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SHORT COMMUNICATION

Clinical performance of Fujirebio Lumipulse G SARS-CoV-2 Ag chemiluminescent immunoassay

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SUMMARY

We evaluated the performance of Fujirebio Lumipulse G SARS-CoV-2 Ag chemiluminescent immunoassay. A nasopharyngeal swab was collected from 160 subjects and assayed simultaneously with Fujirebio Lumipulse G SARS-CoV-2 Ag and Altona Diagnostics RealStar SARS-CoV-2 RT-PCR assays. Using 0.60 pg/mL diagnostic threshold, Fujirebio Lumipulse G SARS-CoV-2 Ag displayed 0.88 area under the curve, 0.88 sensitivity and 0.75 specificity compared to molecular testing. The area under the curve increased to 1.00 after excluding samples with low viral load (i.e., cycle threshold values between 25-37). Thus, this chemiluminescent immunoassay could be used for rapid identification of many subjects with high nasopharyngeal SARS-CoV-2 viral load.

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Although molecular testing, especially reverse-transcription polymerase chain reaction (RT-PCR) assays, remains the gold standard for diagnosing coronavirus disease 2019 (COVID-19), the use of antigen immunoassays, particularly those characterized by high analytical sensibility, is becoming a valuable and almost always reliable perspective for purposes of rapid COVID-19 screening and/or diagnosis (Lippi G *et al*., 2022a). We therefore planned this study to evaluate the clinical performance of Fujirebio Lumipulse G SARS-CoV-2 Ag chemiluminescent immunoassay.

Our study population involved a cohort of subjects screened for suspected COVID-19 (for having clinical symptoms or being close contacts of COVID-19 patients) at the Service of Laboratory Medicine of Pederzoli Hospital (Peschiera del Garda, Verona, Italy), from August 16 to September 15, 2021. The local routine diagnostic practice encompasses the collection of a nasopharyngeal swab (Virus swab UTM Copan, Brescia, Italy), which is then analyzed in duplicate with SARS-CoV-2 antigen and molecular assays. SARS-CoV-2 antigen testing was performed locally with the Fujirebio Lumipulse G SARS-CoV-2 Ag, whose technical and analytical characteristics have been extensively described elsewhere (Lippi *et al*., 2022b). According to the manufacturer's specifications, the analytical sensitivity (Limit of Detection; LoD) is 0.19 pg/mL and test results are available in 30 min. For analytical purposes, we used both low and high diagnostic cut-offs (i.e., 1.0 and 50.0 pg/mL), but we also recalculated the optimal local diagnostic threshold using receiver operating characteristics (ROC) curve analysis (Analyse-it software; Analyse-it Software Ltd, Leeds, UK) on all samples, as well as on those with high viral load, i.e., displaying cycle threshold (Ct) values <25. Molecular testing was simultaneously carried out as the reference technique using the Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany), a RT-PCR assay encompassing amplification and detection of two SARS-CoV-2 (*E* and *S*) genes. The assay is routinely performed using the Bio-Rad CFX96™ Deep Well Dx Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), and test results were considered positive in this investigation when the Ct value of both genes were <37, as suggested by expert consensus (Yang *et al*., 2020). This study was performed as part of routine clinical laboratory operations for diagnosing SARS-CoV-2 at the local laboratory and thereby patient informed consent and Ethical Committee approval were not required. The study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation.

We included a total 160 subjects in our study (me-

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Table 1 - *Clinical performance of the rapid and high-throughput Fujirebio Lumipulse G SARS-CoV-2 Ag chemiluminescent immunoassay.*

Ct values	*AUC*	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Lumipulse cut-off					
<0.60 pg/mL					
- All samples	0.88 (0.83-0.92)	0.75(0.65-0.84)	1.00 (0.95-1.00)	1.00	0.76
- Ct values <25	1.00 (1.00-1.00)	1.00 (0.89-1.00)	1.00 (0.95-1.00)	1.00	1.00
<1.0 pg/mL	0.85 (0.81-0.90)	0.71 (0.60-0.80)	1.00 (0.95-1.00)	1.00	0.73
<50.0 pg/mL	0.70 (0.65-0.75)	0.39 (0.29-0.50)	1.00 (0.95-1.00)	1.00	0.57

AUC, area under the curve; Ct, cycle threshold; PPV, positive predictive value; NPV, negative predictive value.

dian age 38 years, interquartile range (IQR) 24-58 years; 43% females). The positive rate for molecular testing was 89/160 (56%). A highly significant Spearman's correlation was observed between values of Fujirebio Lumipulse G SARS-CoV-2 Ag and measurable Ct values of SARS-CoV-2 of both the *S* (r=-0.94; -0.96 to -0.91; p<0.001) and *E* (r=-0.95; 95% CI, -0.96 to -0.92; p<0.001) genes. The performance of Fujirebio Lumipulse G SARS-CoV-2 Ag is summarized in *Table 1*. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) at the 1.0 pg/mL cut-off were 0.71, 1.00, 1.00 and 0.73, respectively, with a cumulative area under the curve (AUC) of 0.85. At the higher 50.0 pg/mL cut-off, the specificity and PPV remained unvaried, but we observed a dramatic decline of sensitivity, NPV and AUC, which decreased to 0.39, 0.57 and 0.70, respectively. Using ROC analysis, we calculated a cumulative AUC of 0.88, also identifying an optimal diagnostic threshold of 0.60 pg/mL. This locally calculated cut-off was cumulatively characterized by 0.88 sensitivity, 0.75 specificity, 1.00 PPV and 0.76 NPV. More interestingly, a sub-analysis excluding those samples testing positive at molecular testing with lower viral load (i.e., Ct values between 25-37), enabled the attainment of almost perfect diagnostic performance on the 103 residual samples using the 0.60 pg/mL locally calculated threshold.

Taken together, the results of our study substantially confirm a good cumulative diagnostic performance of Fujirebio Lumipulse G SARS-CoV-2 Ag (Lippi *et al*., 2022b). Nonetheless, we also provided additional evidence in support of previous works. First, we showed that our locally-calculated cut-off (i.e., 0.60 pg/mL) displayed better performance than that earlier used in other studies (i.e., 1.0 pg/mL) (Salvetti *et al*., 2022), and definitely outperformed the higher 50.0 pg/mL diagnostic threshold, which is characterized by comparable specificity but dramatically lower sensitivity (i.e., 0.39 *vs* 0.75). In addition, we also showed that the diagnostic performance of Fujirebio Lumipulse G SARS-CoV-2 Ag in samples with high viral load (i.e., Ct value <25) is almost perfect (AUC, 1.00). This essentially means that this accurate, rapid and high-throughput chemiluminescent immunoassay could be used for timely identification of a large number of subjects with high nasopharyngeal viral load, which are those universally considered more infective and responsible for the majority of all SARS-CoV-2 contagions (Yang *et al*., 2021).

Conflicts of interest

None declared.

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