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Clinitest rapid COVID-19 antigen test for the diagnosis of SARS-CoV-2 infection: A multicenter evaluation study

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ABSTRACT

Objectives: RT-PCR assay is the reference method for diagnosis of COVID-19, but it is also a laborious and time-consuming technic, limiting the availability of testing. Rapid antigen-detection tests are faster and less expensive; however, the reliability of these tests must be validated before they can be used widely. The objective of this study was to determine the performance of the Clinitest Rapid COVID-19 Antigen Test (ClinitestRT) (SIEMENS) for SARS-CoV-2 in nasopharyngeal swab specimens.

Methods: This prospective multicenter study was carried out in three Spanish university hospitals including individuals with clinical symptoms or epidemiological criteria for COVID-19. Only individuals with ≤7 days from the onset of symptoms or from exposure to a confirmed case of COVID-19 were included. Two nasopharyngeal samples were taken to perform the ClinitestRT, as a point-of-care test, and a diagnostic RT-PCR test.

Results: Overall sensitivity and specificity for the ClinitestRT among the 450 patients studied were 93.3% (CI 95%: 89.7–96.8) and 99.2% (CI 95%: 97.2–99.8), respectively. Sensitivity in participants with ≤5 days of the clinical course was 93.6% (CI 95%: 89.2–96.3), and in participants who had a C_{T}<25 for the RT-PCR test was 98.4% (CI 95%: 94.5–99.6). Agreement between techniques was 96.7% (kappa score: 0.93; CI 95%: 0.90–0.97).

Conclusions: The ClinitestRT provides good clinical performance, with more reliable results for patients with a higher viral load. The results must be interpreted based on the local epidemiological context.

1. Introduction

There are already multiple CE-marked commercial rapid antigen-detection tests (RADT) for the diagnosis of SARS-CoV-2 infection [1]. RADT detect SARS-CoV-2 proteins produced by replicating viruses in respiratory secretions [2], and they are compatible with both laboratory and near-patient use. RADT allow the generalization of decentralized diagnostic tests for SARS-CoV-2 infection, which are one of the key points for the control of the COVID-19 pandemic. However, independent evaluation studies comparing RADT to the RT-PCR, using different swabs and strictly following manufacturers’ instructions, are frequently lacking [1].

Clinitest Rapid COVID-19 Antigen Test (ClinitestRT) (Siemens, Healthineers, Erlangen, Germany) is a rapid in vitro test for the qualitative detection of the SARS-CoV-2 nucleocapsid protein in nasopharyngeal or nasal swabs to diagnose COVID-19 within the first week after symptoms onset.

ClinitestRT is a lateral-flow-format test that uses
immunochromatography with colloidal gold, and it is designed to be performed at the patient care site by trained healthcare personnel, with results in 15 min.

The objective of this study is to determine the performance of this test, with CE marking, in nasopharyngeal swabs.

2. Materials and methods

2.1. Hospitals and individuals participating

Between November 2020 and January 2021, we carried out an independent, prospective multicenter diagnostic evaluation study across three independent university hospitals in two Spanish autonomous communities (Madrid and Galicia). The hospitals participating in the study were Hospital Clínico Universitario San Carlos and Hospital Universitario La Paz from Madrid, and Complejo Hospitalario Universitario de A Coruña from Galicia.

Individuals with clinical symptoms or epidemiological criteria (asymptomatic close contacts) for COVID-19 in whom a diagnostic RT-PCR test was indicated were offered to participate in this study. All participants were reported as part of the study and verbal informed consent was obtained prior to their inclusion. The ClinitestRT result did not influence the clinical management of the patients, who were informed and treated only based on the RT-PCR result. The symptoms, number of days since the onset of symptoms or exposure, threshold cycle (C_T) values for PCR, and demographic data were collected for all participants. Participant’s data were coded and no samples were stored after the ClinitestRT was performed. Only individuals with ≤7 days from the onset of symptoms or from exposure to a confirmed case of COVID-19 were included, according to recommendations of the WHO [2]. The study was presented to the Research Ethics Committee, which responded favorably.

2.2. SARS-CoV-2 testing

Two nasopharyngeal samples were taken per patient, one in each choana. One of them was taken with the swab provided by ClinitestRT, and the other one with a suitable swab for taking a virus sample including a universal transport medium for RT-PCR (Copan flocked swabs with UTM™, Universal Transport Medium). ClinitestRT was performed immediately, under point-of-care conditions (regulations of quality systems ISO 15,189) [3,4], and according to the manufacturer’s instructions (lot number 2011072) by physicians and nurses from emergency and primary care services trained by microbiology specialists. The second swab was used for a molecular diagnostic (RT-PCR) by each hospital according to its standard procedures for COVID-19 diagnosis. The commercial RT-PCR methods used for the patients hospitals in this study were AlplexTM-2019-nCoV Assay (Seegene, Seoul, South Korea), GENOMICA S.A.U. (Madrid, Spain), TaqPath COVID-19 Combo Kit (Thermo Fisher, Waltham, MA, USA), GeneXpert (Cepheid, Sunnyvale, CA, USA), and Cobas 6800 (Roche, Indianapolis, USA) Due to the high diagnostic demand, in some hospitals different PCR techniques were used.

2.3. Statistical analyses

Specificity and sensitivity, with 95% confidence intervals (CI), of ClinitestRT were calculated using the RT-PCR results as the standard; or which is the same as proportion of negative and positive agreement, respectively. Sensitivity was calculated for all patients and for specific groups of patients according to the time of onset of symptoms or exposure, RT-PCR C_T values and symptoms. The level of agreement between the tests was evaluated using Cohen’s kappa score [5]. Statistical analyses were performed using GraphPad Prism software v.7.02 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

A total of 450 individuals who had at least one symptom compatible with COVID-19 (n = 410) or had been in close contact with a diagnosed COVID-19 patient (n = 40) were included in this study (Table 1). There were between 124 and 200 individuals from each participating hospital, with a median age of 65 years (interquartile range: 44); 58.4% were women (Table 1). There were not cases in pediatric patients (<14 years old).

RT-PCR was positive in 192 (42.7%) and negative in 258 (57.3%); while ClinitestRT was positive in 181 (40.2%) and negative in 269 (59.8%) (Table 2) The agreement between both methods was 96.7% (kappa score: 0.93; CI 95%: 0.90–0.97). In 15 patients the results differed between the two tests, 13 of them were false negatives results with ClinitestRT (2.9% of total cases) (Table 2). Twelve (92.3%) false negatives had C_T ≥ 25 values for RT-PCR.

Based on these data, the overall sensitivity and specificity of the ClinitestRT were 93.3% (CI 95%: 89.7–96.8) and 99.2% (CI 95%: 97.2–99.8), respectively (Table 3). Sensitivity was higher in patients who had a C_T < 25 for the RT-PCR (98.4%; CI 95%: 94.5–99.6) (Table 3), than in those with C_T ≥ 25 (80%; CI 95%: 69.8–91.1). Sensitivity was also higher in patients with ≤5 days of the clinical course of the disease (93.6%; CI 95%: 89.2–96.3) (Table 3) than in those with 6–7 days of clinical evolution (66.7%; CI 95%: 13.3–120); although only three positive cases were included in this last group.

Among the 40 asymptomatic participants, 23 (92%) of the 25 positive by RT-PCR were also positive by ClinitestRT, and there was full agreement in the 15 that were negative.

The negative predictive value (NPV) and positive predictive value (PPV) in the study cohort, were 95.2% and 98.9%, respectively (Table 3).

4. Discussion

This study showed very good clinical performance values of the ClinitestRT, with 93.3% sensitivity and 99.2% specificity; moreover, sensitivity was even improved in samples with C_T < 25 (98.4%) which, is probably closer to the limit of infectivity, as previously reported [6].

The strengths of this study include the large study size and the prospective nature of the study, the inclusion of multiple centers, and its use according to the manufacturer’s instructions under point-of-care conditions. A limitation of the study may be the use of different RT-PCR protocols because the C_T values can vary slightly between techniques; however, all of them are used for routine diagnosis in participating hospitals, and all of them are validated and widely used worldwide.

The gap between the number of diagnostic needs and the testing capacity are still evident in epidemic waves [7]. RADT are simple to perform and interpret by minimally trained health workers at the point-of-care, they do not require specific equipment, they are less expensive than RT-PCR, and they improve the turnaround time for results [8,9]. Although during the first wave of the pandemic, RADT were

Table 1

| Days from symptom onset or from exposure [mean (N)] | Days ≤ 5 [n/N (%)] | Days 6–7 [n/N (%)] |
|---------------------------------------------------|--------------------|--------------------|
| 2.9 (450)                                         | 431/450 (95.8%)    | 19/450 (4.2%)      |

| PCR C_T (n) | C_T ≥ 25 [n, (%)] | C_T < 25 [n, (%)] |
|-------------|------------------|------------------|
| (178)       | [50, (28.1%)]    | [128, (71.9%)]   |

(continued)
Table 2
Summary of the results of the Clinitest Rapid COVID-19 Antigen Test compared to RT-PCR.

|                | RT-PCR | Positive | Negative | TOTAL |
|----------------|--------|----------|----------|-------|
| Positive       |        | 179      | 13       | 192   |
| Negative       |        | 2        | 256      | 258   |
| TOTAL          |        | 181      | 269      | 450   |

Table 3
Estimation of clinical performance of the Clinitest Rapid COVID-19 Antigen Test compared to RT-PCR.

|                              | Clinical sensitivity (95% CI) | Sensitivity days ≤ 7 (95%) | Sensitivity days > 7 (95%) | Specificity (95%) | Agreement (kappa index; 95%) | Positive predictive value (95%) | Negative predictive value (95%) |
|------------------------------|------------------------------|----------------------------|---------------------------|------------------|-----------------------------|-------------------------------|--------------------------------|
| Relative Sensitivity (95%)   | 93.3% (89.7-96.8)            | 93.6% (89.2-96.3)          | 98.4% (94.5-99.8)         | 99.2% (97.2-99.8) | 96.7% (0.93-0.9-0.97)       | 98.9% (96.1-99.7)             | 95.2% (91.9-97.2)               |

not recommended due to poor reliability [10,11], recent studies have shown a significant improvement in the performance of newly developed RADTs [6,12–15]. In a recent Spanish study with ClinitestRT, including 178 symptomatic patients with suspected COVID-19 and 92 asymptomatic close contacts [15], the number of participants with symptoms saturation at the peaks of epidemic waves. However, the results of this group. In general, previous studies have shown a lower sensitivity of RADTs in asymptomatic close contacts than in patients with symptoms [16,17]. This is consistent with the advice from the WHO against using RADT for screening asymptomatic individuals in populations with low COVID-19 prevalence [2] due to the potential increase of higher incidence of false positives.

The performance of an RADT may depend on the epidemiological situation of the population being tested; therefore, how the test is used and how the results are interpreted will depend on local epidemiological factors [2]. As with all diagnostic tests, to correctly interpret and act on the results of the RADT, the prevalence of disease must be previously estimated, since this determines the PPV and NPV values. In an epidemiological setting of high prevalence, the pre-test probability of COVID-19 disease is relatively high, and positive test results have a high predictive value. On the contrary, if the NPV is low, it should advise patients with respiratory symptoms to exercise infection control practices, and consider repeat RADT if symptoms persist or progress [2].

In conclusion, this study showed that the ClinitestRT provides very good clinical performance as a point-of-care test. The use of RADT can have an undoubted impact on diagnostic strategies for COVID-19, helping to prevent an overload on health care services and laboratory saturations at the peaks of epidemic waves. However, the results of RADT performance must be interpreted based on the local epidemiological context.

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CRediT authorship contribution statement

Paloma Merino-Amador: Data curation, Writing – original draft, Investigation, Writing – review & editing. Patricia González-Donapetry: Investigation, Data curation. Mercedes Domínguez-Fernández: Investigation, Data curation. Fernando González-Romo: Investigation, Data curation. Miguel Ángel Sánchez-Castellano: Investigation, Data curation. Alejandro Seoane-Estevéz: Investigation, Data curation. Alberto Delgado-Iribarren: Investigation. Julio García: Investigation. German Bou: Investigation. Manuel Cuenca-Estrella: Conceptualization, Supervision, Investigation. Jesús Oteo-Iglesias: Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

Authors have none to declare.

References

[1] Foundation for Innovative New Diagnostics. SARS-CoV-2 diagnostic pipeline 2020. Available from: https://www.finnovdx.org/covid-19-pipeline/ (date last accessed, April 2021).
[2] World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunodiagnostic assays. Interim Guid., September 11th, 2020. Available from: https://apps.who.int/iris/handle/10665/331509.
[3] Rodríguez Domínguez M., Franco Álvarez de Luna F., Goyanes Galán M.J., García Rodriguez J. Diagnostico microbiológico en el lugar de asistencia al paciente. Procedimientos En Microbiología Clinica. Sociedad Española De Enfermedades Infecciosas y Microbiología Clinica (SEIMC). 2019. Available from: https://seimc.org/contenidos/documentoscientificos/procedimientosmicrobiologia/seimc-procedimiento microbiologia06.pdf.
[4] T. Nguyen, Duong Bang D. A, Wolf, Novel coronavirus disease (COVID-19): paving the road for rapid detection and point-of-care diagnostics, Micromachines (Basel) 2020 11 (2019) 306, https://doi.org/10.3390/mi11030306.
[5] M.L. McHugh, Interntaler reliability: the kappa statistic. Biochem. Med. (Zagreb) 22 (2012) 276–292.
[6] World Health Organization, Laboratory testing strategy recommendations for COVID-19. Interim Guid., March 21st, 2020. Available from: https://apps.who.int/iris/handle/10665/331509.
[7] S. Lambert-Nicolot, A. Caffel, S. Le Pape, C. Vauloup-Fellous, L. Morand-Joubert, A. M. Roque-Afonso, et al., Evaluation of a rapid diagnostic assay for detection of SARS-CoV-2 antigen in nasopharyngeal swabs, J. Clin. Microbiol. 58 (2020), https://doi.org/10.1128/JCM.00977-20 e00977-20.
[8] S.K. Vashist, In vitro diagnostic assays for COVID-19: recent advances and emerging trends, Diagnostics 10 (2020) 202, https://doi.org/10.3390/diagnostics10040202.
[9] Vanderberg, D. Martiny, O. Rochas, A. van Belkum, Z. Koulakis, Considerations for diagnostic COVID-19 tests, Nat. Rev. Microbiol. 19 (2020) 171–183, https://doi.org/10.1038/s41579-020-00461-z.
[10] A. Scohy, A. Anantharajah, M. Bodeux, B. Kabamba-Mukadi, A. Verroken, H. Rodriguez-Villalobos, Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis, J. Clin. Virol. 129 (2020), 104455, https://doi.org/10.1016/j.jcv.2020.1044555.
[11] World Health Organization, Advice on the use of point-of-care immunodiagnostic tests for COVID-19, (n.d.). https://www.who.int/news-room/commentaries/detail/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-covid-19, (Accessed 19 August 2020).
[12] J. Dínnes, J.J. Deeks, A. Adriano, S. Berhanie, C. Davenport, S. Dittrich, D. Emperador, Y. Yakwojini, J. Cunningham, S. Bese, J. Dretze, L. Ferrante di Ruffano, I.M. Harris, M.J. Price, S. Taylor-Phillips, L. Hooft, M.M. Leeflang, R. Spijker, A. Van den Brule, Cochrane COVID-19 diagnostic test accuracy group, Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection, Cochrane Database Syst. Rev. (B) (2020), CD013705, https://doi.org/10.1002/14651858.CD013705.
[13] Young S., Taylor S.N., Cammarata C.L., Varnado K.G., Roger-Dalbert C., Montano A.et al. Clinical evaluation of BD Veritor SARS-CoV-2 point-of-care test performance compared to PCR-based testing and versus the Sofía 2 SARS SARS-CoV Antigen point-of-care test. J. Clin. Microbiol. 2020. 10.1128/JCM.02338-20.
[14] Merino P., Guinea J., Muñoz-Gallego L., González-Donapetry P., Galán J.C., Antona N., Cilla G., Hernández-Gre spo S., Díaz-de Tuesta J.L., Guadí-de Torellla A., González-Romo F., Escriche P., Sánchez-Castellano M.A., Sota-Busse M., Delgado-
Iribarren A., García J., Cantón R., Muñoz P., Folgueira M.D., Cuenca-Estrella M., Otero-Iglesias J.; Spanish Panbio™ COVID-19 validation group. Multicenter evaluation of the Panbio™ COVID-19 rapid antigen-detection test for the diagnosis of SARS-CoV-2 infection. Clin. Microbiol. Infect. 2021 Feb 16;S1198-743X(21)00076-8. doi: 10.1016/j.cmi.2021.02.001. Epub ahead of print. PMID: 33601009; PMCID: PMC7884234.

Torres I., Poujois S., Albert E., Álvarez G., Colomina J., Navarro D. Point-of-care evaluation of a rapid antigen test (CLINITEST® Rapid COVID-19 Antigen Test) for diagnosis of SARS-CoV-2 infection in symptomatic and asymptomatic individuals. J. Infect. 2021 Feb 12;S0163-4453(21)00075-X. doi: 10.1016/j.jinf.2021.02.010. Epub ahead of print. PMID: 33587922; PMCID: PMC7879056.

European centre for disease prevention and control. Options for the use of rapid antigen tests for COVID-19 in the EU/EEA and the UK. Technical Report 19, November 19th, 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Options-use-of-rapid-antigen-tests-for-COVID-19.pdf.

J. Dinnes, J.J. Deeks, S. Berhane, M. Taylor, A. Adriano, C. Davenport, S. Dittrich, D. Emperador, Y. Takwoingi, J. Cunningham, S. Beese, J. Domen, J. Dretzke, L. Ferrante di Ruffano, I.M. Harris, M.J. Price, S. Taylor-Phillips, L. Hooft, M. Leeflang, M.D. McInnes, R. Spijker, A. Van den Bruel. Cochrane COVID-19 diagnostic test accuracy group. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection, Cochrane Database Syst. Rev 3 (2021), CD013705, https://doi.org/10.1002/14651858.CD013705.pub2. Mar 24PMID: 33760236.