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

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Abstract:	Background In the context of the Covid-19 pandemic, the development and validation of rapid and easy-to-perform diagnostic methods are of high priority to enable the required testing scale up in most countries. We evaluated a novel rapid antigen detection test (RDT) for SARS-CoV-2 in respiratory samples. Methods A new fluorescence immunochromatographic SARS-CoV-2 antigen detection test (Bioeasy Biotechnology Co., Shenzhen, China) was evaluated in nasophayngeal (NP) and oropharyngeal (OP) swabs from patients with suspected Covid-19 in Santiago, Chile. Diagnostic accuracy was determined in comparison to SARS-CoV-2 real time (RT)-PCR, using the same material (universal transport medium with NP and OP swab). Findings A total of 127 samples were included. The median patients' age was 38 years (IQR 29·5–44), 53·5% were male, and 93·7% were in the first week after symptom onset. Among 82 RT-PCR positive specimens, 77 were correctly detected by RDT. All 45 RT-PCR negative samples were correctly identified. The overall sensitivity and specificity of the RDT 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 increased viral loads. Interpretation The 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, despite the use of a non-validated sample. The assay was easy to use and provided results in a timely manner. It has the potential to become an important tool for 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

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

Methods. A new fluorescence immunochromatographic SARS-CoV-2 antigen detection test (Bioeasy Biotechnology Co., Shenzhen, China) was evaluated in nasophayngeal (NP) and oropharyngeal (OP) swabs from patients with suspected Covid-19 in Santiago, Chile. Diagnostic accuracy was determined in comparison to SARS-CoV-2 real time (RT)-PCR, using the same material (universal transport medium with NP and OP swab).

Findings. A total of 127 samples were included. The median patients' age was 38 years (IQR 29·5–44), 53·5% were male, and 93·7% were in the first week after symptom onset. Among 82 RT-PCR positive specimens, 77 were correctly detected by RDT. All 45 RT-PCR negative samples were correctly identified. The overall sensitivity and specificity of the RDT 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 increased viral loads.

Interpretation. The 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, despite the use of a non-validated sample. The assay was easy to use and provided results in a timely manner. It 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.[1] Timely detection and isolation of cases and their contacts are considered crucial to help curtail this unprecedented pandemic.[2] 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,[3] and is now applied using WHO or CDC protocols,[4,5] as well as various commercial assays.[6]

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.[7] 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.[7] Tests detecting SARS-CoV-2-specific antigen have recently been developed and many of them are now commercially available.[6] However, the real-world performance of these assays is uncertain and their validation is therefore of high priority.[8] Other options include serological tests, but due to their diagnostic limitations in early infections, these tests are currently not recommended for case detection.[7,8] 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).[9] 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,[10] 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 (Primer Design Ltd., Chander´s Ford, UK) after RNA extraction with the Magna Pure Compact system (Roche Molecular Systems Inc., Pleasanton, Ca, USA). 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. 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.[11] Sensitivity was further analysed for certain subgroups such as gender, days of symptoms at sampling, and RT-PCR Ct values.

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 4^{th} 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 higher Ct values and a steeper positive trend line slope over time of infection compared to male patients (Fig. 2).

Discussion

The novel SARS-CoV-2 antigen test kit from Bioeasy is among the growing number of diagnostic assays available for Covid-19, which have received regulatory approval either in Europe or in the USA.[6] The test has a cassette format with an external reader and is approved to be used with oropharyngeal swabs, nasopharyngeal swabs, and sputum. In our experience, the system was easy to use and gave a qualitative result for an individual sample in approximately 15 minutes. Depending on the reading mode, the analyser permits a throughput of approximately 5 (standard mode with incubation within the device) to >50 (rapid mode with incubation outside

the device) samples per hour. This significant throughput is encouraging given the large number of samples processed in many Covid-19 testing points. However, the inherent biological hazard requires the handling of specimens in a biosafety cabinet,[12] hence slowing down the process and reducing the sample number per hour.

Within our panel of clinical samples, the novel assay proved to be highly sensitive and specific. 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.[13] 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.[14,15] 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.

Positive and negative predictive values (PPV and NPV) for the evaluated assay could not be calculated, since the study population only included a fraction of the total samples examined in our laboratory during the study period. Still, if the sensitivity (93·9%) and specificity (100%) are extrapolated on the total of 1,453 samples tested, of which 134 were positive, the PPV and NPV would be 100% and 99·4%, respectively.

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

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

[1]at time of sampling

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	

Ct, cycle threshold of RT-PCR

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.

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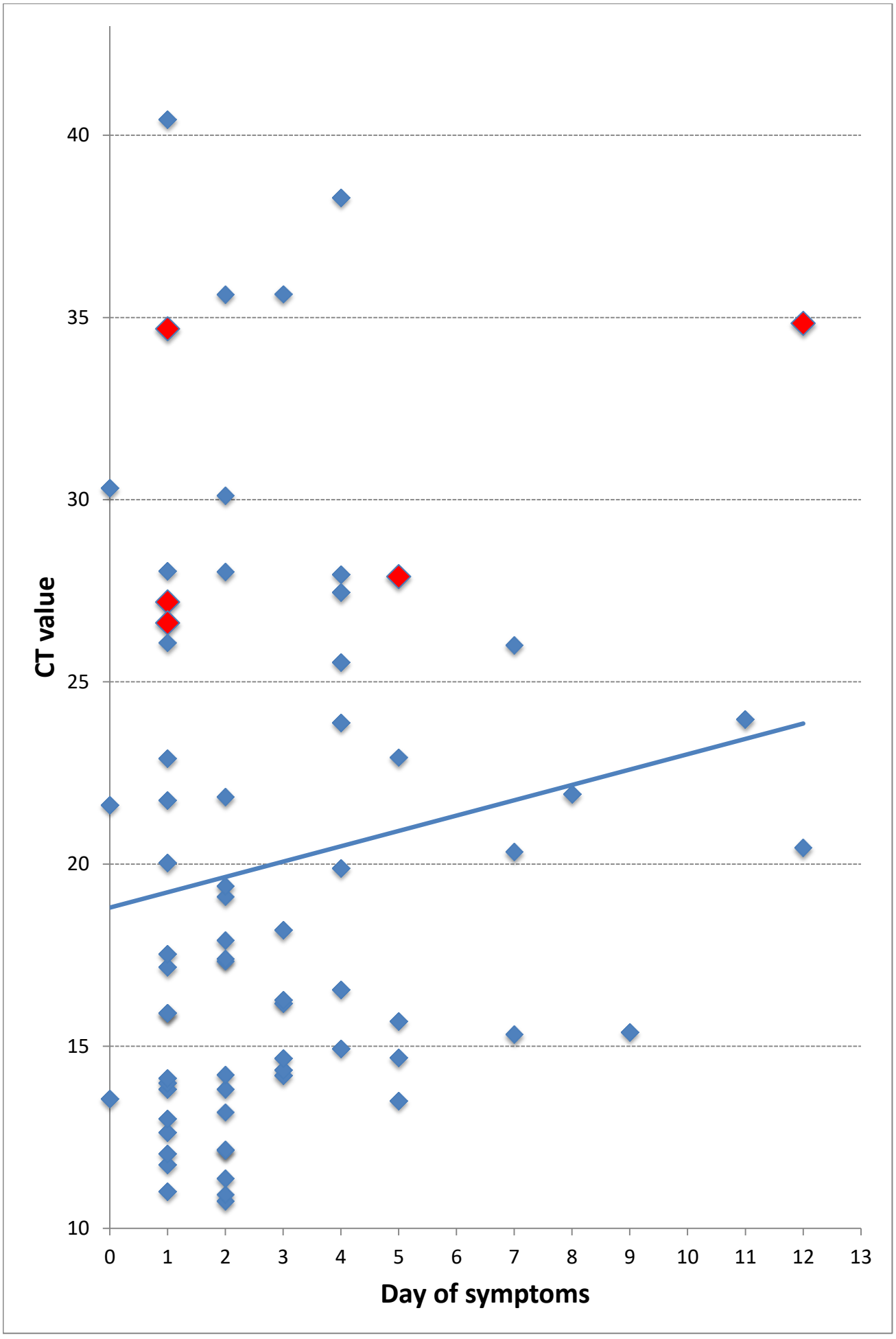
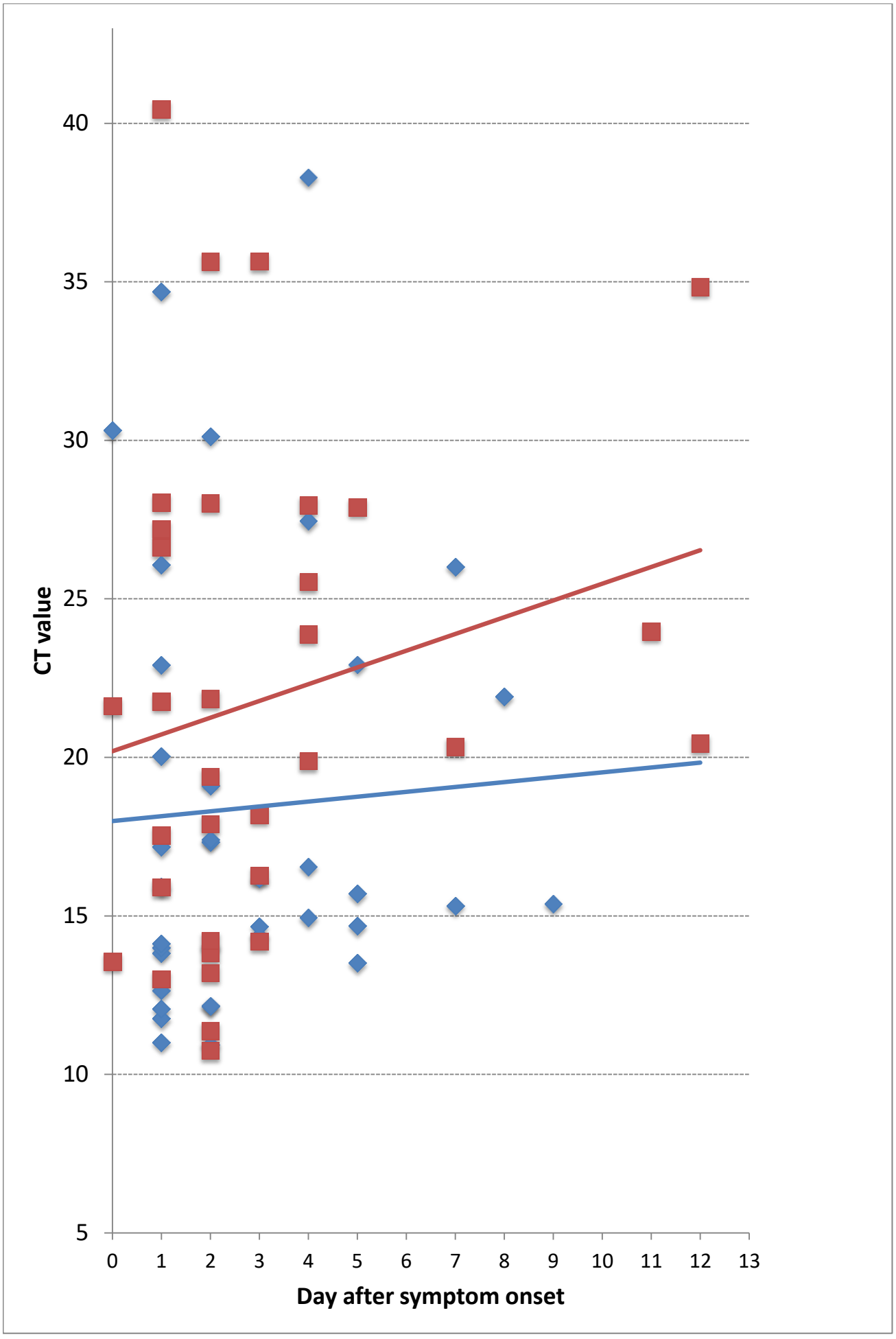
Figure



Figure



Research in context

Evidence before this study

Since its first occurrence in December 2019, the rapidly emerging SARS-CoV-2 pandemic is causing tremendous public health challenges worldwide. Timely detection and isolation of cases and their contacts are crucial. To date, the only recommended diagnostic method for SARS-CoV-2 infection is real-time reverse-transcription polymerase chain reaction (RT-PCR). According to WHO, there is a critical demand for alternative assays such as antigen detection tests, which should be validated with high priority. A literature search with the terms (“SARS-CoV-2” OR “Covid-19”) AND (“Rapid diagnostic test” OR “antigen test” OR "antigen detection") retrieved 0 hits in PubMed and 168 hits in Google Scholar. Among those, only one preprint report provided data on antigen detection tests for SARS-CoV-2.

Added value of this study

To our best knowledge, this study presents the first independent evaluation for a near-patient antigen test for SARS-CoV-2. The data demonstrated that the rapid antigen detection had a high diagnostic sensitivity and specificity in respiratory samples obtained from patients who mainly presented during the first week of Covid-19.

Implications of all the available evidence

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. Our study provides new information, which is critical for decision making in all countries heavily affected by the pandemic and might be useful for global agencies and governments around the world in their procurement decisions and implementation of diagnostic algorithms.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	3
	4	Study objectives and hypotheses	4
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	4
Participants	**6**	Eligibility criteria	4
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	4
	8	Where and when potentially eligible participants were identified (setting, location and dates)	4
	9	Whether participants formed a consecutive, random or convenience series	5
Test methods	**10a**	Index test, in sufficient detail to allow replication	4
	10b	Reference standard, in sufficient detail to allow replication	4
	11	Rationale for choosing the reference standard (if alternatives exist)	4
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	5
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	4
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	5
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	4
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	5
	15	How indeterminate index test or reference standard results were handled	5
	16	How missing data on the index test and reference standard were handled	5
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	5
	18	Intended sample size and how it was determined	4
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Separate document
	20	Baseline demographic and clinical characteristics of participants	6
	21a	Distribution of severity of disease in those with the target condition	Not applicable
	21b	Distribution of alternative diagnoses in those without the target condition	Not applicable
	22	Time interval and any clinical interventions between index test and reference standard	Not applicable
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	11
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	11
	25	Any adverse events from performing the index test or the reference standard	Not applicable
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	8
	27	Implications for practice, including the intended use and clinical role of the index test	9
OTHER INFORMATION			
	28	Registration number and name of registry	Not applicable
	29	Where the full study protocol can be accessed	4
	30	Sources of funding and other support; role of funders	9

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

STARD diagram

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