; Earle et al., 2021; Toor et al., 2021). Several studies have

found that binding and neutralizing antibodies are correlated to each other – both potential correlates of protection. Recent studies using vaccine trial data have found a high correlation between anti-RBD IgG and neutralizing antibody titers (Lustig et al., 2021), and between neutralizing antibodies and vaccine efficacy, and/or between binding antibodies and vaccine efficacy (Earle et al., 2021; Feng et al., 2021; Khoury et al., 2021). A positive relationship was found between vaccine efficacy and neutralizing antibody (S protein IgG) titers in an evaluation of results from seven different vaccine Phase III trials – despite the different vaccine technologies, and other variables (Earle et al., 2021). Calibration to the WHO IS will provide confidence when comparing serology results across studies that use various lab-developed and commercial serology assays around the world due to traceability to a single standard reference material.

In conclusion, this study has demonstrated traceability of index values for two common assays to WHO IS in BAU/mL (sCOVG, 1.00 Index = 45.1 BAU/mL; and COV2T, 1.00 Index = 6.70 BAU/mL). Calibration of these two common SARS-CoV-2 serology assays to the WHO IS reference material will help efforts to define antibody correlates of immune protection and determine usefulness of serology thresholds as surrogates of neutralization activity and vaccine efficacy.

Data availability

Data will be made available on request.

Author contributions

JF designed and supervised the study and drafted the manuscript. JF and JC and were involved with sample processing, project coordination, testing, analysis of data, and interpretation. Both authors contributed to proof-reading the manuscript and approved the final manuscript.

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Patient consent for publication

Patient sera samples (128) RT-PCR positive for SARS-CoV-2 were purchased from Cerba Research (Ghent, Belgium), Discovery Life Sciences (Huntsville, AL), and Boca Biolistics (Pompano Beach FL, USA) which had relevant institutional ethics agreements and donor consent

for the collection of specimens.

Declaration of Competing Interest

The authors are full-time employees of Siemens Healthineers, Tarrytown, New York, United States.

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