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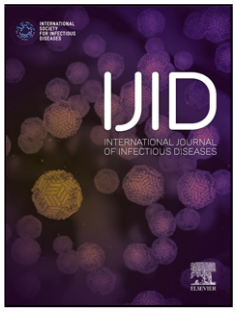

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Highlights

- Due to the rapidly emerging SARS-CoV-2 pandemic and its tremendous public health challenges worldwide, there is a critical demand for rapid and easy to perform diagnostic assays
- The evaluated rapid antigen detection test had a high diagnostic sensitivity and specificity in respiratory samples obtained from patients who mainly presented during the first week of Covid-19
- Rapid antigen detection has the potential to become an important tool for the early diagnosis of SARS-CoV-2, particularly in situations with limited access to molecular methods

Evaluation of novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples

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Abstract

Objectives. In the context of the Covid-19 pandemic, the development and validation of rapid and easy-to-perform diagnostic methods are of high priority. We evaluated a novel rapid antigen detection test (RDT) for SARS-CoV-2 in respiratory samples.

Methods. The fluorescence immunochromatographic SARS-CoV-2 antigen test (Bioeasy Biotechnology Co., Shenzhen, China) was evaluated using universal transport medium with nasopharyngeal (NP) and oropharyngeal (OP) swabs from suspected Covid-19 cases. Diagnostic accuracy was determined in comparison to SARS-CoV-2 real time (RT)-PCR.

Results. A total of 127 samples were included; 82 were RT-PCR positive. Median patients' age was 38 years, 53.5% were male, and 93.7% were from the first week after symptom onset. Overall sensitivity and specificity were 93.9% (CI95% 86.5–97.4) and 100% (CI95% 92.1–100), respectively, with a diagnostic accuracy of 96.1% and Kappa coefficient of 0.9. Sensitivity was significantly higher in samples with high viral loads.

Conclusions. The evaluated RDT showed a high sensitivity and specificity in samples mainly obtained during the first week of symptoms and with high viral loads, despite the use of a non-validated sample material. The assay has the potential to become an important tool for early diagnosis of SARS-CoV-2, particularly in situations with limited access to molecular methods.

Key words: Coronavirus; SARS-CoV-2; Covid-19; diagnosis; rapid diagnostic test; antigen

Introduction

Since its first occurrence in December 2019, the rapidly emerging SARS-CoV-2 pandemic is causing tremendous public health challenges worldwide (WHO 2020a). Timely detection and isolation of cases and their contacts are considered crucial to help curtail this unprecedented pandemic (Nguyen et al., 2020). This strategy relies on robust, rapid, and easy-to-perform diagnostic tools that can be used to test large numbers of samples in a short period of time. To date, the recommended diagnostic method for SARS-CoV-2 infection (known as Covid-19) is real-time reverse-transcription polymerase chain reaction (RT-PCR), which was introduced in January 2020 (Corman et al., 2020), and is now applied using WHO or CDC protocols (WHO 2020b; CDC 2020b) as well as various commercial assays (FIND 2020).

The enormous gap between the large number of patients/contacts and the laboratory capacities to perform RT-PCR in a timely manner is a mayor limitation of current public health containment strategies (WHO 2020c). Therefore, there is a critical demand for alternative assays such as antigen detection tests, which, in contrast to antibody tests, can detect the presence of the virus itself in respiratory samples (WHO 2020c). Tests detecting SARS-CoV-2-specific antigen have recently been developed and many of them are now commercially available (FIND 2020). However, the real-world performance of these assays is uncertain and their validation is therefore of high priority (ECDC 2020). Other options include serological tests, but due to their diagnostic limitations in early infections, these tests are currently not recommended for case detection (WHO 2020c; ECDC 2020). Among possible test formats, rapid diagnostic tests (RDTs) should be prioritized, since they are timely, easy to perform, and can serve as point-of-care testing (POCT) (Patel et al., 2020). Here we present the evaluation of a novel antigen-based RDT for the detection of SARS-CoV-2 in respiratory specimens from suspected Covid-19 cases.

Material and Methods

We conducted a study of the diagnostic accuracy of a rapid SARS-CoV-2 antigen detection test compared to RT-PCR. Samples derived from patients with respiratory symptoms and/or fever and an epidemiological risk factor for SARS-CoV-2 infection (travel or contact with case), attending Clínica Alemana, a private medical centre in Santiago, Chile (Weitzel et al., 2020), during the first weeks of the outbreak in Chile. Specimens were obtained by trained personnel in a newly created "Respiratory Emergency Room" at our hospital and consisted of a nasopharyngeal (NP) and an oropharyngeal swab (OP), which were placed together in a 3 mL tube of universal transport medium (UTM-RT® System, Copan Diagnostics, Murrieta, CA, USA). Samples were initially examined for SARS-CoV-2 by COVID-19 Genesig® Real-Time PCR assay (Primerdesign Ltd., Chander´s Ford, UK) after RNA extraction with the Magna Pure Compact system (Roche Molecular Systems Inc., Pleasanton, Ca, USA). The Primerdesign RT-PCR was the first European SARS-CoV-2 assay, which was commercialized; it received FDA Emergency Use Authorization (EUA) and is among the WHO Emergency Use Listing (EUL) tests eligible for procurement (https://www.who.int/diagnostics_laboratory/eual/listing/en). The assay includes a positive control template and a RNA internal extraction control. Its target gene is the RNA-dependent RNA polymerase (RdRp); the detection limit reported by the manufacturer is 0.58 copies/µL. Samples showing an exponential growth curve and a Ct value ≤40 were considered as positive. PCR characterized samples (UTM with swabs) were kept at 4°C and tested within 48 hours by the "Diagnostic Kit for 2019-Novel Coronavirus (2019-nCoV) Ag Test (Fluorescence Immunochromatographic Assay)" (Bioeasy Biotechnology Co., Shenzhen, China; Cat. N° YRLF04401025, lot N° 2002N408), detecting SARS-CoV-2

nucleocapsid protein by lateral flow technique. The test uses Europium-labelled chicken anti-SARS-CoV-2 IgY antibodies for primary binding and mouse anti-SARS-CoV-2 antibodies for capture; goat anti-chicken IgY antibodies are used for the internal control (Diao et al., 2020).

The manufacturer's instruction for use (IFU) recommends direct testing from OP or NP swabs as well as sputum. Our approach using UTM was chosen since it permitted the rapid evaluation of a large number of previously RT-PCR characterized clinical samples. For this procedure, the manufacturer permitted the application of 100 µL of UTM directly into the cassette (Peter Zhong, personal communication).

Positive and negative samples were selected by convenience among the 1,453 respiratory specimens processed for SARS-CoV-2 in the clinical lab during the study period (March 16-21, 2020). Due to the shortage of available test kits, a 2:1 distribution of positive to negative samples was chosen. The technician performing the RDT was blinded to the RT-PCR results.

UTM tubes were first vortexed for 20 seconds; then, 100 µL of the UTM solution were placed into the sample port of the cassette, incubated at room temperature for 10 minutes, and placed into the fluorescence immunoassay analyser EASY-11 (Bioeasy Biotechnology Co.) The instrument automatically delivers a positive or negative qualitative result with a detection limit of 0.005 ng/mL, according to the manufacturer. All test procedures except the reading of the cassette were performed under a BSL2 cabinet. Results of the RDT were compared to those of RT-PCR as reference method; for samples with discordant result, tests were repeated. The demographic and clinical data were obtained from the mandatory national Covid-19 notification forms and were analysed in an anonymized manner. Statistical analysis considered the calculation of sensitivity, specificity, diagnostic accuracy, and Kappa coefficient using standard formulas, and Wilson score Confidence Interval at 95% (OpenEpi version 3.01). Test

performance was analysed as recommended by current CLSI guidelines (CLSI 2008). Sensitivity was further analysed for certain subgroups such as gender, days of symptoms at sampling, and RT-PCR Ct values.

Test kits used for this evaluation were bought from the local distributor using funds for routine diagnostics of the Clinical Laboratory of Clínica Alemana. The study was approved by the local Institutional Review Board (Comité Etico Científico, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile) and need for informed consent was waived.

Results

A total of 127 samples were included. Of those, 82 were RT-PCR positive for SARS-CoV-2 RNA, representing 61% of total positive samples during the study period, and 45 samples were RT-PCR negative. Among tested cases, 53.5% were male and the median age was 38 years. Most samples were taken during the initial phase of the disease with a median duration of symptoms of 2 days (IQR 1–4) (Table 1). The median cycle threshold (Ct) value of positive RT-PCR samples was 17.7 (IQR 14.2–25.1) (Table 1).

The overall sensitivity and specificity of the evaluated RDT were 93.9% (CI95% 86.5–97.4) and 100% (CI95% 92.1–100), respectively (Table 2). The diagnostic accuracy was 96.1% with a Kappa coefficient of 0.9. Sensitivity was significantly reduced in the subgroup of samples with Ct values >25.1, which represented the 4th quartile of Ct values in our population, indicating lower viral loads. No significant difference within other subgroups (Table 2) was identified. All false negative results (n=5) corresponded to samples with PT-PCR Ct values >26 (Table 3). Ct values of true positives and false negatives and their relation to the duration of symptoms are shown in Fig. 1. A subgroup analysis of Ct values revealed that samples of female patients had

163 higher Ct values and a steeper positive trend line slope over time of infection compared to male
164 patients (Fig. 2).
165
166 **Discussion**
167 The novel SARS-CoV-2 antigen test kit from Bioeasy is among the growing number of
168 diagnostic assays available for Covid-19 with CE marking (FIND 2020), which is based on self-
169 reporting of the manufacturers and can be misused. The challenge of this procedure in light of
170 the rapidly evolving Covid-19 pandemic has recently been addressed by the European
171 Commission (European Commission 2020).
172 The test has a cassette format with an external reader and is approved to be used with
173 oropharyngeal swabs, nasopharyngeal swabs, and sputum. In our experience, the system was
174 easy to use and gave a qualitative result for an individual sample in approximately 15 minutes.
175 Depending on the reading mode, the analyser permits a throughput of approximately 5 (standard
176 mode with incubation within the device) to >50 (rapid mode with incubation outside the device)
177 samples per hour. This significant throughput is encouraging given the large number of samples
178 processed in many Covid-19 testing points and the potential use of RDTs as a large scale
179 decentralized screening tool, e.g. in resource poor settings. However, the inherent biological
180 hazard requires the handling of specimens in a biosafety cabinet (WHO 2020d), hence slowing
181 down the process and reducing the sample number per hour. This problem could be overcome by
182 the use of extraction buffers or solutions with inactivating capacities.
183 Within our panel of clinical samples, the novel assay proved to be highly sensitive and specific.
184 Interestingly, a similarly high sensitivity (94%) was reported for the detection of nucleocapsid
185 antigen in early infections with SARS-CoV in a study from 2004 (Che et al., 2004). The

sensitivity in our study (93.9%) was higher than reported by the manufacturer in the package insert for nasopharyngeal swabs (85.5%) and more than three times higher than the accuracy values reported in the grey literature for a related test with visual read-out. A preprint report from China with participation of the manufacturer found an overall sensitivity of 68% in 208 RT-PCR positive nasopharyngeal swabs from patients from the Hubei province in China (Diao et al., 2020). However, when analysing the subgroup of samples with Ct values ≤30, the sensitivity of the assay increased to 98%. In our study we also observed a reduction of the sensitivity to 72% in samples with higher Ct values. First information on the dynamics of SARS-CoV-2 demonstrated that viral replication in the pharynx is highest during the first days of clinical disease and declines afterwards (Wölfel et al., 2020; Zou et al., 2020). This phenomenon was also observed in the analyses of our Ct values (Fig. 1). Interestingly, the decline in viral load seemed more pronounced in female patients (Fig. 2). Accordingly, antigen tests from upper respiratory swabs should be more sensitive in the initial phase of symptomatic infection. Although we could not prove this effect in our study, it is important to highlight that the vast majority of our samples corresponded to subjects in the early stages of infection (median duration of symptoms 2 days) and patients consulting in the late of Covid-19 were largely underrepresented. Furthermore, we detected several samples from early stage infection with low virus concentration. This might be explained by variations associated to sampling technique or by inaccurate data collection regarding symptom onset. However, the higher overall sensitivity in our study compared to the analysis from China is most probably related to the fact that the majority of samples (93.7%) were from patients during their first week of clinical disease. The high sensitivity of SARS-CoV-2 antigen detection in early infection might be a crucial finding for the design of new RDT-based algorithms, which are particularly important in weaker health

systems and low resource settings, where other high burden diseases, like malaria, also need to be considered.

The assay´s positive and negative predictive values (PPV and NPV) were not calculated for the study population. However, a test with a sensitivity of 93.9% and specificity of 100% would have a PPV and NPV of 100% and 99.4%, respectively, if applied for a population with a prevalence of 9%, as observed in our institution during the study period.

The presented data are critical, not only to support local decision making, but also for global agencies and governments worldwide in the procurement of simpler, scalable diagnostic tests, as an answer to the global call for "test, test, test" (Tedros Adhanom Ghebreyesus, Director General, World Health Organisation, 16 March 2020).

However our study also had some limitations, namely the use of a sample type not specifically permitted in the IFU. The advantage of our adapted sample use was that it allowed the comparison of RT-PCR and RDT from the same material, without possible distribution errors of using separate swabs. The UTM volume of 3 mL could have led to a dilution of the antigen and reduction of sensitivity (the manufacturer recommends using a single swab and elute it in 0.5 mL of buffer solution). Another limitation was the retrospective use of clinical data, which were collected under stressful routine work conditions within the ongoing outbreak. Finally, it is important to note that this evaluation was performed during a period of time (late summer in Chile) with a low circulation of other frequent respiratory viruses; therefore the performance of the antigen-based RDT might change in different epidemiological conditions.

In conclusion, the evaluated antigen-based immunofluorescence RDT showed a high sensitivity and specificity in respiratory samples obtained from patients who mainly presented during the first week of Covid-19. The assay was easy to use and provided results in a timely manner.

Hence, it has the potential to become an important tool for the early diagnosis of SARS-CoV-2, particularly in situations with limited access to molecular methods.

Contributors

LP and TW conceived the study and wrote the first draft. LP, TW, and GP curated the data. LP, PL, XA, and TW analysed the data. LP, PL, GP performed the investigation. TW and LP administered the project. LP, VV, PL, and TW supervised the study. LP, JMM, RA, XA, PV, MI, SD, and TW validated the data. All authors contributed in reviewing and editing later drafts, and approved the final version.

Declaration of interests

All authors declare no competing interests.

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248

249 **Table 1.** Demographic, clinical, and laboratory features of included cases; data represent

250 absolute numbers (%)

		All	PCR pos.	PCR neg.
Total		127	82	45
Gender	Male	68 (53.5)	44 (53.7)	24 (53.3)
	Female	59 (46.5)	38 (46.3)	21 (46.7)
Age (years)	Median	38	38	38
	IQR	29.5–44	31–46.3	29–44
	Range	1–91	1–73	2–91
	0 to 17	16 (12.6)	11 (13.4)	5 (11.1)
	18 to 59	102 (80.3)	66 (80.5)	36 (80.0)
	≥60	9 (7.1)	5 (6·1)	4 (8.9)
Days post symptom onset[1]	Median	2	2	2
	IQR	1–4	1–4	1–4
	Range	0–12	0–12	0–12
	Day 0-3	91 (72.2)	59 (72.8)	32 (71.1)
	Day 4-7	27 (22.4)	17 (21)	10 (22.2)
	Day ≥8	8 (6.3)	5 (6.2)	3 (6.7)
Clinical features[1]	Cough	94 (74.6)	63 (77.8)	31 (68.9)
	Fever	77 (61.1)	57 (70.4)	20 (44.4)
Ct value	Median		17·7	
	Interquartile		14.2–25.1	
	Mean		20	

251 IQR, interquartile range; Ct, cycle threshold of RT-PCR

252 [1]at time of sampling

253

254 **Table 2.** Sensitivity and specificity of antigen detection test in total and in different subgroups of samples

				Antigen detection test				
				Positive	Negative	Sensitivity		Specificity
Samples		**RT-PCR**	n	n	N	%	CI95%	%
All		Positive	82	77	5	93.9	86.5–97.4	100%
		Negative	45	0	45			
Gender	Male	Positive	44	43	1	97.7	88.2–99.6	100%
		Negative	24	0	24			
	Female	Positive	38	34	4	89.5	75.9–95.8	100%
		Negative	21	0	21			
Days post symptom onset	0-7	Positive	76	72	4	94.7	87.2–97.9	100%
		Negative	42	0	42			
	8-12	Positive	5	4	1	80.0	37.6–96.4	100%
		Negative	3	0	3			
Ct values	Quartile 1-3	Positive	52	52	0	100	89.8–100	
	Quartile 4	Positive	18	13	5	72.2	49.1–87.5	

255 Ct, cycle threshold of RT-PCR

256 **Table 3.** Characteristics of RDT false negative samples

N°	Gender	Age (years)	Days of symptoms	Fever	Cough	RT-PCR	Ct	RDT
4	Male	1	1	+	+	Pos.	34.7	Neg.
6	Female	51	12	+	+	Pos.	34.8	Neg.
35	Female	41	1	+	+	Pos.	26.6	Neg.
79	Female	32	1	-	+	Pos.	27.2	Neg.
117	Female	73	5	-	+	Pos.	27.9	Neg.

257 +, present; -, not present; Pos, positive; Neg, negative; Ct, cycle threshold; RDT, rapid diagnostic

258 test

Figure 1. Cycle threshold (Ct) values and lineal trend line of 70 RT-PCT positive samples taken on different days after symptom onset. Dots colours represent false negative (red) and true positive (blue) results by antigen detection test.

Figure 2. Cycle threshold (Ct) values and lineal trend lines of 33 samples of female patients (red) and 37 male patients (blue) taken on different days after symptom onset.

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