Letter to the Editor

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Diagnostic accuracy of the ultrasensitive S-PLEX SARS-CoV-2 N electrochemiluminescence immunoassay

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To the Editor,

We read with interest the recent article of Ren et al. [1], who described the accuracy of an ultrasensitive electrochemiluminescence immunoassay for saliva-based Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nucleocapsid protein (N) detection based on the S-PLEX platform (S-PLEX SARS-CoV-2 N Kit; Meso Scale Discovery, Rockville, MD, United States). This method has been specifically developed for detecting and quantifying the SARS-CoV-2 N antigen in a variety of human specimens, including serum, plasma, saliva and nasopharyngeal swabs (NPS). Briefly, either S-PLEX 96-Well SECTOR or QuickPlex plates coated with streptavidin for binding biotin-conjugated capture anti-SARS-CoV-2 N antibodies are challenged with human samples. After this step, “TURBO-BOOST”-labeled detection antibodies react with the N antigen bound to the solid phase and, after addiction of a specific reagent, an electrochemiluminescent signal is generated and read by the specific instrument. The signal produced is proportional to the concentration of N antigen present in the test sample. A preliminary evaluation of this assay revealed that the limit of detection is 0.16 pg/mL, with a diagnostic threshold set at 0.32 pg/mL and a total imprecision ranging between 7.0 and 7.7% [2]. The sample volume is only 25 μL, with total turnaround time between 4–5 h.

Since this novel technique displayed remarkable diagnostic performance in saliva samples in the hands of Ren and colleagues, exhibiting up to 100% specificity with 92% sensitivity [1], we provide here a critical literature review and pooled analysis of studies which addressed the accuracy of S-PLEX SARS-CoV-2 N Kit for diagnosing acute SARS-CoV-2 infections.

We carried out a digital search in the two scientific databases Medline (PubMed interface) and Scopus, using the following keywords: “S-PLEX” AND “COVID-19” OR “SARS-CoV-2”, without no language or date (i.e., up to February 17, 2022) restrictions. The initial screening of documents was conducted by G.L. and M.M., aimed at selecting studies were the diagnostic accuracy of S-PLEX SARS-CoV-2 N Kit was assessed against a reference molecular technique for diagnosing acute SARS-CoV-2 infections, and with sufficient extrapolable information for construction of a 2×2 table. A pooled analysis, based on the Mantel-Haenszel method and random effects model, was employed for estimating the diagnostic sensitivity, specificity and accuracy (reported as Summary Receiver Operating Characteristic Curve [SROC] and agreement) of this method. The inter-study heterogeneity was also assessed with χ² test and I² statistic. The statistical analysis was performed with Meta-DiSc 1.4 (Unit of Clinical Biostatistics team of the Ramón y Cajal Hospital, Madrid, Spain) [3]. The analysis was carried out in accordance with the Declaration of Helsinki and within the terms of
local legislation. No Ethical Committee approval was necessary, as this is a systematic literature review.

Our electronic search identified six articles after duplicates elimination. One article was excluded as it did not present data on S-PLEX SARS-CoV-2 and another because containing insufficient information for constructing a 2×2 table, thus four studies (totaling 657 clinical samples) were included in our analysis (Table 1) [1, 2, 4, 5]. An additional search, using the key word “Meso Scale”, allowed to detect 12 more articles, but none of these presented data on S-PLEX SARS-CoV-2 N Kit.

All four studies were from North America (three from the US and one from Canada), with a sample size comprised between 105 and 226 clinical specimens and cumulative disease prevalence of 53.4% (95%CI, 49.5–57.3%). The population from which the specimens were collected was only specified in two instances (Table 1). SARS-CoV-2 diagnosis was made by using multiple molecular assays in two cases, and a commercial and an in-house molecular technique in the remaining two studies. Overall, molecular detection of SARS-CoV-2 was carried out in NPS in three investigations and saliva in the remaining study. As concerns S-PLEX, SARS-CoV-2 antigen detection was conducted in NPS in two studies, and in saliva or plasma in the remaining two investigations. In three cases, a cut-off of ≤0.32 pg/mL was used, whilst the remaining investigation applied a different threshold.

The cumulative diagnostic accuracy of S-PLEX for diagnosing acute SARS-CoV-2 infections patients is summarized in Figure 1. The diagnostic sensitivity was 0.87 (95% CI, 0.83–0.90), the specificity 0.92 (95%CI, 0.89–0.95), the area under the curve (AUC) 0.955 (95%CI, 0.944–0.967), whilst the accuracy was 89.5% (95%CI, 86.9–91.7%). Similar results were obtained when our analysis was restricted to the three studies which evaluated SARS-CoV-2 in NPS or saliva, in that the sensitivity was 0.86 (95%CI, 0.81–0.89), the specificity 0.92 (95%CI, 0.88–0.95), the AUC 0.950 (95% CI, 0.934–0.966) and the accuracy 88.7% (85.7–91.3%), respectively.

The results of our critical literature review and pooled analysis confirm that the ultrasensitive S-PLEX SARS-CoV-2 N electrochemiluminescence immunoassay displays excellent performance for detecting SARS-CoV-2 N antigen in a vast array of clinical specimens (NPS, saliva and even plasma), exhibiting a cumulative diagnostic sensitivity as high as 87%, which would make it one of the most sensitive SARS-CoV-2 antigen tests currently available in the market. As comparison, the cumulative diagnostic sensitivity of this method was found to be substantially better than that found with laboratory-based chemiluminescent techniques such as DiaSorin LIAISON SARS-CoV-2 Ag test (i.e., 31%) [6] and Roche Elecsys SARS-CoV-2 antigen electrochemiluminescence immunoassay (i.e., 68%) [7], slightly higher than that reported for Ortho VITROS SARS-CoV-2 antigen test (i.e., 82%) [8], and exactly identical to that of the other high-sensitivity immunoassay Fujirebio Lumipulse SARS-CoV-2 Ag test (i.e., 87%) [9].

In conclusion, the relatively low rate of false negative test results (i.e., 13%) in high-prevalence settings (i.e., disease prevalence around 50%) would make the ultrasensitive S-PLEX SARS-CoV-2 N electrochemiluminescence immunoassay a good surrogate of molecular testing for initial screening of patients with high clinical suspicion of SARS-CoV-2 infection. It is also important to mention here, that this method has also been validated for measuring SARS-CoV-2 N antigen in serum or plasma, thus paving the way to improve prognostication of COVID-19. In fact, the serum concentration of SARS-CoV-2 N protein is significantly associated with disease severity, as mirrored by higher risk of hospitalization and longer hospital stay [10], but also display high diagnostic sensitivity in specimens with high viral load (i.e., up to 100% in those with cycle threshold value <33) [11].

Table 1: Summary of studies which explored the diagnostic accuracy of S-PLEX SARS-CoV-2 N electrochemiluminescence immunoassay for diagnosing acute SARS-CoV-2 infections.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Sample size</th>
<th>Disease prevalence</th>
<th>Sample</th>
<th>Presumptive S-PLEX cut-off</th>
<th>COVID-19 diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollock et al. [2]</td>
<td>USA</td>
<td>226</td>
<td>60.1% (95%CI, 53.5–66.6%)</td>
<td>NPS</td>
<td>≤0.32 pg/mL</td>
<td>Multiple molecular assays in NPS</td>
</tr>
<tr>
<td>Ren et al. [1]</td>
<td>Canada</td>
<td>105</td>
<td>48.0% (95%CI, 38.0–58.2%)</td>
<td>Saliva</td>
<td>≤0.32 pg/mL</td>
<td>In-house molecular assay in saliva</td>
</tr>
<tr>
<td>Wang et al. [4]</td>
<td>USA</td>
<td>200</td>
<td>50.0% (95%CI, 42.9–57.1%)</td>
<td>NPS</td>
<td>≤0.28 pg/mL</td>
<td>Panther Fusion RT-PCR in NPS</td>
</tr>
<tr>
<td>Wang et al. [5]</td>
<td>USA</td>
<td>126</td>
<td>58.2% (95%CI, 49.6–67.4%)</td>
<td>Plasma</td>
<td>≤0.32 pg/mL</td>
<td>Multiple molecular assays in NPS</td>
</tr>
</tbody>
</table>

95%CI, 95% confidence interval; IQR, interquartile range; NPS, nasopharyngeal swab; RT-PCR, real-time polymerase chain reaction.
Figure 1: Cumulative diagnostic accuracy of S-PLEX SARS-CoV-2 N electrochemiluminescence immunoassay for diagnosing acute SARS-CoV-2 infections. 95% CI, 95% confidence interval.
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References


