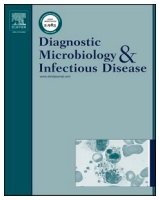
Diagnostic Microbiology & Infectious Disease 110 (2024) 116466

Contents lists available at ScienceDirect

Diagnostic Microbiology & Infectious Disease

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



Evaluation of STANDARD™ M10 SARS-CoV-2 from bronchoalveolar lavage samples

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ARTICLE INFO

Keywords:
BAL
LRTI
COVID-19
SARS-CoV-2

ABSTRACT

Detection of SARS-CoV-2 in bronchoalveolar lavage (BAL) is considered as a promising alternative method to detect COVID-19 infection. STANDARD™ M10 SARS-CoV-2 assay on 150 negative and 50 positives BAL samples for SARS-CoV-2 showed 96 % sensitivity, 100 % specificity compared to Allplex™ SARS-CoV-2 assay and a 31.25 genomic copies/mL limit of detection.

1. Introduction

The rapid and accurate detection of SARS-CoV-2 during the COVID-19 pandemic has been subjected to numerous studies to identify the best methods for infection diagnosis [1,2]. The gold standard for the diagnosis of SARS-CoV-2 infection is Reverse Transcription Polymerase Chain Reaction (RT-PCR) starting primarily from respiratory specimens such as oropharyngeal (OP) and nasopharyngeal (NP) swabs. Bronchoalveolar lavage (BAL) and bronchial aspirate have been considered as possible alternatives [2,3], since negative results may occur when testing OP and NP swabs, due to sampling errors or low viral load [4]. In fact, NP swabs and BALs have been reported to present an overall good agreement for the detection of viral respiratory infections, but patients with negative results for SARS-CoV-2 in NP samples, diagnosed with SARS-CoV-2 by BAL testing, have been described [4-6]. Indeed, SARS-CoV-2 diagnosis from OP and NP samples has been shown to present a limited sensitivity (32 % and 63 %, respectively), while positivity for SARS-CoV-2 in BAL samples was found in 93 % of patients [7]. These data have cast doubt that a negative NP swab may not be sufficient to rule out SARS-CoV-2 disease and BAL analysis would therefore help for definitive diagnosis, in particular, in case of clinical and radiographic suspect of COVID-19 [4]. BAL analysis is mainly recommended for diagnosis in immunocompromised patients if there is a strong suspicion of superinfection or in case of severe disease in life-saving conditions [4]. Moreover, BAL analysis could be useful to monitor disease staging in intensive care units, where it is commonly collected to detect possible coinfections [2]. Similarly, in cases of lung transplantation, testing for SARS-CoV-2 in BAL from donor lung (sometimes prescribed by internal or local procedures) could significantly reduce the risk of transmission [8]. The evolving pandemic situation has shown the importance of accurate and rapid diagnostic tests for the detection of SARS-CoV-2, to minimize the spread of the disease; for this reason, the US Food and Drug Administration (FDA) has approved several rapid tests under the Emergency Use Authorization (EUA) for the detection of SARS-CoV-2 [9]. However, SARS-CoV-2 testing in BAL samples has limited application due to its invasiveness along with the limited number of available validated assays.

The aim of this study was to evaluate the clinical sensitivity and specificity of SARS-CoV-2 detection from BAL samples with the rapid STANDARD™ M10 SARS-CoV-2 cartridge assay (SD Biosensor Inc, Suwon, Republic of Korea) compared with the CE-IVD marked Allplex™ SARS-CoV-2 assay (Seegene Inc, Seoul, Republic of Korea).

2. Material and methods

A total of 200 residual anonymized BAL samples were collected

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https://doi.org/10.1016/j.diagmicrobio.2024.116466
Received 30 May 2024; Received in revised form 8 July 2024; Accepted 22 July 2024
Available online 31 July 2024

Table 1
Comparison of results obtained with prospectively collected bronchoalveolar lavage samples with STANDARD™ M10 SARS-CoV-2 and Allplex™ SARS-CoV-2 assays.

		STANDARD™ M10 SARS-CoV-2		
Allplex™ SARS-CoV-2		POS	NEG	TOT
	POS	48	2	50
	NEG	0	150	150
	TOT	48	152	200

during January and February 2022 from SARS-CoV-2 positive and negative patients and stored at -80°C. Nucleic acid extraction was performed with STARMag™ 96×4 Universal Cartridge kit on the automated system NIMBUS (Seegene), as well as the one-step RT-PCR setup using Allplex™ SARS-CoV-2 assay (ASCV) (Seegene). RT-PCR was performed on CFX96 Real-time PCR System with the CFX Manager™ Software-IVD (v1.6) (Bio-Rad, Hercules, CA, USA) and interpreted with Seegene Viewer Software V3, according to manufacturer's instructions. ASCV detects SARS-CoV-2 *E, RdRp* and *N* genes and includes an exogenous internal control (IC). The assay requires a sample load of 200 μL, with a detection limit of 50 copies/reaction (approximately 1000 copies/mL, Cq value = 40) and a running time of approximately 4.5 h, including hands-on time of about 20-30 min.

The STANDARD™ M10 SARS-CoV-2 assay (SMSCV) is a sample-to-result multiplex RT-PCR test intended for use with STANDARD™ M10 system. The SMSCV test contains primers and probes for *E* and *Orf1ab* genes for the *in vitro* qualitative detection of SARS-CoV-2 RNA in nasopharyngeal or oropharyngeal swab specimens and an exogenous IC. The assay was performed using 600 μL of sample, obtained by 1:4 dilution of the BAL with Sputasol (Oxoid™ Thermo Scientific™, Waltham, MA, USA); the solution was incubated at room temperature for 10 min and vortexed for 30 s, before being loaded into the cartridge. In case of invalid results, testing was repeated using samples further diluted 1:2 in Sputasol. SMSCV has a detection limit of 100 copies/mL (Cq value = 38) and a running time of approximately 60 min including hands-on time. ASCV and SMSCV were performed simultaneously from frozen BAL samples, to avoid multiple freeze-thawing steps.

Discrepancies resolution was carried out with SARS-CoV-2 ELITe MGB® Kit on ELITe InGenius® automated system (ELITechGroup, Paris, France).

Limit of detection (LoD) of SMSCV was evaluated with AccuPlex™ SARS-CoV-2 Molecular Controls Kit - Full Genome (0505-0159) (SeraCare part of LGC Clinical Diagnostics, Milford, MA, USA), which provides positive control vials with SARS-CoV-2 concentrated at 5000 copies/mL (cp/mL) as previously reported and recommended by the Manufacturer [10]. Four residual anonymized BAL samples (previously reported as negative for SARS-CoV-2) were pooled and diluted 1:4 in Sputasol. Starting from a positive control vial, a stock solution at 1000 cp/mL was prepared, followed by seven 2-fold dilutions up to 15.625 cp/mL. Each dilution was analysed 6 times with SMSCV, for a total of 45 samples. At the same time, 200 μL of each dilution (including the positive control vial and BAL pool) was subjected to nucleic acids extraction and ASCV as previously described. This study was approved by the local Ethics Committee (number 23421_DM, January 17th, 2023).

3. Results

A total of 50 SARS-CoV-2 positive and 150 negative BAL samples

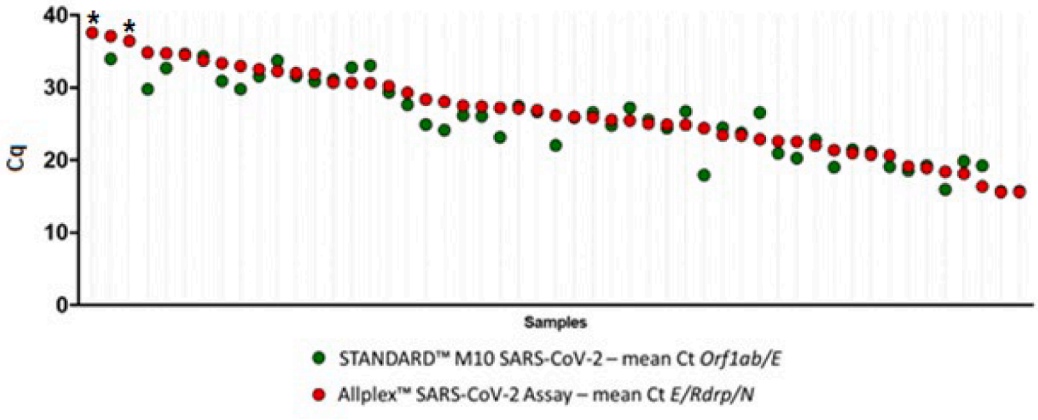

Fig. 1. Comparison of Ct values obtained with prospectively collected bronchoalveolar lavage samples with STANDARD™ M10 SARS-CoV-2 and Allplex™ SARS-CoV-2 Assays. "*" Indicates two specimens positive by Allplex™ SARS-CoV-2 Assay and negative by STANDARD™ M10 SARS-CoV-2 which were rested with SARS-CoV-2 ELITe MGB® Kit reported as positive with Cq of 35.0 and 35.6 calculated on the *rdrp* and *orf8* genes, respectively.

Table 2
Limit of detection of STANDARD™ M10 SARS-CoV-2 assay. SD: standard deviation; "-": not evaluable; N/A: not amplified.

	STANDARD™ M10 SARS-CoV-2 assay						Allplex™ SARS-CoV-2 assay			
	E			*Orf1ab*						
copies/mL	Positivity (%)	Cq[a]	SD	Positivity (%)	Cq[a]	SD	*E* Cq	*RdRP/S* Cq	*N* Cq	Cq[b]
1000	6/6 (100)	33.45	1.21	6/6 (100)	32.77	0.20	34.42	33.31	34.93	34.22
500	6/6 (100)	33.98	0.80	6/6 (100)	33.74	0.86	34.61	33.99	35.44	34.68
250	3/6 (50)	34.20	0.27	3/6 (50)	34.08	0.43	37.12	36.79	38.76	37.56
125	2/6 (33)	35.91	1.00	2/6 (33)	34.86	0.33	36.70	37.76	37.40	37.29
62.5	3/6 (50)	34.93	0.09	0/6 (-)	N/A	-	N/A	39.02	N/A	39.02
31.25	0/6 (-)	N/A	-	0/6 (-)	N/A	-	N/A	N/A	N/A	N/A
15.625	0/6 (-)	N/A	-	0/6 (-)	N/A	-	N/A	N/A	N/A	N/A

[a] mean of six repetitions
[b] mean of *E, Rdrp, N* genes Cq in a single experiment

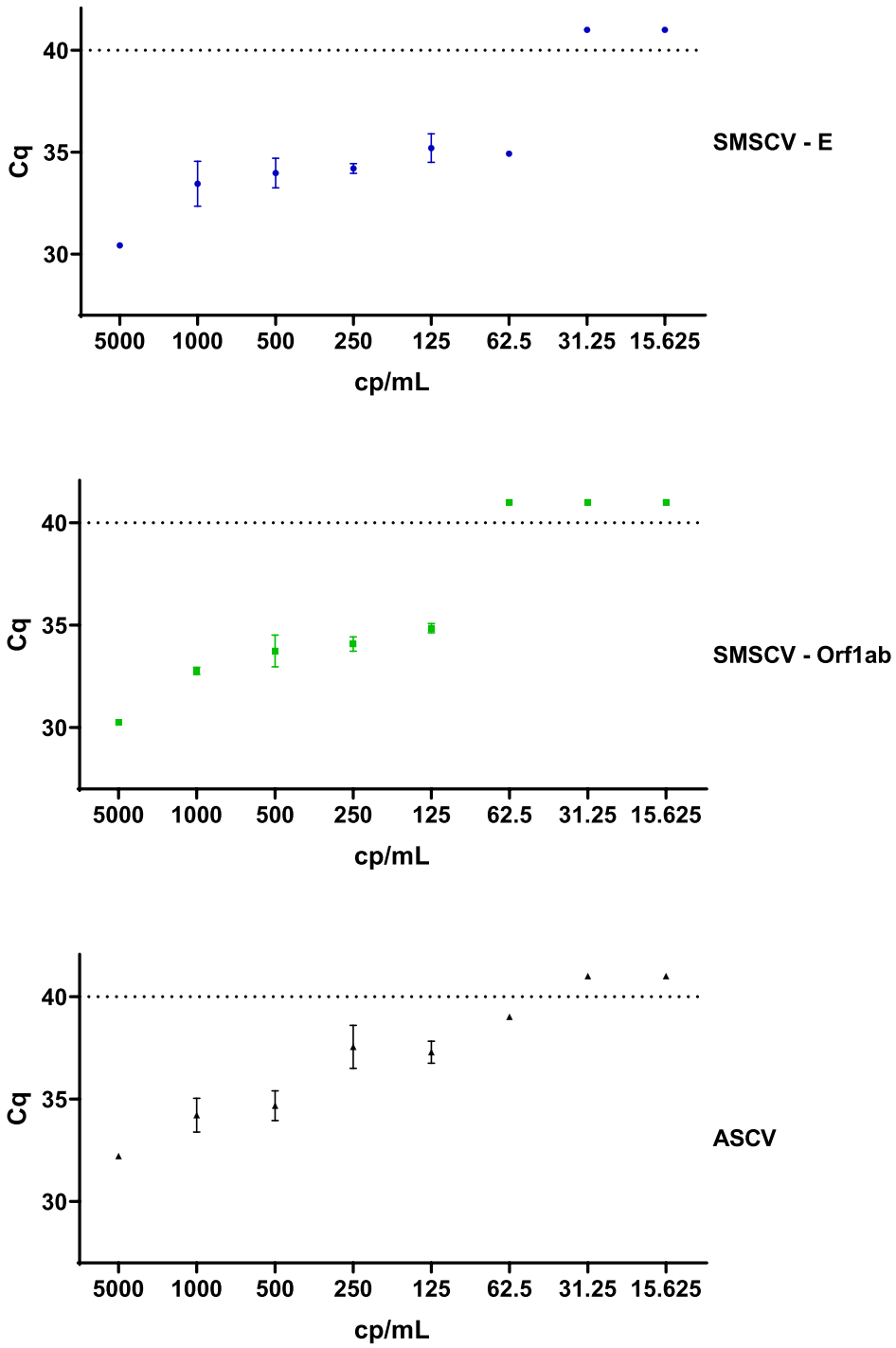

Fig. 2. Cq mean revealed after six repetitions of each dilution for STANDARD™ M10 SARS-CoV-2 assay (SMSCV) target *E* and *Orf1ab* and Allplex™ SARS-CoV-2 Assay (ASCV) (mean of four targets performed in one experiment). Result of positive control at 5000 copies/mL (cp/mL) is indicated. Negative samples were plotted as Cq > 40.

were analyzed with both ASCV and SMSCV assays. A detailed comparison of the assay results is shown in Table 1. Eight of the samples yielded an invalid result with SMSCV at a first analysis (invalid ratio 4 %), all of which were solved by repeating the test with a further two-fold dilution of the specimens in Sputasol. Overall, SMSCV showed a high sensitivity (48/50 - 96 %; 95 %CI 87.8-99.2) and specificity (150/150 - 100 %; 95 %CI 98.3-100) compared with ASCV. The false negative results yielded by SMSCV assay were observed with positive samples that exhibited high Cq values with the ASCV (37.31 and 37.00 for the *E* gene, 37.83 and 35.92 for the *N* gene and negative result for the *RdRP* gene target samples, respectively). These samples were also tested by a third method which confirmed positivity in both cases. The mean Cq difference of the two assays showed slightly lower values for SMSCV compared to ASCV (ΔCq genes -0.71, Fig. 1). LoD analysis of SMSCV revealed a value of 62.5 cp/mL (3/6 – 50 % repetitions were positive only for the *E* gene) with a Cq mean of 34.93. The same LoD was achieved also by ASCV, which revealed only the *Rdrp/S* genes with a Cq of 39.02 (lower than declared LoD of 1000 cp/mL). The *Orf1ab* target was revealed by SMSCV up to 125 cp/mL in 2/6 – 33 % repetitions, with an overall Cq mean of 34.86 (Table 2 and Fig. 2).

4. Discussion

RT-PCR is considered the gold standard in clinical diagnosis of COVID-19. Upper respiratory tract samples (NP and OP swabs) have been widely used, given their low invasiveness and ease of collection when compared with other specimens such as BAL [11], which use has been restricted to the evaluation of SARS-CoV-2 and secondary infections in patients with invasive mechanical ventilation mainly in critical care wards [12]. The advantage of BAL compared to other fluids is mainly in increasing diagnostic accuracy as it can be performed with greater precision, possibly in accordance with radiological results, despite the high invasiveness of the procedure [13]. In case of critical patients, with an involvement of the lower-respiratory tract, but a negative test on NP, a rapid method to detect SARS-CoV-2 nucleic acids from BAL could be of interest to reduce any further diagnostic delay. A rapid and simple SARS-CoV-2 test performed on lower respiratory tract specimens is also recommended for thoracic organ donors, despite the difficulty in specimens collection [13].

Determination of LoD revealed that SMSCV is able to identify positive samples with at least 62.5 cp/mL in 50 % of cases, a result comparable to reference molecular assays with ASCV (Table 2). Other commercial assays have been validated with BAL samples, including systems presenting a higher throughput but a slower turnaround time (more than 3 h) [14,15] or rapid point-of-care (POC) systems, which are generally more expensive than SMSCV [16,17], but can be reliable and helpful in long-term or rehabilitation facilities.

5. Conclusions

In conclusion, the SMSCV represents a novel diagnostic tool for the rapid detection of SARS-CoV-2 in BAL samples with high sensitivity and specificity. The lower throughput of this POC method compared to other analytic systems (each instrument module can perform one sample at a time) meets the limited number of BAL sample requests compared with upper respiratory tract samples. Furthermore, this method could be useful in case of suspect of COVID-19 infection in critical patients admitted to critical care wards, and to prevent disease transmission in patients undergoing lung transplantation.

Authors statement

This work is original and has not been published nor is it currently under consideration for publication elsewhere. All authors contributed significantly to the work, approved the manuscript, and agree with its submission to *Diagnostic Microbiology & Infectious Disease*.

CRediT authorship contribution statement

Andrea Bartolini: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Fabio Morecchiato:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Alberto Antonelli:** Writing – original draft, Methodology, Data curation, Conceptualization. **Francesca Malentacchi:** Writing – review & editing, Supervision, Conceptualization. **Gian Maria Rossolini:** Writing – review & editing, Supervision, Conceptualization. **Simona Pollini:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Outside of the submitted work, AA and GMR received honoraria from SD Biosensor for presentations at meetings within educational workshops.

Funding

SD Biosensor supported part of the study by providing STANDARD™ M10 instrument, reagents and a research grant to the laboratory.

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