Clinical Performance of the Roche SARS-CoV-2 Serologic Assay

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To the Editor:

In a study published in *Clinical Chemistry*, we compared the clinical performance of two serologic assays (Abbott and EUROIMMUN (EI)) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) *(1)*. Here, we characterize the clinical performance of the Roche Elecsys Anti-SARS-CoV-2 assay using the same specimens and validation protocol.

The Roche assay measures total immunoglobulins directed towards a recombinant nucleocapsid protein from SARS-CoV-2. The assay reports a ratio of specimen electrochemiluminescent signal to calibrator. The final interpretation of positivity is defined as a cut-off index (ratio) ≥1.0.

Compared to SARS-CoV-2 PCR, the specificity of the Roche SARS-CoV-2 assay was 98.69% (95% CI; 95.63-99.84) (Abbott: 99.34%; 96.41-99.98. EI: 94.77%; 89.96-97.72.) **(Fig. 1A)**. The sensitivity of the Roche assay at >14d post-onset of symptoms was 89.36% (76.9-96.45) (Abbott: 93.76%; 82.8-98.69. EI: 85.42%; 72.24-93.93). The sensitivity ranged from 50.0% (95% CI; 34.19-65,81) at <3d post-PCR testing to 75.0% (47.62-92.73%) at >14d **(Fig. 1B)**. Relative to the Abbott assay, the total concordance was 0.89 (95% CI; 0.81-0.95), the positive percent agreement was 96.0% and the negative percent agreement was 95.2%. Relative to the EI assay, the total concordance was 0.84 (0.77-0.92), the positive percent agreement was 95.8% and the negative percent agreement was 88.2% if borderline results were considered positive. Among patients with PCR confirmed SARS-CoV-2, at <14d of symptom onset, the Roche assay detected 6 more true positives than the Abbott assay and 5 more true positives than the EI assay. However, at 14d+ of symptom onset, the Roche assay detected 2

fewer positives when compared to Abbott. Three patients with immunodeficiencies were negative by all three assays after day 14 from symptom onset. Two patients were negative by molecular testing but positive by the Roche assay; one was clinically adjudicated as COVID-19+ (open circle) and positive by all three assays while the second was negative by the Abbott and EI assays and not clinically adjudicated as COVID-19+.

Time from symptom onset to positive result could not be calculated due to a lack of discernable kinetic pattern when the signal from serial specimens was examined **(Fig. 1C)**. The Roche assay demonstrated a wider analytic measuring range (ratio 0.089-112.0) relative to Abbott (0.01-7.6) and EI (0.2-10.0). However, the signal diminished over time in several patients with serial specimens **(Fig. 1D)**. Specimen pools at three concentrations (ratio 0.09, 1.29, and 54.42) had repeatability and total imprecision of <3.9%. No interference was noted up to a hemolysis index of 1000, lipemic index of 1000, or icteric index of 60 and no carryover was observed.

The Abbott assay demonstrated the highest specificity and sensitivity for detection of antibodies to SARS-CoV-2 >14d after onset of symptoms compared to Roche and EI, but these findings were not statistically different. Importantly, the three assays evaluated do not have sufficient clinical sensitivity for detection of SARS-CoV-2 prior to 14d from symptom onset to be useful for acute diagnosis. Our results *(1)* demonstrate inferior clinical performance (particularly with regards to sensitivity) of SARS-CoV-2 immunoassays than that reported by the manufacturers. Our studies used specimens from acutely ill hospitalized patients, which may have differed from the patient populations used in studies performed by the manufacturers. Nonetheless, serological

assays will likely be used for both acutely ill and recovered healthy patients and assay validations should be performed using both patient populations *(2)*.

Despite the Abbott and Roche assays both targeting the SARS-CoV-2 nucleocapsid protein, modest differences in sensitivity and specificity were noted, possibly reflecting variation in assay design. The Roche assay is unique since it measures total immunoglobulin (i.e., IgG, IgA, and IgM) as opposed to IgG (Abbott and EI). This likely explains why the Roche assay was able to detect antibodies to SARS-CoV-2 earlier than the EI and Abbott assays. Furthermore, the potential to detect multiple classes of immunoglobulins likely underlies the lack of discernable pattern in the kinetics of serial specimens. Paradoxically, fewer positive results were observed in patients with >14d of symptoms on the Roche assay relative to the Abbott assay. All three specimens that were positive by Abbott and negative by Roche had a modest signal on the Abbott assay (ratio 3-4). Therefore, the antibodies detected in these patients may have lower affinity and are unable to bind to the antigen targets in the Roche assay. To this end, Roche has stated in publications distributed to users that the assay primarily targets high-affinity antibodies to increase specificity. However, the robust signal detected in several patients early in symptomatology (<7d) and rapidly decreasing signal from days 7-28 may be more consistent with lower affinity IgM antibody binding on the Roche assay. Larger studies must be performed to confirm this finding.

In conclusion, the Abbott assay demonstrated the fewest false negative results >14d post-symptom onset and the fewest false positive results. While the Roche assay detected more positive results earlier after onset of symptoms than the other assays,

none of the assays demonstrated high enough clinical sensitivity before day 14 from symptom onset to diagnose acute infection. Nonetheless, the clinical performance between the Roche, Abbott, and EI SARS-CoV-2 assays are similar and can detect antibodies to SARS-CoV-2 in a majority of patients 14d after the onset of symptoms.

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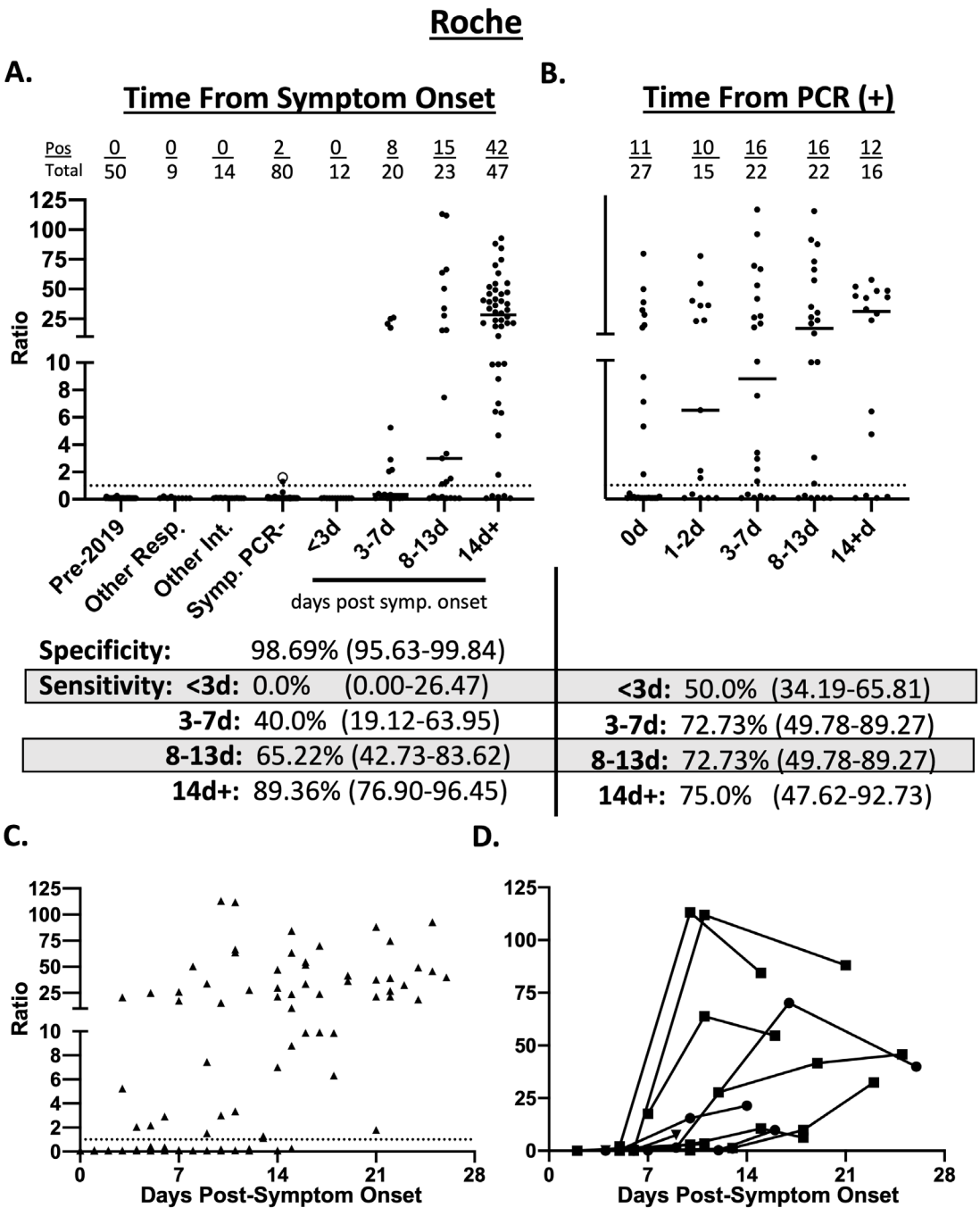
2. Farnsworth CW, Anderson NW. Sars-cov-2 serology: Much hype, little data. [Epub ahead of print] Clin Chem 2020 Apr 28 as doi: 10.1093/clinchem/hvaa107.

FIGURE LEGEND

Clinical performance of Roche SARS-CoV-2 Immunoassay. **(A)** Seropositivity in 153 expected negative specimens and 102 specimens from 48 patients with PCR-positive COVID-19 relative to days from onset of symptoms. **(B)** Seropositivity in 102 specimens from 48 patients with PCR-positive COVID-19 relative to days from testing positive by PCR. *Pre-2019* -- 50 specimens collected in 2015 and stored at -80◦C. *Other Resp.* -- specimens from patients with PCR confirmed Influenza A (n=2), influenza B (n=2), other non-COVID-19 coronaviruses (n=5, including Coronaviruses HKU1, NL63, and 229E). *Other Int.* -- specimens from patients with positive CMV IgG (n=5), EBV VCA IgG (n=5), EBV VCA IgM (n=3), Rheumatoid factor (n=1). *Symp. PCR*- -- specimens from 80 patients presenting to the hospital with symptoms of respiratory infection and PCR negative for COVID-19. The large open circle represents a patient who was PCR negative but had symptoms consistent with COVID-19 and prolonged exposure to a family member with PCR confirmed COVID-19. Values in parentheses represent 95% confidence interval. **(C)** Seropositivity relative to days of symptom onset. Dotted line represents the cutoff off for positivity (Ratio ≥1.0). **(D)** Kinetics of the antibody response in 12 patients with serial samples.

Figure 1


Roche
A.
Time From Symptom Onset
B.
Time From PCR (+)
Pos 0 0 0 2 0 8 15 42
Total 50 9 14 80 12 20 23 47
11 10 16 16 12
27 15 22 22 16
Ratio
Pre-2019
Other Resp.
Other Int.
Symp. PCR-
<3d
3-7d
8-13d
14d+
days post symp. onset
0d
1-2d
3-7d
8-13d
14+d
Specificity: 98.69% (95.63-99.84)
Sensitivity: <3d: 0.0% (0.00-26.47)
3-7d: 40.0% (19.12-63.95)
8-13d: 65.22% (42.73-83.62)
14d+: 89.36% (76.90-96.45)
<3d: 50.0% (34.19-65.81)
3-7d: 72.73% (49.78-89.27)
8-13d: 72.73% (49.78-89.27)
14d+: 75.0% (47.62-92.73)
C.
D.
Ratio
Days Post-Symptom Onset
Days Post-Symptom Onset