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Journal of Clinical Virology 137 (2021) 104782

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Handling and accuracy of four rapid antigen tests for the diagnosis of SARS-CoV-2 compared to RT-qPCR

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ARTICLE INFO

Keywords:
Accuracy
Handling
SARS-CoV-2
Rapid antigen test AgPOCT

ABSTRACT

Background: SARS-CoV-2 molecular diagnostics is facing material shortages and long turnaround times due to exponential increase of testing demand.
Objective: We evaluated the analytic performance and handling of four rapid Antigen Point of Care Tests (AgPOCTs) I-IV (Distributors: (I) Roche, (II) Abbott, (III) MEDsan and (IV) Siemens).
Methods: 100 RT-PCR negative and 84 RT-PCR positive oropharyngeal swabs were prospectively collected and used to determine performance and accuracy of these AgPOCTs. Handling was evaluated by 10 healthcare workers/users through a questionnaire.
Results: The median duration from symptom onset to sampling was 6 days (IQR 2–12 days). The overall respective sensitivity were 49.4 % (CI95 %: 38.9–59.9), 44.6 % (CI95 %: 34.3–55.3), 45.8 % (CI95 %: 35.5–56.5) and 54.9 % (CI95 %: 43.4–65.9) for tests I, II, III and IV, respectively. In the high viral load subgroup (containing $>10^6$ copies of SARS-CoV-2 /swab, n = 26), AgPOCTs reached sensitivities of 92.3 % or more (range 92.3 %–100 %). Specificity was 100 % for tests I, II (CI95 %: 96.3–100 for both tests) and IV (CI95 %: 96.3–100) and 97 % (CI95 %: 91.5–98.9) for test III. Regarding handling, test I obtained the overall highest scores, while test II was considered to have the most convenient components. Of note, users considered all assays, with the exception of test I, to pose a significant risk for contamination by drips or spills.
Discussion: Besides some differences in sensitivity and handling, all four AgPOCTs showed acceptable performance in high viral load samples. However, due to the significantly lower sensitivity compared to RT-qPCR, a careful consideration of pro and cons of AgPOCT has to be taken into account before clinical implementation.

Abbreviations: RT-qPCR, Reverse transcription-polymerase chain reaction, AgPOCT.

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https://doi.org/10.1016/j.jcv.2021.104782
Received 4 December 2020; Received in revised form 28 January 2021; Accepted 28 February 2021
Available online 3 March 2021

1. Introduction

The SARS-CoV-2 pandemic continues to spread at accelerating pace [1], posing an unprecedented challenge for health care systems around the globe. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis remains the gold standard for diagnosis of SARS-CoV-2 infection [2]; however, limited supply of material and specialized personnel, as well as frequent reporting delays bring about the need for alternative testing formats. Rapid Antigen Point of Care Tests (AgPOCTs) represent one such alternative and have been pushed into the market by countless distributors in recent months. The tally of CE-cleared AgPOCT products has mounted to just over 200 during this time [3], leaving potential users mostly in the dark about their performance in real-world situations as independent comparison studies were unable to keep pace with the flood of novel tests [4,5].

2. Objective

The aim of this study was to evaluate and compare handling, analytical- and clinical performance of four commercial rapid antigen point of care tests (AgPOCT) distributed by established diagnostics firms.

3. Methods

3.1. Clinical samples and ethics

Single center non-interventional diagnostic multiple-gate study. Respiratory samples were oropharyngeal or nasopharyngeal swabs collected using Universal Transport Medium (UTM) based collection kits by Copan (Italy, Brescia) or Iclean (Shenzhen, China) and prospectively collected following routine diagnostics at the University Medical Center Hamburg-Eppendorf (during August through November 2020) from patients hospitalized with suspected or known COVID-19. Once the required number of positive RT-qPCR SARS-CoV-2 samples was collected, a further 100 RT-qPCR negative SARS-CoV-2 samples were randomly selected to serve as negative control. This work was conducted in accordance with §12 of the Hamburg hospital law (§12 HmbKHG). The use of anonymized samples was approved by the ethics committee, Freie und Hansestadt Hamburg, PV5626.

AgPOCT and RT-qPCR procedures: Four different CE-labeled AgPOCT kits were compared (Table 1). Swabs supplied with the AgPOCT kits were immersed in patient oropharyngeal/nasopharyngeal samples for approximately 10 s before all further steps of the tests were carried out according to instructions by manufacturers. The results of the AgPOCTs were read by two unblinded operators. It has to be noted, that this particular protocol of dipping nasopharyngeal swabs into UTM samples is a deviation from the recommended sample matrix and or handling recommendations by the manufacturer.

Based on previous experience, we postulated that roughly 100 µL of UTM sample are carried over by the procedure described above. To allow for a meaningful clinical correlation, 'effective' viral loads/swab were calculated by applying a factor of 0.1 to all PCR measurements from original samples. This was done in an effort to generate results more representative of a real-life testing procedure. A commercial SARS-CoV-2 RT-qPCR assay (Roche cobas SARS-CoV-2 IVD) was used as reference, performed directly from UTM samples.

For limit of detection experiments, a serial dilution of cell-free virus solution containing SARS-CoV-2 strain HH-1 [6] in UTM was prepared and subjected to all four tests with five repeats per dilution step. As RT-qPCR reference method, the cobas6800 SARS-CoV-2 IVD assay was used in conjunction with quantitative external control material by Instand e.V. (Düsseldorf, Germany) to allow for absolute quantification, as previously described [7,8]. To evaluate the ease of handling and implementation of AgPOCTs into clinical routine we conducted a user survey employing a questionnaire (see supplementary Table S1) with 10 participants representing different clinical specialties and professions (3 Intensive Care Unit (ICU) medical doctors, 2 ICU nurses, 3 microbiologists and 2 lab technicians). All users had no prior experience with SARS-CoV-2 AgPOCT, but have handled other lateral flow tests in the past. Every user received the complete set of materials for every test, including the instructions manual. Questionnaires were filled in anonymously and independently by each participant. A 5-point Likert scale (1 = "do not agree at all" to 5 = "absolutely agree") was used to evaluate for different test dimensions. Statistical analysis was performed with STATA (version 15) and GraphPad Prism (version 86 9.0.0).

Table 1
Main characteristics of four rapid SARS-CoV-2 rapid antigen tests (according to the manufacturer).

	Test I	Test II	Test III	Test IV
Description	SARS-CoV-2 Rapid Antigen Test (Roche)	COVID-19 Rapid Test Device (Abbott)	MEDsan SARS-CoV-2 Antigen Rapid Test	CLINITEST Rapid COVID.19 Antigen Test
Distributor	Roche Diagnostics	Abbott Rapid Diagnostics	MEDsan GmbH	Siemens
Manufacturer	SD Biosensor	Panbio Ltd.	MEDsan GmbH	Zhejiang Orient Biotech Co.
Country of origin	South Korea	Australia	Germany	China
Certification	CE-IVD	CE-IVD	CE-IVD	CE-IVD
Specimen	NP	NP	NP	NP
Limit of detection (manufacturer)	$3{,}1 \times 10^{2{,}2}$ TCID50/mL	$2{,}5 \times 10^{2}$ TCID50/mL	$1{,}4 \times 10^{2}$ TCID50/mL	$1{,}1 \times 10^{2}$ TCID50/mL
Volume applied into cassette	3 drops	5 drops	2 drops	4 drops
Incubation	15−30 minutes	15−20 minutes	15−20 minutes	15−20 minutes
Readout	Visual: colored band	Visual: colored band	Visual: colored band	Visual: colored band
UTM protocol	Reported	Not specified	Not specified	Not specified
Cross-reactivity against other human respiratory virus evaluated and excluded	Reported	Reported	Reported	Reported

N-protein: Nucleocapsid protein Ag.
NP: nasopharyngeal swab.
TCID50: Median tissue culture infective dose.

4. Results

4.1. Analytical and clinical performance

In total 100 RT-qPCR negative and 84 positive respiratory samples were included in this study (test IV was performed on only 72 positive respiratory samples; Fig. 1).56.5 % (26/ 46, unknown 38) of specimens were taken within one week of symptom onset. The median duration from symptom onset to sampling was 6 days (IQR 2–12 days). Median viral load/ swab for the AgPOCT was 3.8×10^5 copies/swab (IQR $5.4 \times 10^3 - 3.8 \times 10^6$ copies/swab). Clinical sensitivity was 49.4 % (CI95: 38.9 %–59.9 %), 44.6 % (CI95: 34.3 %–55.3 %), 45.8 % (CI95: 35.5 %–56.5 %) and 54.9 % (CI95: 43.4 %–65.9 %) for the tests I, II, III and IV, respectively (Table 2). Limit of Detection (LOD) using cell culture derived SARS-CoV-2 virus was in line with these results showing similar analytical performance with best performance for test IV (see suppl. Fig. S1). All assays showed a specificity of 100 %, with the exception of test III (97 % (CI95: 91.5 %–98.9 %), see Table 2). In a set of samples that all contained $> 10^6$ copies/swab (n = 32 for test I,II and III and n = 28 for

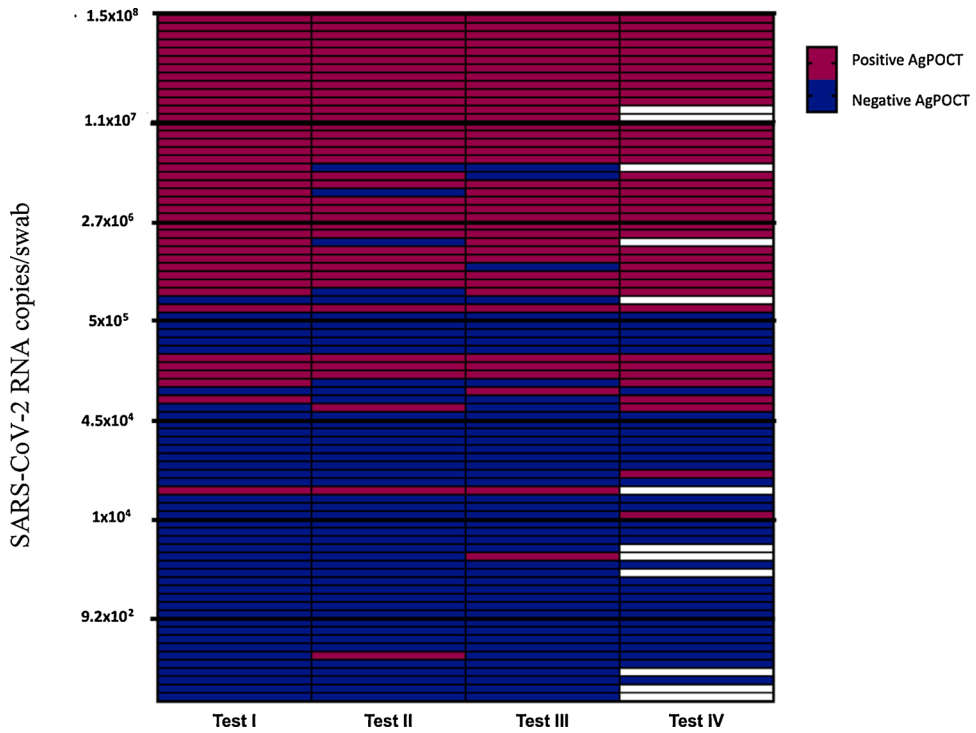

Fig. 1. AgPOCT results in relation to SARS-CoV-2 RNA copies/swab. Purple fields represent positive and blue fields negative AgPOCT results. Blank fields correspond to cases where samples were not tested for the corresponding assay (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2
Performance of four rapid antigen tests for SARS-CoV-2 compared to RT-qPCR.

Test	Sensitivity % (CI95)	Specificity % (CI95)	Accuracy %	Kappa coefficient
I (n positive = 84; n negative = 100)	49.4 (38.9–59.9)	100 (96.3–100)	77.1	0.52
II (n positive = 84; n negative = 100)	44.6 (34.3–55.3)	100 (96.3–100)	74.8	0.46
III (n positive = 84; n negative = 100)	45.8 (35.5–56.5)	97 (91.5–98.9)	73.7	0.45
IV (n positive = 72; n negative = 100)	54.9 (43.4–65.9)	100 (96.3–100)	81.2	0.58

test IV; median duration from symptom onset to sampling 6 days (IQR 2–9)), clinical sensitivity rose to 100 % (CI95: 87 %–100 %), 92.3 % (CI95: 75.8 %–97.8 %), 92.3 % (CI95: 75.8 %–97.8 %) and 100 % (CI95: 85.7 %–100 %) for tests I, II, III and IV, respectively.

4.2. Handling

The median of the results for each item is illustrated in Fig. 2. Test I was considered the overall easiest to usefollowed by test II. Test III scored lowest overall. Of note, users considered all assays to pose a significant risk for contamination by drips or spills (see Fig. 2).

5. Discussion

Here we report a head-to-head comparison of performance and handling for four rapid AgPOCTs of several major distributors in Europe, using the latest version of CE-labeled kits. All tests were able to detect 10^6 or more copies/swab with high reliability (95 %), implying that patients with high viral loads can be identified with acceptable accuracy. This is in line with previous studies [4,5].

Relative clinical sensitivity of rapid SARS-CoV-2 antigen tests has repeatedly fallen short of optimistic manufacturer claims [4,9–11], in large parts due to differences in patient characteristics in respective cohorts and artificial conditions when performing AgPOCT. This also applies to this study, as e.g. antigen tests were performed from clinical UTM samples. Still, the lack of an amplification step inherently entails significantly higher analytic limits when compared to RT-qPCR, which is expected to be 100–1000 times more sensitive than an AgPOCT. However, in the context of increased frequency of testing, lower sensitivity tests may be just as effective as highly sensitive ones in identifying individual infections and outbreaks, as suggested by mathematical modeling studies [12].

All AgPOCT evaluated in this study showed largely comparable clinical and analytical sensitivity, with the exception of Test IV (Siemens). The slight edge Test IV had in LoD experiments translated into a moderately increased positive agreement with RT-qPCR (of 54 %) compared to the other tests (lower than 50 %). It has to be noted that fewer clinical samples were tested with Test IV as it only became available when experiments were already underway, thus providing only an incomplete dataset to compare with the other tests. Furthermore, the limited sensitivity of the assays can also be justified by the fact that half of the samples were collected at least one week after symptom onset, as shown in other studies [13].

Besides analytical properties, handling and practical application were evaluated as part of this study. While all AgPOCTs were deemed “easy to use”, Test I was considered slightly better than the rest, while Test II was considered to have the most convenient components. We were surprised to notice that most users reported a high risk of contamination (by drips or spills). This aspect should not be underestimated in particular considering AgPOCT are to be used outside the health care sector and might also be performed by untrained personnel lacking experience in dealing with infectious materials. Especially in a context where test performance is largely identical, practical aspects

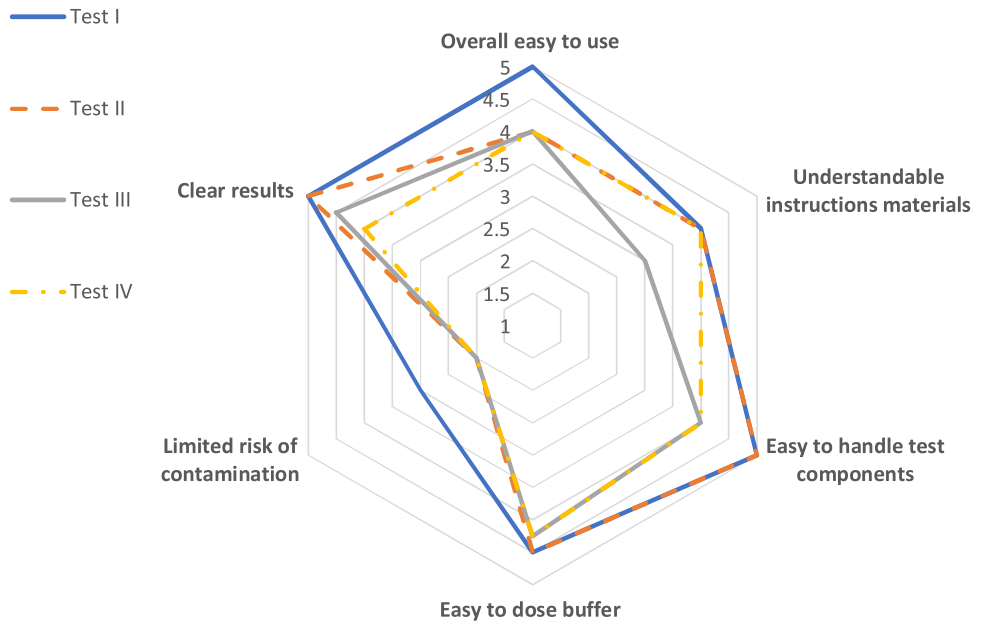

Fig. 2. Evaluation of the handling of four AgPOCTs. Scores vary from 1 ("do not agree at all") to 5 ("absolutely agree").

should not be neglected when choosing which test to employ, as minor inconveniences in performing a single test can add up to massive delays when performing them in large numbers.

In conclusion, we present analytical and clinical evaluation of four rapid SARS-CoV-2 antigen tests, as well as a user survey for practical application. All tests demonstrated largely similar performance and are expected to detect high viral loads with adequate reliability. RT-qPCR remains the gold standard to definitively confirm or rule out infections due to its significantly higher sensitivity and specificity.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2021.104782.

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