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Evaluation of three immunoassays for the rapid detection of SARS-CoV-2 antigens

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ABSTRACT

Three assays for SARS-CoV-2 antigen detection in nasopharyngeal swabs (Lumipulse® G SARS-CoV-2 Ag [LPG], STANDARD™ F COVID-19 Ag FIA [STF] and AFIAS COVID-19 Ag [AFC]) were evaluated. Compared to RT-PCR, LPG, AFC and STF showed a variable sensitivity (87.9%, 37.5%, and 35.7%, respectively) and an overall high specificity (> 95%).

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread worldwide. Rapid and accurate diagnosis of infected patients is essential for their management and for control of viral spread (Karimi et al., 2020; Li et al., 2020).

To-date, the reference method for diagnosing SARS-CoV-2 infection is based on detection of viral RNA in clinical specimens by nucleic acid amplification technologies, and numerous commercial kits are now available (Van Kasteren, 2020). Those tests, however, require expensive equipment, may take up to several hours, and are relatively expensive.

In this scenario, rapid diagnostic assays for the detection of SARS-CoV-2 antigens in nasopharyngeal (NP) swabs or saliva have been developed as an interesting alternative to current molecular techniques (Liotti et al., 2020; Porte et al., 2020). Among the assays for SARS-CoV-2 antigen detection, some are based on visual reading of lateral flow immunochromatography strips by the operator, while others require instrumental reading. The latter approach facilitates interpretation of results by returning a numerical value rather than a subjective interpretation, although these may require more technical expertise and steps.

In this study we evaluated the performance of three SARS-CoV-2 antigen assays based on instrumental reading, including a chemiluminescent enzyme immunoassay (CLEIA), namely Lumipulse® G SARS-CoV-2 Ag (LPG) (Fujirebio, Tokio, Japan) and two lateral flow fluorescence immunoassays (LIA), namely STANDARD™ F COVID-19 Ag FIA (STF) (SD Biosensor; Suwon-si, South Korea), and AFIAS COVID-19 Ag (AFC) (Menarini; Florence, Italy). All three assays are validated for in vitro diagnostic use. The LPG assay requires a machine (Lumipulse 1200® G system) in which the nasopharyngeal swab eluate, in UTM medium, is directly loaded in random access, after centrifugation at 5000 g for 5 minutes. Results are quantitatively reported in pg/ml in 30 minutes, and the machine can process up to 120 specimens per hour. According to the manufacturer, positivity is considered for an antigen concentration ≥ 1.34 pg/ml, but a grey zone is considered for an antigen concentration between 1 and 10 pg/ml. The STF and AFC assays consist of lateral flow cartridges where the specimens are manually loaded. Results are read after 20 or 12...
Performance of Lumipulse® G SARS-CoV-2 Ag, AFIAS COVID 19 Ag and STANDARD™ F COVID-19 Ag FIA on UTM swabs.

| Total sample | Sensitivity (%) | 95% CI-Sensitivity | Specificity (%) | 95% CI-Specificity | PPV (%) | 95% CI-PPV | NPV (%) | 95% CI-NPV |
|--------------|----------------|---------------------|-----------------|-------------------|---------|------------|---------|------------|
| Lumipulse® G SARS-CoV-2 Ag | 201 | 29/33 (87.9) | 73.7-95.8 | 161/168 (95.8) | 92-98.1 | 80.5 | 65.6-90.9 | 97 | 93.7-98.8 |
| AFIAS COVID 19 Ag* | 81 | 9/24 (37.5) | 20.4-57.4 | 57/57 (100) | 95.7-100 | 100 | 76.2-100 | 79.2 | 68.8-87.3 |
| STANDARD™ F COVID-19 Ag FIA | 93 | 10/28 (35.7) | 20.1-54.2 | 65/65 (100) | 96.2-100 | 100 | 78.3-100 | 78.3 | 68.8-86.1 |

*42 invalid samples were excluded from the analysis.

minutes respectively, with the help of a small portable fluorescence reader. These assays can be run in the diagnostic laboratory but are also amenable to point-of-care testing. For analysis with AFC and STF, the swab eluate in UTM medium (450 µL and 350 µL respectively) was diluted in the extraction buffer provided by the manufacturer and processed with the AFIAS-6 (this procedure is not yet CE-IVD validated) or with the STANDARD F-2400 Analyzer instrument respectively. With both tests the result was interpreted as positive when the cut-off Index (COI) was ≥ 1 and reported qualitatively as detected or not detected.

NP swabs, collected in UTM medium (3 ml, COPAN; Brescia, Italy) were anonymized residual routine diagnostic samples from inpatients in three Tuscan hospitals (Careggi University Hospital and Santa Maria Annunziata Hospital in Florence, and San Giuseppe Hospital in Prato, Italy). All samples were also tested with the Allplex™ SARS-CoV-2 Assay (Seegene; Seoul, Korea). Samples were considered negative when the Ct values were ≥ 40 for all targets.

Percentages with their 95% confidence intervals (CI) were calculated using the “Jeffreys” method (http://www.ausvet.com.au).

A total of 201 NP swabs in UTM were processed with LPG, including 33 from SARS-CoV-2 positive and 168 from SARS-CoV-2 negative patients, as confirmed by molecular testing carried out on the same sample. Results showed a sensitivity of 87.9% (29/33; CI 73.7-95.8) and a specificity of 95.8% (161/168; CI 92-98.1).

A subset of 93 of these NP (including 28 from SARS-CoV-2 positive and 65 from SARS-CoV-2 negative patients) were also analyzed with the AFC and STF systems. With AFC, 12 samples tested invalid and the final number of analyzed samples was therefore 81 (including 24 and 57 SARS-CoV-2 positive and negative, respectively). The STF system yielded no invalid results. The overall sensitivity rates of AFC and STF assays were 37.5% (9/24; CI 20.4-57.4) and 35.7% (10/28; CI 20.1-54.2), respectively, with a 100% specificity in both cases (Table 1).

Positive predictive values (PPV) were 80.5% (CI 65.6-90.9) for LPG and 100% (CI 76.2-100; 78.3-100) for both AFC and STF, while negative predictive values (NPV) were 97% (CI 93.7-98.8), 79.2% (CI 68.8-87.3) and 78.3% (CI 68.8-86.1), for LPG, AFC and STF, respectively (Table 1).

Considering the correlation of antigen detection with estimated viral load measured by Ct positivity for the N gene (used as reference since no significant differences were detected with the Ct values for other genes), the sensitivity was 100% with all systems for samples showing a Ct value ≤ 25. AFC and STF called positive only 4 of 9 and 5 of 9 samples with a Ct value > 25 and ≤ 30, respectively, and none of the samples with a Ct value > 30. On the contrary, LPG called positive almost all samples with a Ct value ≤ 35 (25/27), and 3 of 5 samples with a Ct value > 35 (Fig. 1; Supplementary Fig. 1).

The spread of COVID-19 pandemic is seriously threatening the public health system, and control measures are urgently needed. In this scenario, even though the “gold standard” for the diagnosis of SARS-CoV-2 infection remains viral RNA detection by molecular assays, antigen detection tests could be a valid alternative due to their rapidity, reduced hands-on time, no need for advanced expertise on molecular biology and, with some systems, the possibility of use also as a point of care test (Candel, 2020).

AFC and STF showed an equally high sensitivity (100%) for samples with a high viral load (Ct ≤ 25), but a decreased sensitivity for samples with lower viral loads, as previously reported (Liotti et al., 2020). On the other hand, LPG showed an overall high sensitivity rate (87.8%), being able to detect also several samples with low viral load, in accordance to previous studies (Aoki et al., 2020; Hirotsu et al., 2020). As a trade-off, specificity of LPG was 95.8%. However, most false positive samples showed an antigen concentration between 1.34 and 10 pg/ml. Moreover, according to previous studies, samples positivity between 1 and 100 pg/ml for UTM swabs might be reconfirmed by RT-PCR in order to increase specificity avoiding false positive results (Ogawa T et al., 2020). However, this could lead to a considerable reduction in the sensitivity rate, further studies will be necessary to better set up the positivity cutoff.

In conclusion, AFC and STF are designed to work with simple rapid bench tools and may be easily applied even for point of-care testing, for instance at airports, prisons and schools (WHO, 2020). On the contrary LPG, which showed a higher sensitivity rate, could be a valid implementation in the laboratory diagnostic routine to reduce costs and turnaround time.

A limitation of this study is represented by the relatively low number of positive patients tested. Further testing with a higher number of samples will allow to expand current knowledge on the potential of these assays.

Fig. 1. Number of positive isolates detected by Lumipulse® G SARS-CoV-2 Ag, AFIAS COVID 19 Ag and STANDARD™ F COVID-19 Ag FIA correlated to the N gene Ct value by RT-PCR. Samples with Ct ≥ 40 have been considered as negative.
Author statements

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Supplementary materials

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