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Diagnostic Microbiology and Infectious Disease 100 (2021) 115340

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Available COVID-19 serial seroconversion panel for validation of SARS-CoV-2 antibody assays.

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ARTICLE INFO

Article history:
Received 17 November 2020
Revised in revised form 27 January 2021
Accepted 2 February 2021
Available online 6 February 2021

Keywords:
COVID-19
SARS-CoV-2
Immunoglobulins
Seroconversion panel

ABSTRACT

Seroconversion panels are an important tool for investigating antibody responses and developing serological assays. A seroconversion panel was generated from a single SARS-CoV-2 positive plasma donor over 87 days. This seroconversion panel was tested against 6 SARS-CoV-2 antibody tests (IgG, IgM, and total Ig). All test kits utilized recombinant antigens that are specific to SARS-CoV-2. The seroconversion panel showed IgG responses for SARS-CoV-2 after day 50. IgM levels peaked on day 50 (prior to IgG) and declined in subsequent samples. This seroconversion panel is a useful tool for validation of SARS-CoV-2 antibody assays.

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1. Introduction

The emergence of the SARS-CoV-2 virus in Wuhan, China (December 2019) (Huang et al., 2020) and the resulting infection (COVID-19) has resulted in a global pandemic with significant morbidity and mortality (Johns Hopkins University Center for Systems Science and Engineering, 2020). The SARS-CoV-2 virus has been completely sequenced and shows substantial homology with SARS-CoV-1, the virus that causes severe acute respiratory syndrome (SARS) (Zhou et al., 2020).

As yet, no specific treatment has been identified to prevent or treat COVID-19. Public health efforts are focused on determination of the prevalence and containment of the spread of COVID-19. Serologic testing is key to providing data not only for estimation of prevalence but also tracking and containment of the virus in the population. Serologic assays for SARS-CoV-2 antibodies can differ in sensitivity and specificity as well as window period of detection. One important tool in studying antibody response for window period detection and validation of an assay is a seroconversion panel collected over time (pre- and postinfection).

Seroconversion panels have been used for multiple purposes including antibody assay development, process and product validation and quality control. Regulatory authorities may require or recommend validation with seroconversion panels. For example, the US Food and Drug Administration (FDA) Guidance for Hepatitis A Virus Serological Assays recommends incorporation of seroconversion panels in the validation testing plan in order to assess the appearance of the analyte and the waning of IgM over time (Food and Drug Administration, 2006). Available seroconversion panels provide patient samples over time for a number of viral infectious diseases including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis A virus (HAV).

Currently, there is no commercial seroconversion panel available for SARS-CoV-2. A seroconversion panel was identified and generated from a single COVID-19 plasma donor. This serial panel represented preinfection, infection, and convalescence over 87 days. Characterization of the antibody response over time was evaluated by enzyme-linked immunosorbent assays (ELISA) and chemiluminescent assays.

2. Materials and methods

Six anti-SARS-CoV-2 antibody tests were used in this study to characterize a COVID-19 seroconversion panel (COVID-19 Seroconversion

Abbreviations: CE-IVD, European Commission approval for *in vitro* diagnostics; CLIA, chemiluminescent assay; ELISA, enzyme-linked immunosorbent assay; EUA, emergency use authorization; FDA, US Food and Drug Administration; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; Ig, immunoglobulin; IgG, immunoglobulin G; IgM, immunoglobulin M; SARS, severe acute respiratory syndrome

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https://doi.org/10.1016/j.diagmicrobio.2021.115340
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Panel: CVD19SCP, Access Biologicals, Vista, CA, USA). The antibody tests used were: the Gold Standard Diagnostics SARS-CoV-2 IgG ELISA and SARS-CoV-2 IgM ELISA test kits (Gold Standard Diagnostics (GSD), Davis, CA, USA; CE-IVD (EUA) submission pending), Vitros® anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 total Ig tests (Vitros® Immunodiagnostic Products, Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA; EUA approved), Liaison® SARS-CoV-2 S1/S2 IgG assay (Diasorin, Inc., Saluggia, Italy; EUA approved) and Progenika anti-SARS-CoV-2 IgG ELISA kit (Progenika Biopharma, Derio, Bizkaia, Spain; CE-IVD certified immunoassays). All the antibody test kits were used according to the manufacturer's instructions. All 4 test kits utilize recombinant antigens specific to SARS-CoV-2 (GSD, antigenic proteins for IgG and IgM; Vitros®, spike protein for IgG and spike protein S1 for IgM, Diasorin, spike protein S1 and S2 for Ig G; and Progenika, spike protein S1 for IgG [data obtained from the kits' manufacturing instructions]). Although the exact sequences of the antigens are not specified, they were independently created and presumed to be nonidentical.

The seroconversion panel consisted of 14 vials of 1.0 mL each of human plasma collected from a single SARS-CoV-2 antibody positive donor. The samples were collected for 87 days (from March 4 to May 29) over the course of the infection at an FDA-licensed plasma donor center (Saturn Biomedical, Indianapolis, IN, USA). The samples were collected with informed consent, under an approved IRB protocol (Advarra, Columbia MD, USA) and in compliance with all applicable regulatory guidelines. The preservative-free plasma samples were collected in 4% sodium citrate and aseptically filtered. Samples were stored at −20°C until use. Prior to use the samples were thawed at room temperature and gently mixed by inversion.

When this frequent plasma donor tested positive for SARS-CoV-2 antibodies upon arrival for a regular donation (Liaison® SARS-CoV-2 S1/S2 IgG assay, Diasorin, Inc.), previous plasma donations from this were isolated as potential preinfection samples and future appointments were scheduled with this donor for plasma collections to cover the timeline of antibody development. Donor plasma collections were ended when the expected decrease in IgM antibodies was observed, i.e., when a negative IgM result was obtained.

This donor was considered to have had a mild COVID-19 infection between plasma collection number 8 (day 36) and collection number 9 (day 50) as the donor reported not feeling well for approximately 2 weeks. As with the vast majority of people with mild or asymptomatic infections, this donor did not seek medical attention and treated their symptoms at home. The donor's convalescent stage was considered to have begun when SARS-CoV-2 antibodies were detected in their plasma—panel member 9 collected on day 50. No respiratory samples were collected from this donor.

Plasma from this single donor was screened and found to be negative for syphilis and antibodies to HIV-1/2, HCV and nonreactive for hepatitis B surface antigen (HBsAg). In addition, nonreactive results were obtained for HIV-1/2 RNA, HBV DNA, and HCV RNA using FDA-approved nucleic acid test assays. All donor plasma samples were also non-reactive for SARS-CoV-2 using a nucleic acid transcription-mediated amplification SARS-CoV-2 assay (Procleix® assay and Procleix Panther® system, Grifols Diagnostic Solutions Inc., San Diego, CA). This SARS-CoV-2 assay is a qualitative in vitro nucleic acid transcription-mediated amplification test for the detection of the SARS-CoV-2 RNA in plasma, serum and respiratory specimens. Based on probit analysis, the 95% limits of detection of heat-inactivated SARS-CoV-2 (NR-52286, BEI Resources, Manassas, VA) diluted in K_2EDTA plasma was estimated to be 10.7 copies/mL with 95% fiducial limits of 8.7 − 14.1 copies/mL (Procleix, 2020).

3. Results

As shown in Fig. 1A, the seroconversion panel showed positive IgG responses for SARS-CoV-2 at times ≥day 50 with all 4 assay kits. Each assay kit expressed the results in arbitrary units. In order to allow comparison across the assay kits, results from all three kits were expressed on a 100-point scale (Fig. 1A). For all 4 assays, based on the manufacturer's cut-off points, all values prior to day 50 were considered nonreactive.

IgG levels increased in all four assays at times ≥day 50. Two of the assays detected peak IgG levels at day 64 and 2 at day 71. After the

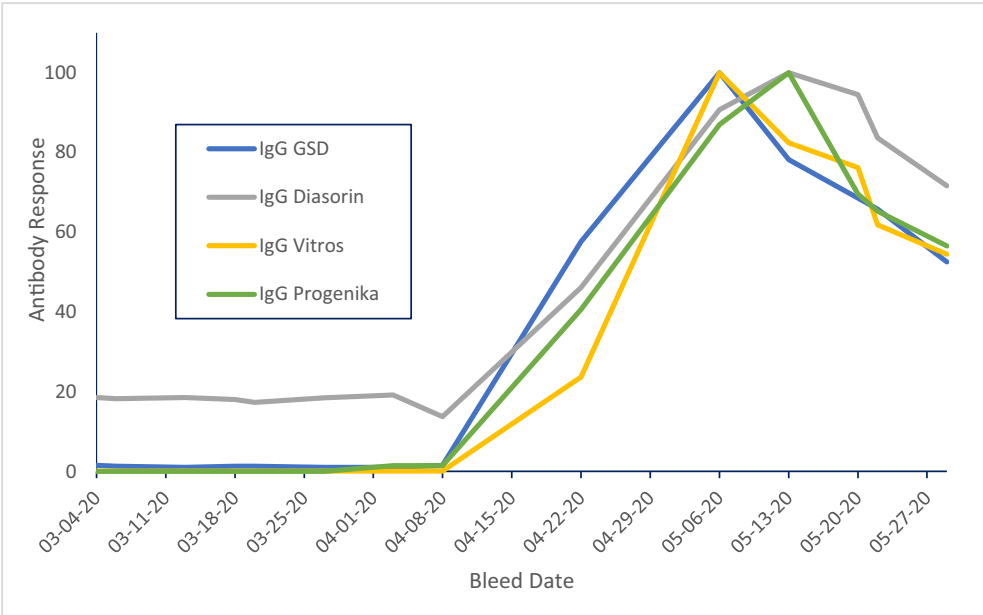

Fig. 1A. Levels of anti-SARS-CoV-2 IgG antibodies in a seroconversion panel as measured by 4 different assay kits. Each assay assigned arbitrary units to IgG, so levels were normalized to a 100-point scale to allow comparison across assays.

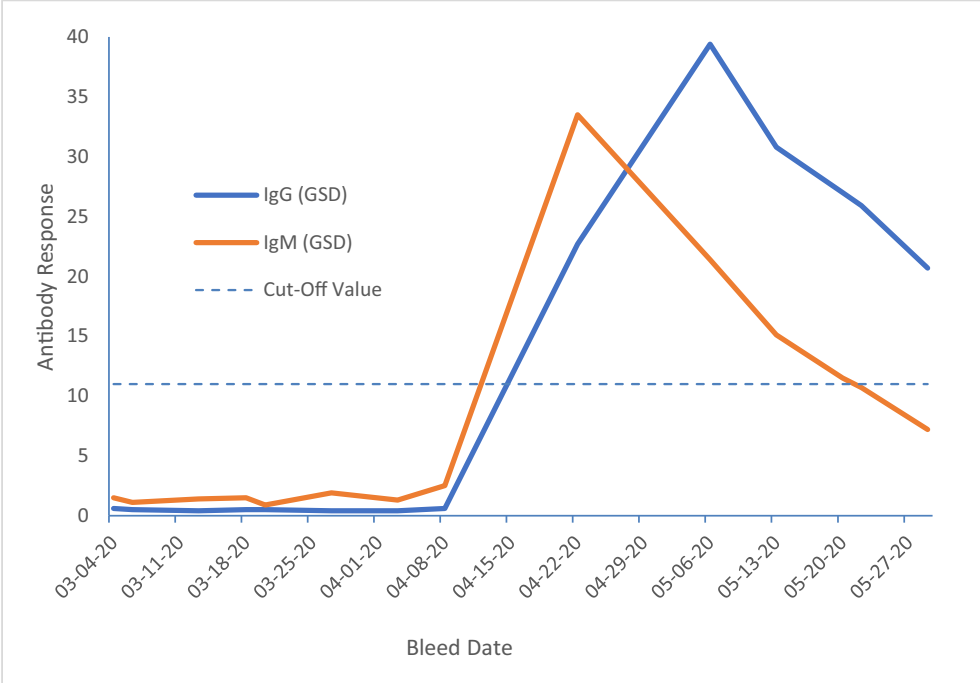

Fig. 1B. Anti-SARS-CoV-2 IgG and IgM antibodies measured in a SARS-CoV-2 seroconversion panel using Gold Standard® Diagnostics (GSD) kits. Values are expressed on an arbitrary scale specified by the manufacturer. Values above 11 are considered reactive (cut-off value). Values between 9 and 11 were considered equivocal and values below 9 were considered nonreactive.

peak response was seen, all 4 kits detected decreased anti-SARS-CoV-2 IgG levels over the remainder of the sample period. At 87 days, the anti-SARS-CoV-2 IgG levels were 60% to 70% of the peak level.

The GSD assay kit measured anti-SARS-CoV-2 IgM as well as IgG. Fig. 1B shows the timeline for development of IgM and IgG for this assay. Antibody levels in this donor behave as expected; anti-SARS-CoV-2 IgM levels peaked prior to IgG levels (on day 50) and declined over the time course of the study. At 87 days IgM levels have fallen below the manufacturers' cut-off and the sample would be considered nonreactive for IgM.

The Vitros® assay measured total anti-SARS-CoV-2 Ig as well as IgG (Fig. 1C). Overall, total anti-SARS-CoV-2 Ig levels increased throughout the assay period peaking on day 87. Expressed relative to anti-SARS-CoV-2 IgG, total Ig was much higher than IgG.

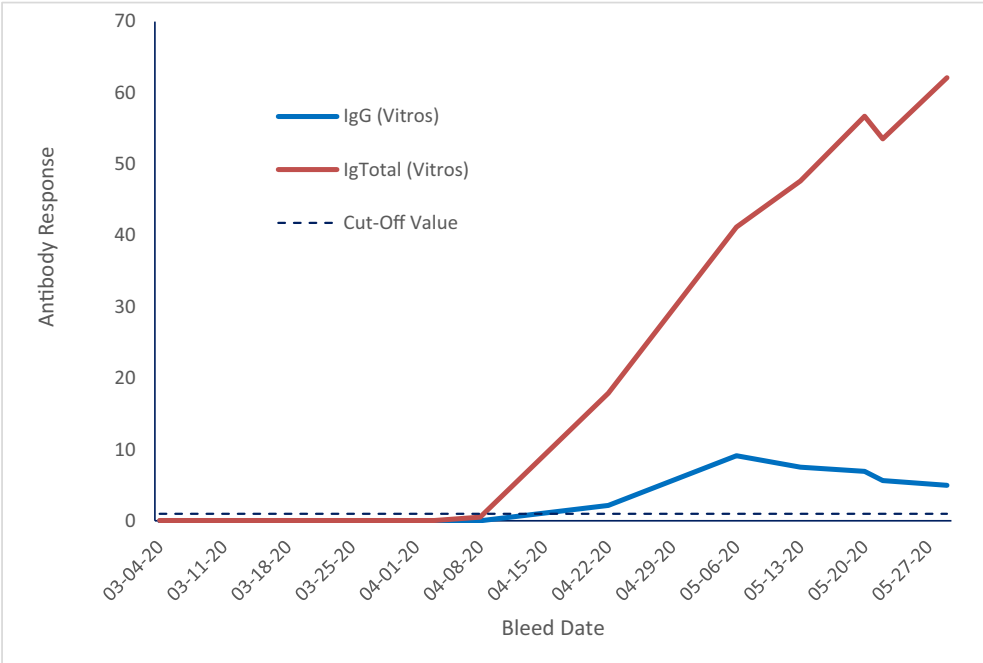

Fig. 1C. Anti-SARS-CoV-2 total immunoglobulins (Ig Total) and IgG measured in a SARS-CoV-2 seroconversion panel using Vitros® kits. Values were expressed on an arbitrary scale relative specified by the manufacturer. For this assay, values ≥1.0 were considered reactive (cut-off value) and values <1.0 were considered nonreactive for SARS-CoV-2.

4. Discussion

Since SARS-CoV-2 is a newly emergent virus, SARS-CoV-2 antibody detection is useful tool for diagnosis of past infections, development of passive immunity treatments, evaluation of vaccine efficacy and development of screening modalities for epidemiological monitoring. Current serological tests vary in their sensitivity, specificity, and practicality, and must be well-characterized to determine which test is most appropriate for use in the settings mentioned above.

It is well characterized that the antibodies responsible for clearance of coronaviruses during infection are those with specific neutralizing activity that blocks entry of the virus into host cells (Qian et al., 2015). SARS-CoV-2 antibodies that target the receptor-binding domains present in the S1 region of viral S protein are the main neutralizing antibodies that block the entry of the virus into host cells (Ou et al., 2020). Monitoring the presence and concentration of these antibodies in plasma is crucial to controlling the COVID-19 pandemic. Assays for these antibodies are an integral part of evaluating vaccine efficacy and developing passive immunity therapeutics such as convalescent plasma and hyperimmune globulin.

In addition, recent studies have shown that virus-specific cellular responses (both T and B cells: T-cell data have not been peer-reviewed) remain detectable after 6 months postinfection, contributing to protection against SARS-CoV-2 infection (Gaebler et al., 2021; Zuo et al., 2020) and bolstering hopes that widespread vaccination and convalescent immunity will help to quell the current pandemic.

In this study, a seroconversion panel obtained from a single patient was tested against four commercially available test kits for detecting anti-SARS-CoV-2 antibodies. This seroconversion panel is one of the first available for SARS-CoV-2. The advantage of this characterized seroconversion panel is the expansive time period (samples taken over almost 90 days) providing an extensive record of seroconversion in this single donor. This is a much longer time course than most seroconversion panels (Hsueh et al., 2004; Louie et al., 2006; Novack et al., 2006).

Similar results were obtained with all four assay kits which employ different recombinant SARS-CoV-2 antigens. Detection of anti-SARS-CoV-2 IgG antibodies showed the same time course in all 4 assays. This time course from infection or symptom development to antibody generation has been found to be highly variable between patients (To et al., 2020). The time course for antibody generation in a different patient could be substantially different. Given that most patients who present with COVID-19 (even mild or asymptomatic cases) would not have preinfection plasma samples available, obtaining the entire time course of pre-infection, infection and convalescent samples has been difficult. For this reason, a comparison of the time course of seroconversion panels from different individuals has not been made.

In this study, a decrease in IgG levels was observed after a peak at 64 to 71 days. IgG levels decreased to 60% to 70% of the peak level. Other studies have not shown this decrease in IgG at later time points, but these studies were shorter in duration which may account for the difference. In addition, IgM and IgG showed the expected temporal relationship in this seroconversion panel, i.e., IgM peaked prior to IgG and declined more steeply than IgG similar to the findings in other studies (Guo et al., 2020; Jin et al., 2020; Padoan et al., 2020). The Ig total Vitros assay detects total antibodies against the SARS-CoV-2 virus, i.e., a combination of IgG, IgM, IgA, and other isotypes. IgA and other isotypes could contribute to the increase in total Ig even while IgG and IgM were declining. Measurements of IgA and other isotypes were not performed in this study.

In conclusion, this seroconversion panel is a useful tool for developers of SARS-CoV-2 antibody detection assays, as well as for a proper validation of existing serological assays and as part of the decision-making when choosing the most reliable serological kit for a specific application. SARS-CoV-2 antibody detection is now and will continue to be a valuable tool for detection of COVID-19 immune responses especially in asymptomatic and convalescent patients.

Funding

These studies were supported by Grifols (Barcelona, Spain) and Access Biologicals (Vista, CA, USA).

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Advarra Institutional Review Board (Columbia, MD, USA). Informed consent was obtained from all individuals included in this study.

Declaration of competing interest

All authors have completed the ICMJE uniform disclosure form. FB, MLM, and NT are employees of Grifols. RC and MC are employees of Access Biologicals.

Acknowledgments

Michael K James, PhD (Grifols) is acknowledged for medical writing and Jordi Bozzo, PhD, CMPP (Grifols) is acknowledged for editorial support in the preparation of this manuscript. Contributions from Norbert Piel, Sansan Lin, Rodrigo Gajardo and Jerry A. Holmberg (Grifols) who provided their expert opinions are also acknowledged.

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