Davide Giavarina* and Mariarosa Carta

Improvements and limits of anti SARS-CoV-2 antibodies assays by WHO (NIBSC 20/136) standardization

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Abstract

Objectives: A few CLIA automated immunoassays for the recognition of anti S1-RBD SARS-CoV-2 antibodies have recently been placed on the market. Preliminary data demonstrate a high correlation between methods but wide differences in absolute concentrations. A new WHO international standard for anti-SARS-CoV-2 immunoglobulin, NIBSC code 20/136, has been recently introduced to reduce the differences. The aim of this study is thus to verify the harmonization made by NIBSC 20/136 on Ab anti S1-RBD measurement on real samples.

Methods: The following assays were studied: LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin); Elecsys® anti-SARS-CoV-2 S (ROCHE); Atellica IM SARS-CoV-2 IgG (sCOVG) (Siemens); MAGLUMI® SARS-CoV-2 S-RBD IgG (Snibe), measuring 210 samples from 70 health workers with no previous SARS-CoV2 infection, during their Pfizer-BioNTech's BNT162b2 vaccination period.

Results: The recalculation of concentrations based on the NIBSC 20/136 standardization improve the analytical and diagnostic comparability but do not cancel this variability between methods: recalibrated results remain different across methods, both in terms of tendency and single data.

Conclusions: The recalculation of concentrations based on the NIBSC 20/136 standardization improves the analytical and diagnostic comparability but does not cancel the differences between methods: recalibrated results remain different across methods, both in terms of tendency and single data.

Keywords: antibody immunoassays; harmonization; NIBSC 20/136; SARS-CoV2; WHO standardization.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the etiological agent of the Coronavirus Disease 2019 (COVID-19). The diagnosis of the infection is based on molecular tests such as reverse transcriptase-polymerase chain reaction (rRT-PCR) or on assays for the search of viral antigens.

The search for antibodies (Ab) to SARS-CoV-2 plays a small role in the diagnosis of the disease, given that the Ab appear only after 8–14 days from the infection, a time too long for diagnostic needs as well as for tracing and isolating possible sources of infection. Furthermore, the Ab response to infection is variable, frequently with very low responses in the asymptomatic forms.

The measurement of Ab to SARS-Cov-2 can instead be important in the epidemiological evaluation of the spread of the disease, precisely because in many subjects the infection can occur in a completely asymptomatic form that can remain undetected. It can be even more important for guiding vaccine administration, identifying possible vaccine failure and estimating the time of protection [1].

The development of analytical methods for the determination of anti-SARS-CoV-2 Ab has been rather tumultuous, leading to the placing of several products on the market which differ in terms of immunoglobulin class, binding epitope and detection method. The first studies showed that the determination of immunoglobulins M (IgM) is not particularly useful, since their increase in serum does not happen at an early stage compared to immunoglobulins G (IgG). Moreover, levels of IgM peak early in the disease course and then decline, whereas IgG peak later and may remain detectable for at least 120 days [2].

Rapid determination methods have been widely used, based on lateral flow immunoassay (LFIA) and directed towards multiple epitopes of the virus, but with very poor analytical performance [3, 4].

Immunoassays based on enzymatic (ELISA) or chemiluminescent (CLIA) techniques on automatic instruments have only become available at a later stage. Furthermore, early Ab immunoassays methods targeted different virus epitopes, mainly anti-nucleocapsid Ab. Immunoassays for Ab against the S1 protein – in whole or in the portion of the

*Corresponding author: Davide Giavarina, MD, Laboratory Medicine, St. Bortolo Hospital, Vial Mons. Rodolfi 37, 36100, Vicenza, Italy, Phone: +39 0444 753254, E-mail: davide.giavarina@aulss8.veneto.it, <https://orcid.org/0000-0001-9589-178X>

Mariarosa Carta, Laboratory Medicine, St. Bortolo Hospital, Vicenza, Italy. <https://orcid.org/0000-0002-7143-4928>

receptor-binding domain (RBD) – have more recently been placed on the market. These assays are interesting as they are closely linked to the neutralizing capacity of the Ab, produced both in response to infections and following vaccination. In fact, the anti S1-RBD Ab bind and mask the virus binding sites to the ACE2 receptors of the target cells. For this reason, all the vaccines currently in use have been developed against the S1 protein of the SARS-CoV-2, particularly towards the RBD epitope [5].

Preliminary data about CLIA immunoassays, applied to automatic instruments for the recognition of anti S1-RBD Ab demonstrate high diagnostic sensitivity and specificity in subjects who were exposed to the virus [6] as well as on vaccinated subjects [7]. Such methods also demonstrate high correlations between them [8]. However, in absolute values, the concentrations measured by the different methods vary widely among each other. Standardization, or at least harmonization, of the measurement methods of these Ab is thus necessary to realise comparable datasets across laboratories, be able to define levels of protective/neutralizing Ab as well as to improve the understanding of the epidemiology of the virus.

For this purpose, the Expert Committee on Biological Standardization of the World Health Organization established the WHO International Standard and Reference Panel for anti-SARS-CoV-2 Ab on December 2020 (WHO/BS/2020.2403) [9]. The First WHO International Standard for anti-SARS-CoV-2 immunoglobulin was a freeze-dried equivalent of 0.25 mL of pooled plasma obtained from 11 individuals recovered from SARS-CoV-2 infection, NIBSC code 20/136 [10]. Results obtained from the methods recalibrated on the NIBSC code 20/136 standard are expressed in binding Ab unit/mL (BAU/mL).

We recently had the opportunity to study four of these CLIA automated immunoassays to monitor the immune response to vaccination. This provided us with the opportunity to evaluate the effects of recalibration on harmonization and results on real samples.

Materials and methods

Samples from 70 health workers with no previous SARS –CoV2 infection who underwent vaccination (Pfizer-Biontech's BNT162b2) were tested at t0, before the first dose of vaccine; at t1, at the moment of the booster dose, and at t2, 15 days later, to evaluate Ab anti S1-RBD response to the vaccination. In parallel, 210 samples were tested for the Ab anti SARS-CoV-2 concentrations by using the four CLIA automated immunoassays listed in Table 1. The results were expressed in their own original units and in BAU/mL, after recalculation based on the WHO standard NIBSC code 20/136 calibration. The standard alignment coefficients, declared by manufacturers to be perfectly

linear, are respectively: LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin) 1.0 AU/mL=2.6 BAU/mL; Elecsys® anti-SARS-CoV-2 S (ROCHE) 1.0 U/mL=1.029 BAU/mL; Atellica IM SARS-CoV-2 IgG (sCOVG) (Siemens) 1.0 index=21.8 BAU/mL; MAGLUMI® SARS-CoV-2 S-RBD IgG (Snibe) 1.0 AU/mL=4.33 BAU/mL.

Passing and Bablok regression as well as the Notched-box-and-Whisker plot were used for comparison between methods (MedCalc Software, ver. 20.014, 2021), using the 140 samples collected at t1 and t2. The study was done in compliance with the World Medical Association Declaration of Helsinki.

Results

All the samples were negative at t0 by all the four evaluated immunoassays.

Figure 1A shows the concentrations of Ab measured on the 140 samples collected after vaccination (t1 and t2 together) using the four methods, expressed in their original units of measurement. Figure 1B shows the same comparison after recalibration according to the manufacturer's declared calibration factor, based on the WHO NIBSC code 20/136 standard. In Figure 1C we can observe the effect of an "internal" harmonization between the four methods studied, using one of the four methods studied as reference (Roche).

Figure 2 shows the correlations between the MAGLUMI, LIAISON and ATELLICA vs. ELECSYS methods, respectively in their original measurement units, in BAU/mL and after harmonization on concentrations with ELECSYS.

Data about slope, intercept and correlation coefficient are summarized in Table 2.

Discussion

In our study, we compared the performance of four different immunoassays, all specifically targeting the spike glycoprotein RBD. These methods showed high clinical sensitivity to vaccination, and high correlation between them, as we reported in a previous study [8].

However, as expected, the absolute values of Ab concentration varied greatly depending on the method used. The immunoassays results correlate, but the slopes of the regression lines are very different from each other if compared with the original units of measurement. Unfortunately, while the comparability improves when the results are expressed as BAU/mL, it remains markedly different depending on the methods used. In fact, our data demonstrate that WHO standardization, while showing perfect linearity for each single method, does not produce the same effect on "real" samples. The concentrations

Table 1: Main characteristics of the four studied immunoassays, for the determination of Ab to SARS-CoV-2 S1-RBD spike protein.

	Atellica IM SARS-CoV-2 IgG (sCOVG)	Elecsys® anti-SARS-CoV-2 S	LIAISON® SARS-CoV-2 Trimerics IgG	MAGLUMI® SARS-CoV-2 S-RBD IgG
Company	Siemens Healthineers, (Tarrytown, NY, USA)	Roche Diagnostics (Basel, Switzerland)	DiaSorin (Saluggia, TO, Italy)	Snibe Co., Ltd., (Shenzhen, China)
Immunoassay	Two-step sandwich immunoassay	One-step double antigen sandwich assay	Two-step sandwich immunoassay	Two-step sandwich immunoassay
Detection of IgG Ab Technology	Qualitative and quantitative Indirect chemiluminescence	Qualitative and quantitative Electrochemiluminescence	Quantitative Indirect chemiluminescence	Quantitative Indirect chemiluminescence
Measuring interval	0.50–150.0 index	0.4–250 U/mL	1.85–800.0 AU/mL	0.180–100 AU/mL
Units	Index	U/mL	AU/mL	AU/mL
Specimen types	Serum, lithium heparin plasma	Serum, lithium heparin and EDTA plasma	Serum, lithium heparin and EDTA plasma	Serum, EDTA plasma
Reagents	Mouse monoclonal anti-human IgG Ab labeled with acridinium ester	Recombinant protein representing the RBD of the S antigen in a double-antigen sandwich assay format (biotinylated RBD antigen- Sample anti-SARS-CoV-2-ruthenylated RBD antigen)	Mouse monoclonal anti-human IgG Ab linked to an isoluminol derivative	Anti-human IgG Ab labeled with N-(4-aminobutyl)-N-ethyl-iso-luminol (ABEI)
Reagents – solid phase	Streptavidin-coated paramagnetic microparticles performed with biotinylated SARS-CoV-2 S1 RBD antigen	Streptavidin-coated microparticles	Paramagnetic particles coated with recombinant trimeric SARS-CoV-2 spike protein	Magnetic microbeads coated with recombinant SARS-CoV-2 S-RBD antigen
Heat inactivation	Not recommended	Not recommended	Not declared	Recommended
Cutoff (reactive)	≥1.00 Index	≥0.80 U/mL	≥13.0 AU/mL	≥1.00 AU/mL
Limit of blank (LoB)	0.40 Index	0.30 U/mL	0.595 AU/mL	0.100 AU/mL
Limit of detection (LoD)	0.50 Index	0.35 U/mL	0.12 AU/mL	0.180 AU/mL
Limit of quantitation (LoQ)	0.50 Index	0.40 U/mL	1.63 AU/mL	=
Reproducibility	≤7.1%	≤6.7%	≤5.8%	≤8.5%
Clinical sensitivity (after 15 days)	96.41%	96.6%	98.7%	100%
Clinical specificity	99.90%	99.98%	99.5%	99.6%
WHO standard (20/136) unit conversion	1 index=21.8 BAU/mL	1 U/mL=1.029 BAU/mL	1 AU/mL=2.6 BAU/mL	1 AU/mL=4.33 BAU/mL

Figure 1: Comparison of Ab anti S1-RBD concentrations on all the 210 samples from 70 subjects, before and after Pfizer-Biontech's BNT162b2 vaccination (before first vaccine dose and 21 and 43 days after) measured by: LIAISON® SARS-CoV-2 Trimeric IgG (DiaSorin); ELECSYS® anti-SARS-CoV-2 S (ROCHE); Atellica IM SARS-CoV-2 IgG (sCOVG) (Siemens); MAGLUMI® SARS-CoV-2 S-RBD IgG (Snibe).

(A) Original concentrations, expressed in Arbitrary Units (U/mL for ELECSYS; AU/mL for LIAISON AND MAGLUMI; Index for ATELICA). (B) Same results, expressed in BAU/mL. After re-calculation according WHO calibration. (C) Same results, after internal harmonization (results expressed in Arbitrary Units).

Figure 2: Correlations between the MAGLUMI, LIAISON and ATELICA vs. ELECSYS methods, calculated on the 140 samples from 70 subjects, collected and 21 and 43 days after Pfizer-Biontech's BNT162b2 vaccination, represented in their original measurement units, in BAU/mL and after “harmonization” on concentrations with ELECSYS, respectively.

between the methods are close, but the single samples can present marked differences, with heterogeneous behavior. Larger differences are observed for higher concentrations, although the differences in these concentrations are probably less critical from a diagnostic and monitoring point of view.

Unsurprisingly, our “internal” harmonization demonstrates an excellent correlation between the methods used to measure Ab concentrations after vaccination with Pfizer-Biontech's BNT162b2. However, even in this case,

methods vary significantly from each other in terms of magnitude.

Our study has the limit of “sample size”, given that the sample was made up of 70 subjects; the methods were compared on only 140 specimens, two samples per subject, collected at t1 and t2. However, our findings seem to confirm that, although all of the studies of immunoassays showed good correlation, their results were not interchangeable, even when converted to BAU per milliliter using the WHO international standard for SARS-CoV-2 immunoglobulin [11].

Table 2: Intercept, slope and Spearman rank correlation coefficient between MAGLUMI, LIAISON and ATELLICA methods vs. ELECSYS method, in the native measure units, after WHO recalibration (BAU/mL), and after harmonization on concentrations with ELECSYS. Confidence intervals between brackets.

	Systematic differences		Proportional differences		Correlation coefficient		
	Intercept	95% CI	Slope	95% CI	95% CI		
Original measure units							
MAGLUMI vs. ELECSYS	24.7	(16.9–30.4)	0.39	(0.36–0.44)	0.95	(0.94–0.97)	P<0.0001
LIAISON vs. ELECSYS	106.1	(82.7–124.1)	0.76	(0.70–0.82)	0.93	(0.90–0.95)	P<0.0001
ATELLICA vs. ELECSYS	4.8	(3.8–5.9)	0.05	(0.05–0.06)	0.94	(0.91–0.96)	P<0.0001
WHO recalibration							
MAGLUMI vs. ELECSYS	79.3	(56.3–119.2)	1.74	(1.60–1.94)	0.95	(0.94–0.97)	P<0.0001
LIAISON vs. ELECSYS	244.1	(196.0–312.4)	1.97	(1.81–2.17)	0.93	(0.90–0.95)	P<0.0001
ATELLICA vs. ELECSYS	82.6	(53.4–103.8)	1.17	(1.07–1.31)	0.94	(0.91–0.96)	P<0.0001
Internal harmonization							
MAGLUMI vs. ELECSYS	53.3	(36.5–71.1)	1.01	(0.94–1.13)	0.95	(0.94–0.97)	P<0.0001
LIAISON vs. ELECSYS	130.1	(98.3–152.2)	0.93	(0.86–1.02)	0.93	(0.90–0.95)	P<0.0001
ATELLICA vs. ELECSYS	62.9	(39.6–78.5)	0.88	(0.81–0.98)	0.94	(0.91–0.96)	P<0.0001

Also noteworthy is the structural difference of the Roche method compared to the others. The MAGLUMI, ATELLICA and LIAISON methods have all been conceived to recognize and measure IgG anti-spike proteins only, while ELECSYS anti-SARS-CoV-2 S are meant for the detection of all SARS-CoV-2 antibodies, mainly anti-SARS-CoV-2 IgG, but also IgA and IgM anti-SARS-CoV-2.

In summary, the standardization of the CLIA assay for detecting S1-RBD SARS-CoV-2 Ab offers a substantial contribution to improving the analytical and diagnostic comparability. However, it may be important to highlight the limitations of this standardization among clinicians given that the results remain different across methods, both in terms of tendency and single data. This can be relevant for monitoring a single patient, determining the adequate levels of Ab as well as for evaluating the permanence of immunity over time. Reporting S1-RBD SARS-CoV-2 Ab as BAU/mL can induce a false perception of interchangeability between methods, which is unfortunately still far from being achieved.

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Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was undertaken by enrolling the health personnel of our own laboratory, concurrently

with our internal vaccination campaign. The personnel participated in the study on a voluntary basis. At the beginning of January of this year the entirety of the health personnel of our Institute have been called for vaccination with the Pfizer vaccine. More than 6,000 employees were vaccinated in 15 days. The inoculation of the second dose of the vaccine took place exactly 21 days after the first dose, according to protocol. Unfortunately, the response time of our Ethical Committee is of two months since the presentation of the proposal, on average. Given the necessity of proceeding promptly and at very specific times to ensure that samples were taken correctly during the phase of mass vaccination, we couldn't pursue the formal authorization, which would have arrived too late in relation to the objectives of our study. We thus followed a fast protocol in full adherence to the Helsinki criteria. Every participant signed the informed consent form for the execution of the venous sampling and the collection of the information relating to their vaccination state and previous SARS-COV-2 infection. In the samples collected we only measured the anti-SARS-COV-2 antibodies, eliminating the remaining sample. The results of the determination with the Roche method (method in use for our outpatients at the moment of the study) were communicated to every single participant, privately, in accordance with the standard reporting procedures of the exams carried out in our laboratory. The results were made anonymous for their successive elaboration. The entirety of the process is compliant with the standards required by our Institute. The health director of our Institute has also been adequately informed of our experimentation. We would be happy to make the informed consent forms available as well as to eventually share the testimonies from every single participant if need be.

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