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Short Communication

Parallel detection of SARS-CoV-2 RNA and nucleocapsid antigen in nasopharyngeal specimens from a COVID-19 patient screening cohort

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Objectives: Reverse-transcription PCR (RT-PCR) is considered the most sensitive method for the detection of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). However, this method is relatively resource- and time-consuming. This study was performed to compare SARS-CoV-2 nucleocapsid antigen (N-Ag) testing using an enzyme-linked immunosorbent assay (ELISA) with SARS-CoV-2 RNA detection.

Methods: Parallel SARS-CoV-2 RT-PCR and quantitative N-Ag ELISA analysis was executed on nasopharyngeal specimens obtained during SARS-CoV-2 screening in a cohort of pre-hospitalization patients.

Results: In total, 277 specimens were examined, including 182 (65.7\%) RT-PCR-positive specimens, which demonstrated a median cycle threshold (Ct) value of 27 (interquartile range (IQR) 23–35). The SARS-CoV-2 N-Ag was detected in 164 of the 182 RT-PCR-positive specimens (overall sensitivity 90.1\%). Among the 95 RT-PCR-negative specimens, 72 were N-Ag-negative (specificity 75.8\%). SARS-CoV-2 RT-PCR and N-Ag ELISA results demonstrated a strong agreement (Cramer’s V = 0.668; P < 0.001). N-Ag concentrations spanned from 5.4 to 296,000 pg/ml (median 901 pg/ml, IQR 43–1407 pg/ml) and were inversely correlated with Ct values (Spearman’s r = −0.720; P < 0.001).

Conclusions: SARS-CoV-2 N-Ag ELISA results were in close agreement with RT-PCR results, and N-Ag concentrations were proportional to viral loads. Thus, SARS-CoV-2 quantitative antigen testing could be an additional diagnostic instrument for SARS-CoV-2.

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Introduction

The ongoing pandemic of coronavirus disease 2019 (COVID–19) caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) poses serious challenges for healthcare. Unprecedented demands on molecular testing have revealed a number of practical limitations of the PCR-based COVID–19 diagnostics. In this situation, additional and less demanding laboratory tools for SARS-CoV-2 detection, such as testing for viral antigens, merit consideration. A series of studies have reported the performance of numerous qualitative immunochromatographic rapid diagnostic tests (Young et al., 2020; Chaimayo et al., 2020; Lanser et al., 2020), as well as several variants of automated quantitative assays (Hirotu et al., 2021; Pollock et al., 2021). In the present study, quantitative SARS-CoV-2 nucleocapsid antigen (N-Ag) testing was performed using an enzyme-linked immunosorbent assay (ELISA) in parallel with SARS-CoV-2 RNA detection in a series of nasopharyngeal specimens obtained during COVID–19 screening.

Materials and methods

Nasopharyngeal specimens (N = 277) were obtained from individuals during routine screening for COVID–19 before planned hospitalization using the Σ-Transwab system (MWE, UK) in October–November 2020. RT-PCR was performed using the SARS-CoV-2/SARS-CoV Multiplex Real-Time PCR Detection Kit (DNA-Technology LLC, Russia) (SARS-CoV-2/SARS-CoV instruction manual, 2021) targeting a conserved E-gene site common to the group of SARS-CoV-like coronaviruses (including SARS-CoV and SARS-CoV-2) and SARS-CoV-2-specific E-gene and N-gene sites;
were PCR-positive. The distribution of N-Ag concentrations with <5 pg/ml was considered negative. A subset of specimens demonstrating an N-Ag concentration >1000 pg/ml were diluted 10- to 1000-fold to obtain a true concentration value.

**Results**

Among the 277 specimens examined, 182 (65.7%) were RT-PCR-positive (Table 1), with a median Ct value of 27 (interquartile range [IQR] 23–35). The SARS-CoV-2 N-Ag was detected in 164 of the 182 RT-PCR-positive specimens, demonstrating an overall sensitivity of 90.1%. Among the 95 RT-PCR-negative specimens, 72 were N-Ag-negative (specificity 75.8%). The Cramer’s V test demonstrated a strong agreement between SARS-CoV-2 RT-PCR and N-Ag ELISA results (V = 0.668; P < 0.001).

At Ct <20, there were no discordant results between the SARS-CoV-2 RT-PCR and N-Ag test (100% sensitivity) (Table 1). Furthermore, >90% of specimens demonstrating a Ct value in the range of 21–30 were also positive in the N-Ag ELISA assay. Higher Ct values resulted in a decreased sensitivity rate of 85.3% and 50% for the Ct range of 31–35 and >35, respectively.

In 82 (50%) RT-PCR-positive/N-Ag-positive specimens, the N-Ag concentration was above the upper detection limit of 1000 pg/ml of the ELISA assay (Figure 1A); therefore a repeated N-Ag measurement was performed in a subset of specimens from this cohort (n = 32) after dilution (Figure 1B). Thus, the true N-Ag concentration was detected in 114 RT-PCR-positive specimens, which spanned from 5.4 to 296 000 pg/ml (median 901 pg/ml, IQR 43–1407 pg/ml). N-Ag concentrations correlated inversely with Ct values (Spearman’s r = −0.720; P < 0.001) suggesting a tight correlation with the viral load.

Among 18 RT-PCR-negative specimens in which the N-Ag was detected, N-Ag concentrations varied between 5.3 and 364 pg/ml (median 11 pg/ml, IQR 6–25 pg/ml).

**Discussion**

This study demonstrated a strong agreement between SARS-CoV-2 RT-PCR and N-Ag testing results. The highest rate of discordant results was observed at Ct ≤35, indicating that the N-Ag test was highly likely to be positive in individuals with a significant viral load, who are considered as key vectors for virus spreading (Bullard et al., 2020). In specimens with Ct >35, much more discordant results with a negative N-Ag test were observed.

Several studies have demonstrated a direct correlation between viral load and SARS-CoV-2 culture positivity. According to Singanayagam et al. (2020), the probability of culturing virus is as low as 8.3% in samples with a Ct >35, while Bullard et al. (2020) did not observe SARS-CoV-2 infectivity at Ct >24. Importantly, compared to RT-PCR, antigen testing could be a better predictor of the presence of cultivable SARS-CoV-2 (Pekosz et al., 2021). Ct values are widely used as a semiquantitative indicator of the SARS-CoV-2 viral load (Singanayagam et al., 2020); thus, the close relationship between SARS-CoV-2 antigen concentrations and RT-PCR Ct values, as observed in the present study and by other authors (Pollock et al., 2021), may explain this finding. The epidemiological significance of specimens demonstrating high RT-PCR Ct values but no detectable SARS-CoV-2 antigen is not clear.

N-Ag ELISA concentrations in RT-PCR-positive specimens were distributed over a wide range (at least five orders of magnitude), which is similar to the findings of Hirotsu et al. (2021). The

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**Table 1**

Parallel detection of SARS-CoV-2 RNA and nucleocapsid antigen in nasopharyngeal specimens.

| N-Ag, SARS-CoV-2 | SARS-CoV-2 RT-PCR |
|------------------|-------------------|
|                  | Positive (by Ct range) | Negative |
|                  | Any Ct | <20 | 21–25 | 26–30 | 31–35 | >35 |
| Total (N=277)    | 182     | 26 | 62   | 42   | 34   | 18  | 95  |
| Positive, n      | 164     | 26 | 61   | 39   | 29   | 9   | 23  |
| Negative, n      | 18      | 0  | 1    | 3    | 5    | 9   | 72  |
| Sensitivity, % (95% CI) | 90.1 (85.1–93.8) | 98.4 (92.7–99.8) | 92.9 (82.1–97.9) | 85.3 (70.7–94.2) | 50 (28.4–71.6) | NA  |
| Specificity, % (95% CI) | NA     | 75.8 (66.5–83.5) |

**Note:** The sensitivity and specificity of the N-Ag test were calculated considering RT-PCR results as the reference. CI, confidence interval; Ct, cycle threshold; NA, not applicable; N-Ag, nucleocapsid antigen; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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Figure 1. Nucleocapsid antigen (N-Ag) SARS-CoV-2 concentration distribution in relation to SARS-CoV-2 RT-PCR cycle threshold (Ct) values. (A) Nasopharyngeal specimens (N = 277) were subjected to SARS-CoV-2 RT-PCR (Ct value of 38 for defining positivity) and N-Ag ELISA (assay range 5–1000 pg/ml) in parallel. All specimens with a concentration of >1000 pg/ml and <5 pg/ml are depicted on the scattergram at 1000 pg/ml and 1 pg/ml, respectively. The dashed line at 5 pg/ml indicates the N-Ag positivity threshold. (B) To obtain the true N-Ag concentration, the measurement was repeated after a 10- to 1000-fold dilution in a subset of specimens with an N-Ag concentration >1000 pg/ml (n = 32).
quantitative SARS-CoV-2 antigen measurement may be instrumental for monitoring viral clearance, demonstrating a gradual decline over a broad concentration range in contrast to abrupt fluctuations in RT-PCR positivity (Hirotu et al., 2021).

A number of RT-PCR-negative specimens in which N-Ag was detected were observed, albeit at low concentrations. Such results were unlikely to be related to the ELISA assay specificity, because it had been tested over a panel of coronavirus strains and no cross-reactivity was detected (CoviNAg instruction manual). The RT-PCR method as a relatively ‘fastidious’ technique could be more demanding in terms of specimen quality and RNA stability compared to antigen testing. Clinical as well as SARS-CoV-2 serology data could be helpful in resolving this situation, but this information was not available for the study.

In conclusion, quantitative SARS-CoV-2 N-Ag ELISA demonstrating a comparable sensitivity with RT-PCR at the epidemiologically important Ct values may be a useful additional instrument for detecting SARS-CoV-2.

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**Ethics statement**

According to national rules and regulations, ethical approval was not required.

**Conflict of interest**

Y.L. is an employee of XEMA Company. All other authors have no competing interests.

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