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# High Incidence of False-Positive Results in Patients with Acute Infections Other than COVID-19 by the Liaison SARS-CoV-2 Commercial Chemiluminescent Microparticle Immunoassay for Detection of IgG Anti-SARS-CoV-2 Antibodies

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Coronavirus disease 19 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a major pandemic (1). Diagnosis is based on detection of viral RNA, whereas the immune response to SARS-CoV-2 is measured by serological tests which detect antibodies (Abs) directed against nucleocapsid and/or spike envelope proteins of the virus. The median day of IgG seroconversion is around 14 days after symptom onset (2), but a high interindividual variation has been reported, as well as contrasting performances between commercial assays (3–6).

We evaluated the first chemiluminescent microparticle immunoassay (CLIA) available for routine use in Saint-Antoine Hospital (Paris, France), the Liaison SARS-CoV-2 S1/S2 IgG assay (DiaSorin, Antony, France), which uses a combination of SARS-CoV-2 recombinant S1 and S2 proteins as capture antigens.

Deidentified specimens were analyzed, as follows. There were 68 specimens from unselected reverse transcription-PCR (RT-PCR)-confirmed COVID-19 patients with estimated date of symptom onset, for whom one unique serum specimen submitted for serological testing was available. There were 76 specimens from 49 RT-PCR-confirmed COVID-19 patients hospitalized in the intensive care unit with estimated date of symptom onset, longitudinally collected from excess plasma specimens used for blood cell count analysis. To minimize the potential bias resulting from several samplings from the same individual patients of this population, we censored the results on the first positive sample when all were reactive ($n = 16$), on the last negative sample when all were nonreactive ($n = 6$), and on the 2 samples framing the seroconversion, when observed ($n = 27$). There were 100 specimens collected before the COVID-19 epidemic in France: 40 unselected serum samples and 60 samples from patients suffering from various infectious conditions (Table 1).

A total of 244 plasma or serum samples stored at −20 or −80°C were tested. Samples were tested as recommended by the manufacturer, and equivocal results were retested (7).

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**TABLE 1** Specificity results

| Sample type or source | No. of samples[a] | | | | % Positivity |
|---|---|---|---|---|---|
| | Total | Negative | Positive | Equivocal | |
| Coronavirus 229E, NL63, and OC43 | 10 | 10 | 0 | 0 | 0 |
| Primary CMV infection | 5 | 4 | 1 | 0 | 20 |
| Primary EBV infection | 10 | 6 | 4 | 0 | 40 |
| Acute HAV infection | 5 | 5 | 0 | 0 | 0 |
| Acute HBV infection | 4 | 1 | 3 | 0 | 75 |
| Acute HCV infection | 3 | 2 | 1 | 0 | 33.3 |
| Acute HEV infection | 5 | 4 | 0 | 1 | 0 |
| Acute HIV infection | 5 | 5 | 0 | 0 | 0 |
| Influenza A/B virus infection | 10 | 10 | 0 | 0 | 0 |
| Acute malaria | 3 | 2 | 1 | 0 | 33.3 |
| Unselected sera | 40 | 40 | 0 | 0 | 0 |
| Total | 100 | 89 | 10 | 1 | 10 |

[a]Negative is defined as <12 absorbance units (AU)/ml, positive as ≥15 AU/ml, and equivocal as 12 to 15 AU/ml. CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

According to delay from symptom onset, clinical sensitivity was 24.1% for 1 to 7 days (7/29), 54.7% for 8 to 14 days (41/75), and 75% after day 15 (30/40).

Sensitivity did not seem to significantly increase over time after day 15, since the test was reactive for 22/28 (78.6%) samples collected between day 15 and day 20 and for 8/12 (66.6%) samples collected between day 21 and day 30.

We observed 27 seroconversions, at a median of 16 days (interquartile range [IQR], 11.5 to 17.5 days). These performances are lower than the sensitivities previously reported (2–6) or described by the manufacturer (7). This could result from the lack of recognition by some Abs of the denatured nonglycosylated S proteins used in the assay (8) and/or from comparing sensitivity according to the date of positive PCR and not before disease onset. Since PCR can be performed several days after disease onset, this may account for an apparent better sensitivity.

Furthermore, we were alerted by a high incidence (10%) of false-positive results, which led our laboratory to stop the routine use of this assay. This specificity issue was consistently observed in patients suffering from acute infectious conditions, especially infection with Epstein-Barr virus (EBV) (4/10) or hepatitis B virus (HBV) (3/4) (Table 1). This suggests that they result from nonspecific immune activation rather than cross-reactivity between non-SARS-Cov-2 Abs and the SARS-CoV-2 proteins used in the assay. This might also explain the difference between our results and the specificity announced by the manufacturer, which was determined by using unselected samples from clinical laboratory or blood banks.

After stopping the use of the Liaison SARS-CoV-2 assay, we changed to the CLIA Alinity I SARS-CoV-2 IgG assay (Abbott Diagnostics, Rungis, France), which detects Ab directed against capsid antigen of the virus (9), and evaluated the performances of this test on the same panel of samples.

According to the delay from symptom onset, clinical sensitivity of the Alinity I assay was better than that of the Liaison assay: 45.2% for 1 to 7 days, 72.6% for 8 to 14 days, and 84.4% after day 15. The median time for seroconversion (13 days; IQR, 10 to 17 days) was also shorter with this assay.

Furthermore, the 100 samples in our specificity panel were negative with the Alinity I SARS-CoV-2 IgG assay, except for one serum sample from a patient with coronavirus 229E infection, which was weakly reactive (signal-to-cutoff index = 1.93; cutoff is 1.4 index), which leads to a specificity of 99%. This last result could mean that the lack of specificity observed with the Liaison assay was linked to the test rather than to the nature of the samples.

While the clinical significance of SARS-Cov-2 antibody detection remains to be determined, our results confirm that careful evaluation of the tests on appropriate samples is required before implementing assays for routine use.

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