Performance Evaluation of the Siemens SARS-CoV-2 Total Antibody and IgG Antibody Test

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Laboratory Medicine 2021;52:e147-e153
DOI: 10.1093/labmed/lmab027

ABSTRACT

Objective: In this study, the performance of 2 commercially available SARS-CoV-2 antibody assays is evaluated.

Methods: The Siemens SARS-CoV-2 Total (COV2T) and IgG (COV2G) antibody tests were evaluated on a Siemens Atellica IM1300 analyzer. Imprecision was assessed with the CLSI EP15 protocol using positive controls. Ninety control group specimens were analyzed for specificity, and 175 specimens from 58 patients with polymerase chain reaction–confirmed SARS-CoV-2 were measured for the sensitivity and kinetics of the antibody response.

Results: Within-run and total imprecision were acceptable for both assays. Both tests showed a specificity of 100%. Sensitivity earlier in the disease state was greater for the COV2T assay than for the COV2G assay, but sensitivity >14 days after onset of symptoms approached 100% for both. For all patients, antibody titers remained above the seroconversion cutoff for all follow-up specimens.

Conclusion: This study shows acceptable performance for both the Siemens COV2T and COV2G test, although seroconversion occurs earlier with the COV2T test.

Keywords: COVID-19, SARS-CoV-2, serology, antibody kinetics, performance evaluation, immunoassay

SARS-CoV-2, a novel coronavirus belonging to the beta-coronaviruses, emerged in December 2019 in the area of Wuhan, China, and spread rapidly all over the world thereafter. On March 11, 2020, the World Health Organization declared the spread of the virus as a pandemic.1 COVID-19, the disease caused by SARS-CoV-2, may trigger a clinical spectrum of symptoms ranging from mild to life-threatening. Furthermore, many patients are asymptomatic and are unconsciously responsible for the further spread of the virus.2 Therefore, timely and precise diagnosis is crucial for adequate treatment and for infection control. Diagnosis is commonly performed by reverse-transcription polymerase chain reaction (RT-PCR) of viral RNA in upper respiratory tract specimens.3 Detection of specific SARS-CoV-2 IgM, IgA, and/or IgG antibodies in serum or plasma may be of added value in patients who present late after-symptom onset with a low viral load, causing the PCR test to be a false negative. In addition, antibody tests may be of use in epidemiological studies to determine antibody prevalence in the universal population or in specific settings, such as health care workers. Furthermore, large-scale vaccine studies are developing worldwide, and (serial) measurement of antibodies may be used for follow-up of vaccine effectiveness.4-6 Previous studies have shown that antibodies typically appear starting 5 to 7 days after infection and are therefore not useful in detection of acute infection. Since the start of the spread of this disease, numerous antibody assays, mainly targeting the nucleocapsid (N) protein or spike (S) protein, have been developed. These assays are lateral flow assays, enzyme-linked immunosorbent assays (ELISAs), and electrochemiluminescent or chemiluminescent immunoassays (CLIAs), compatible with high-throughput analyzers.7-13 Siemens Healthineers developed two CLIA-based SARS-CoV-2 antibody tests directed against the spike 1 protein receptor binding domain (S1-RBD): a total antibody test (COV2T) detecting both IgM and IgG antibodies, and an...
IgG antibody test (COV2G) detecting solely IgG antibodies. To date, 2 other studies have described the performance of the COV2T test, but no other studies have evaluated the COV2G antibody test.\textsuperscript{9,14} It was the aim of this study to evaluate both antibody assays and to describe the kinetics of antibody response in patients with COVID-19 with specimens measured with both assays.

**Materials and Methods**

**Patient Selection and Study Design**

In this retrospective study, specificity was evaluated using residual pre-pandemic serum specimens from healthy volunteers (n = 34) and random patients (n = 22). In addition, specimens from patients with potential cross-reacting antibodies, including antinuclear antibodies (n = 5), rheumatoid factors (n = 5), Epstein-Barr virus (n = 5) and cytomegalovirus (n = 5) IgM-positive specimens, paraproteins (n = 5), and PCR-confirmed acute infections with other coronavirus strains (NL63: n = 3; HKU-1: n = 3; OC43: n = 3) were analyzed. For sensitivity, 175 follow-up routine serum specimens from 58 hospitalized patients (median age 80 years) with confirmed detection of SARS-CoV-2 RNA by RT-PCR on nasopharyngeal swab were measured. Specimens were drawn between 0 and 109 days after PCR positivity. Sensitivity was calculated for different time frames: day < 4, day 4–7, day 8–10, day 11–14 and day > 14, starting from the time to the first positive PCR result and starting from the time of symptom onset. Calculation of 95% confidence intervals (CI) was performed with MedCalc Statistical Software (MedCalc Software, Ostend, Belgium). Information about the start of symptoms was derived from the medical records.

For calculation of sensitivity compared to symptom onset, 18 specimens from 8 patients were excluded because these patients were asymptomatic. The median time between a positive PCR test or symptom onset and serum specimen collection was 8 days (interquartile range, 4–13 days) and 12 days (interquartile range, 6.5–18 days), respectively. With the same specimens, the kinetics of antibody response were assessed for both assays. A method comparison was performed against the Euroimmun Anti-SARS-CoV-2 IgG ELISA (Euroimmun AG, nr. EL2606-9601G), targeted against the S1 protein, was carried out on a BEP 2000.

**Analytical Performance**

Intra- and interassay imprecision were assessed according to the CLSI EP15-A3 protocol with positive control material provided by the manufacturer by measurement of quintuplicates during 5 successive days.\textsuperscript{15} An analysis of variance was used to calculate the repeatability and intermediate precision.

**Laboratory Measurements**

The COV2T test (Siemens Healthineers nr. 11206711, Munich, Germany)\textsuperscript{16} is an automated 1-step antigen chemiluminescent sandwich immunoassay for total antibodies, and the COV2G (Siemens Healthineers nr. 11206997)\textsuperscript{17} is an automated 2-step chemiluminescent sandwich immunoassay for IgG antibodies against the SARS-CoV-2 virus. Both assays target the S1-RBD. The COV2T assay is designed as a qualitative assay, whereas the COV2G test is used for the semi-quantitative detection of IgG antibodies. The performance of both assays was assessed on a Atellica IM1300 analyzer (Siemens Healthineers, Erlangen, Germany). Characteristics of these assays, as stated in the product leaflet,\textsuperscript{16,17} are summarized in Table 1.

For the method comparison, the anti-SARS-CoV-2 ELISA IgG test (Euroimmun AG, nr. EL2606-9601G), targeted against the S1 protein, was carried out on a BEP 2000.

| Assay   | Detection | Sensitivity (d after PCR positivity) | Specificity | Measuring Interval (index value) |
|---------|-----------|--------------------------------------|-------------|----------------------------------|
|         |           | < 6 d | 7–13 | > 13 |                           |
| COV2T   | IgM and IgG | 60.7% | 97.3% | 100% | 0.05–10                   |
|         | IgG       | 56.0% | 92.2% | 100% | 0.5–20                     |
| COV2G   | IgG       | 60.7% | 97.3% | 100% | 0.05–10                   |

COV2T, SARS-CoV-2 Total antibody assay; COV2G, SARS-CoV-2 IgG antibody assay; PCR, polymerase chain reaction.

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Table 1. Characteristics of Siemens COV2T and COV2G, According to Manufacturer’s Specifications
Advance system (Siemens Healthineers). All specimens were stored at −80°C until the time of analysis. Specimens were analyzed by trained laboratory staff in the general hospital AZ Sint-Lucas in Bruges, Belgium, in accordance with the manufacturer’s protocol.

Results for COV2T and COV2G were reported as index values and considered as nonreactive (index < 1.0 for Siemens and index < 0.8 for Euroimmun), borderline (0.8 < index < 1.1 for Euroimmun) or reactive (index ≥ 1.0 for Siemens and index ≥ 1.1 for Euroimmun) according to the manufacturer’s instructions. The measuring interval reported by the manufacturer was 0.05 to 20.00 for COV2T and 0.50 to 20.00 for COV2G.

Results

Results for imprecision are given in Table 2. Intra-assay imprecision for COV2T and COV2G assays revealed coefficients of variations of 2.81% and 2.35%, respectively, both lower than the manufacturer’s claim of 10%. Total imprecision was 7.12% and 5.81%, respectively, also remaining below the manufacturer’s claim of 12%.

Specificity, calculated on 90 specimens, was 100% for the COV2T assay and for the COV2G assay (95% CI, 96.4–100.0%). There was no cross-reactivity found with possible interfering antibodies, including the specimens from other common-cold coronavirus strains.

Table 3 gives an overview of results for the sensitivity of both assays, calculated from the days since positive PCR result and days since symptom onset. Overall, calculated sensitivities were lower than the sensitivities stated in the product leaflets (Table 1). As expected, the sensitivity of both assays increased gradually with disease progression, reaching 100% (95% CI, 89.7–100.0%) for the COV2T assay 14 days after PCR positivity. For the COV2G assay, sensitivity 14 days after PCR positivity was 97.1% (95% CI, 84.7–100.0%) because of 1 specimen with a result just below the cutoff value. Sensitivity 14 days after disease onset was 94.6% (95% CI, 85.1–98.9) and 91.1% (95% CI, 80.4–97.0) for the COV2T and COV2G assay, respectively, because of 3 and 4 specimens (respectively) that remained seronegative after 14 days. However, follow-up specimens from these patients showed seroconversion later in the disease progression. Sensitivity in the different time frames was lower for COV2G than for COV2T. Sensitivities were lower when calculated for days after symptom onset because symptoms were typically already present a few days before PCR testing was performed.

The kinetics of antibody response on different days from symptom onset were evaluated on the same specimen set from hospitalized patients, with specimens taken up to 109 days after the start of symptoms. Figures 1A and 1B show the results for the COV2T and COV2G assays. The median time for seroconversion, after the start of

| Table 2. Intra- and Interassay Imprecision of Siemens SARS-CoV-2 Antibody Assays, Assessed on Quality Control Material Provided by Manufacturer |
|---|---|---|---|---|
| Assay | Mean | Intra-Assay Imprecision | Total Imprecision |
| | | SD | CV (%) | SD | CV (%) |
| COV2T | 2.44 | 0.07 | 2.81 | 0.17 | 7.12 |
| COV2G | 2.57 | 0.06 | 2.35 | 0.15 | 5.81 |

**Table 3. Sensitivity of COV2T and COV2G Tests Calculated Against Day of Positive PCR Result and Day of Symptom Onset**

| Days After PCR Positivity | Days After Symptom Onset |
|---|---|
| COV2T | COV2G | COV2T | COV2G |
| < 4 d | 25.0% (10/40; 12.7–41.2) | 17.5% (7/40; 7.3–32.8) | 5.9% (1/17; 0.2–28.7) | 5.9% (1/17; 0.2–28.7) |
| 4–7 d | 39.0% (16/41; 24.2–55.5) | 31.7% (13/41; 18.1–48.1) | 19.4% (6/31; 7.5–37.5) | 12.9% (4/31; 3.63–29.8) |
| 8–10 d | 62.1% (18/29; 42.3–79.3) | 44.8% (13/29; 26.5–64.3) | 57.1% (12/21; 34.0–78.2) | 38.1% (8/21; 18.1–61.6) |
| 11–14 d | 90.3% (28/31; 74.3–98.0) | 64.5% (20/31; 45.4–80.8) | 84.4% (27/32; 67.2–94.7) | 56.3% (18/32; 37.7–73.6) |
| > 14 d | 100.0% (34/34; 89.7–100.0) | 97.1% (33/34; 84.7–100.0) | 92.9% (53/56; 85.1–98.9) | 91.1% (51/56; 80.4–97.0) |

COV2T, SARS-CoV-2 Total antibody assay; COV2G, SARS-CoV-2 IgG antibody assay; PCR, polymerase chain reaction.

Numbers in parentheses are absolute specimen numbers, followed by calculated 95% confidence intervals for sensitivity.
symptoms, was 11 days for the COV2T assay. For the COV2G assay, the median time to seroconversion was only 1 day later, at 12 days after symptom onset. The median antibody levels reached the upper limit of the manufacturers’ measuring range 14 days and 16 days after symptom onset for the COV2T and COV2G assays, respectively. For
all but 2 patients, after reaching the maximum antibody levels, these levels were held with all further follow-up specimens. For those 2 patients, COV2T and COV2G antibody levels declined at 51 and 109 days, respectively, but values remained above the cutoff for seropositivity.

One hundred ninety-five specimens from health care workers working at the AZ Sint-Lucas hospital in Bruges, Belgium, and with presumptive or PCR-confirmed COVID-19 disease were analyzed with the Siemens COV2T assay and the Euroimmun IgG ELISA (Table 4). Twenty-four specimens were positive with the Siemens COV2T assay and negative with the Euroimmun assay, whereas 3 specimens were negative with the COV2T assay but positive or borderline with the Euroimmun ELISA. The results of this specimen set suggest a higher sensitivity of the Siemens COV2T assay compared to the Euroimmun ELISA. The percentage agreement between both assays, when borderline results were considered positive, was 86.2%. Only 94 of 195 specimens could be compared to the COV2G test, 34 were found to be seropositive with COV2T but negative with COV2G, resulting in a sensitivity of 66% of the COV2G assay compared to COV2T.

### Discussion

Serologic testing for SARS-CoV-2 may be of added value in the diagnosis of COVID-19. Furthermore, serologic assays can be used in epidemiological studies. However, it is not yet clear what the presence of anti-SARS-CoV-2 antibodies means for protection against reinfection. The International Federation of Clinical Chemistry recently published interim guidelines for the serologic testing of antibodies against SARS-CoV-2. The guidelines make recommendations about clinical indications for serologic testing and suggest a framework for assay selection and verification protocols for commercially available assays. Fully automated assays permit rapid identification of the SARS-CoV-2 immune status. In this study, the COV2T and COV2G assays were evaluated. Imprecision, as tested by the CLSI EP15-A3 protocol, was found to be adequate. These results were in line with what was found earlier by Hörber et al, who described an intra-assay imprecision of 3.4% and an interassay imprecision of 4.9%.

Specificity for COV2T and COV2G assays was both 100%. No cross-reaction was found for specimens with other infectious diseases, including the specimens from common-cold coronavirus strains. This finding is in line with the manufacturer claims and previous studies that found a specificity of 99.9% and 100% for the COV2T assay. A drawback of this study was that no SARS-CoV specimens could be analyzed, but this missing information is of less importance because that virus is known to be eradicated. An excellent specificity is of great importance for assay performance because the positive predictive value of a test decreases greatly when the prevalence of the tested disease is low. The current SARS-CoV-2 seroprevalence is still relatively low—the latest numbers vary at approximately 10% seropositivity, so a high specificity is required when conducting large population-scale studies.

The sensitivity of a SARS-CoV-2 antibody assay depends on both the assay setup and the moment of testing in the disease course. Whereas assays that target the nucleocapsid protein are typically more sensitive earlier in the disease phase, antibodies targeting the spike protein are more specific and may have a neutralizing effect.

### Table 4. Method Comparison of COV2T and COV2G Assays Against Euroimmun SARS-CoV-2 IgG ELISA, Performed on Specimens from Health Care Workers

|          | COV2T   |            | COV2G   |            |
|----------|---------|------------|---------|------------|
|          | +       | –          | Total   | +          | –          | Total   |
| Euroimmun| 145     | 2          | 147     | 61         | 17         | 78      |
| ±        | 16      | 1          | 17      | 1          | 5          | 6       |
| –        | 24      | 7          | 31      | 0          | 10         | 10      |
| Total    | 185     | 10         | 195     | 62         | 32         | 94      |

COV2T: SARS-CoV-2 Total antibody assay; COV2G: SARS-CoV-2 IgG antibody assay; ELISA, enzyme-linked immunosorbent assay.
Muecksch et al\textsuperscript{24} found a significant correlation of the Siemens COV2T assay with neutralizing antibody levels, but this correlation was highest earlier in the disease state and declined thereafter. To date, an antibody cutoff for protective immunity has not yet been defined. In addition, the durability and nature of immunity conferred by these antibodies remains unclear. Moreover, because antibody titers tend to be associated with clinical severity, assay performance may depend on disease severity.\textsuperscript{18} In this study, sensitivities calculated for timepoints < 14 days were lower than those claimed by the manufacturer. In general, clinical sensitivity in validation studies may be lower than stated in the product leaflet, whereas clinical specificity tends to be more stable.\textsuperscript{18} The sensitivity for the COV2T assay found in this study earlier in the disease stage was lower than that found by Hörber et al., who found a sensitivity of 56.5\% 6 days after PCR positivity. For the later timepoints, sensitivity was comparable to the study of Hörber et al. This result was also in line with a study from the national SARS-CoV-2 serology assay evaluation group in the United Kingdom that found a sensitivity of 98.1\% in specimens collected > 20 days post-symptom onset.\textsuperscript{9}

The median time to seroconversion in this study was 8 days after PCR positivity for both assays and 11 and 12 days after symptom onset for the COV2T and COV2G assay, respectively. This median time may have lagged behind the true time to seroconversion because patients were not tested on a daily basis. Although the median time until seroconversion did not differ significantly for both assays, the sensitivity of the COV2T assay was higher compared to the COV2G assay in the different time frames up to 14 days after PCR positivity or disease onset. This finding may be explained by the complementary detection of IgM antibodies in the COV2T assay. However, contradictory studies have been published regarding the sensitivity of different antibody isotypes: Some studies have shown that the time to seropositivity for IgM or IgA is not significantly shorter than that for IgG, whereas others have reported a faster seroconversion for IgM and/or IgA.\textsuperscript{18}

A drawback to this study was that this specimen set only included specimens from hospitalized patients and healthcare workers but none from outpatients, children, or asymptomatic patients. Because the antibody titer of almost all of the patients reached the upper limit of the dynamic range at a certain timepoint, no comparison could be made with regard to disease severity.

The kinetics of the antibody response were evaluated using the same specimen set. Only in 2 out of 50 patients did antibody levels decline, but the results remained above the seropositivity cutoff index. This decline started at 51 days for 1 patient and 109 days for the other. Earlier studies have shown that antibody titers may decline and that this decline also depends on the assay used. Muecksch et al\textsuperscript{24} found that antibody titers globally decreased over time in a serologic Abbott assay, increased in the assays from manufacturers Roche and DiaSorin, and remained constant in the Siemens COV2T assay.

The method comparison against the Euroimmun anti-SARS-CoV-2 IgG ELISA, performed on samples from healthcare workers, showed a lower sensitivity compared to the Siemens COV2T assay, but a greater sensitivity when compared to the COV2G assay. The improved sensitivity of the Siemens total antibody assay may be attributable to the concomitant detection of IgM (and possibly also IgA) antibodies. A possible explanation for the lower sensitivity of the COV2G assay compared to the Euroimmun IgG assay is that the latter assay detects antibodies directed against the full-length S1 protein, whereas the COV2G assay only detects antibodies against the S1-RBD. However, not all of the specimens were from healthcare workers who were confirmed PCR-positive, so these results must be interpreted with caution. More studies are necessary to confirm this observation. Hörber et al\textsuperscript{14} showed that the Siemens COV2T assay was slightly more sensitive than the Euroimmun IgG ELISA and the Roche Elecsys anti-SARS-CoV-2 antibody assay, which targets total antibodies against the nucleocapsid protein. Diagnostic specificity was comparable for all 3 assays. In addition, in the study from the U.K. national SARS-CoV-2 serology assay evaluation group, the Siemens COV2T assay showed superior sensitivity compared to the other tested commercially available assays of Roche, DiaSorin, and Abbott.\textsuperscript{9} Finally, the study by Muecksch et al\textsuperscript{24} indicated a comparable performance of the Roche, Abbott, and Siemens assays, but only specimens starting from 21 days after a PCR-positive test were included.

**Conclusion**

The Siemens COV2T and COV2G assays for the detection of antibodies against SARS-CoV-2 are highly specific.
Sensitivity 14 days after COVID-19 symptom onset approaches 100%. The assays are reliable and accurate for routine clinical use. LM

Acknowledgments

The authors thank Siemens for kindly providing the COV2T and COV2G reagents for this study.

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