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Multicenter clinical evaluation of a novel transcription-mediated amplification assay for SARS-CoV-2 molecular testing

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ABSTRACT

Introduction: The onset and spread of COVID-19 pandemic has forced clinical laboratories to rapidly expand testing capacity for SARS-CoV-2. This study evaluates the clinical performance of the TMA Procleix SARS-CoV-2 assay in comparison to the RT-PCR assay Allplex™ SARS-CoV-2 for the qualitative detection of SARS-CoV-2 RNA.
Methods: Between November 2020 and February 2021, 610 upper-respiratory specimens received for routine SARS-CoV-2 molecular testing were prospectively collected and selected at the Hospital Universitari Vall d'Hebron and the Hospital Universitari Bellvitge in Barcelona, Spain. All samples were processed in parallel with the TMA and the RT-PCR assays, and results were compared. Discrepancies were retested by an additional RT-PCR method and the clinical history of these patients was reviewed.
Results: Overall, the level of concordance between both assays was 92.0% (κ, 0.772). Most discordant results (36/38, 94.7%) corresponded to samples testing positive with the TMA assay and negative with the RT-PCR method. Of these discrepant cases, most (28/36, 77.8%) were finally classified as confirmed or probable SARS-CoV-2 cases according to the discrepant analysis.
Conclusion: In conclusion, the TMA Procleix SARS-CoV-2 assay performed well for the qualitative detection of SARS-CoV-2 RNA in a multisite clinical setting. This novel TMA assay demonstrated a greater sensitivity in comparison to RT-PCR methods for the molecular detection of SARS-CoV-2. This higher sensitivity but also the qualitative feature of this detection of SARS-CoV-2 should be considered when making testing algorithm decisions.

Evaluación clínica multicéntrica de un nuevo ensayo de amplificación mediada por transcripción para la detección molecular de SARS-CoV-2

Palabras clave:
SARS-CoV-2
COVID-19
Amplificación mediada por transcripción

RESUMEN

Introducción: El inicio y la expansión de la pandemia por COVID-19 han forzado a los laboratorios clínicos a ampliar rápidamente la capacidad de detección de SARS-CoV-2. Evaluamos el rendimiento clínico del ensayo de TMA Procleix SARS-CoV-2 en comparación con el ensayo de RT-PCR Allplex™ SARS-CoV-2 para la detección cualitativa de ARN de SARS-CoV-2.

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Métodos: Entre noviembre de 2020 y febrero de 2021 se seleccionaron prospectivamente 610 muestras del tracto respiratorio superior recibidas de rutina en el Hospital Universitario Vall d'Hebron y el Hospital Universitario de Bellvitge en Barcelona, España, para el diagnóstico molecular de SARS-CoV-2. Todas las muestras fueron procesadas en paralelo con los ensayos de TMA y RT-PCR, y se compararon los resultados. Las discrepancias se estudiaron por un método adicional de RT-PCR y se revisaron las historias clínicas de los pacientes.
Resultados: En general, la concordancia entre ambos ensayos fue del 92,0% (κ, 0,772). La mayoría de los casos discrepantes (36/38, 94,7%) correspondían a muestras positivas con el ensayo de TMA y negativas con el método de RT-PCR. De estos, la mayoría (28/36, 77,8%) fueron finalmente clasificados como casos confirmados o probables de SARS-CoV-2 de acuerdo al análisis de discrepantes.
Conclusión: El ensayo de TMA Procleix SARS-CoV-2 funcionó bien para la detección cualitativa de ARN de SARS-CoV-2 en un entorno clínico multicéntrico. Este ensayo TMA demostró una mayor sensibilidad en comparación con métodos de RT-PCR para la detección molecular de SARS-CoV-2. Esta mayor sensibilidad, pero también el carácter cualitativo de esta detección de SARS-CoV-2, se deben considerar en el diagnóstico de la infección.

Introduction

The onset and rapid spread of COVID-19 pandemic has forced clinical laboratories to rapidly expand testing capacity for SARS-CoV-2.[1,2] As quarantine measures are gradually being relaxed and novel disturbing SARS-CoV-2 variants are emerging, there is potential risk for upsurge in cases and rates of viral transmission. Accurate and sensitive viral detection methods with high-throughput diagnostic capability are essential for rapid clinical decision-making, implementing infection prevention practices and guiding public health responses. In this regard, numerous nucleic acid amplification tests (NAATs), granted with emergency-use authorization (EUA) by the Food and Drug Administration (FDA), are available for the molecular detection of SARS-CoV-2 in upper respiratory specimens.[3]

Most SARS-CoV-2 NAATs are based on real-time reverse transcription PCR (RT-PCR) techniques, such as the RT-PCR assay Allplex™ SARS-CoV-2 (Seegene, Inc., South Korea). Nevertheless, in accordance with the need for pandemic-scale diagnostic testing, transcription-mediated amplification (TMA) methods like the TMA Procleix SARS-CoV-2 assay (Grifols International, S.A., Spain) also emerged as part of the diagnostic arsenal against COVID-19.[4]

If appropriately optimized, NAATs are highly sensitive and specific. Nevertheless, in real-life clinical performance several pre-analytical and analytical issues may occasionally affect sensitivity and specificity of NAATs. Thus, the quick communication of these experiences is urgently required to better understand the potential benefits and limitations of the implementation of each technique into SARS-CoV-2 testing strategies.

Herein, we evaluate the clinical performance of the TMA Procleix SARS-CoV-2 assay in comparison to the RT-PCR assay Allplex™ SARS-CoV-2 for the qualitative detection of SARS-CoV-2 RNA. A comprehensive analysis of discrepant results between both methods is conducted to assess the relevance of these cases in the management of SARS-CoV-2 infected individuals.

Methods

Sample selection and laboratory procedures

Between November 2020 and February 2021, 154 and 456 upper-respiratory specimens received for routine SARS-CoV-2 molecular testing were prospectively collected and randomly selected for the current evaluation at the Hospital Universitari Vall d'Hebron (HUVH) and the Hospital Universitari Bellvitge (HUB) in Barcelona, Spain; respectively. The selection included nasopharyngeal and nasal swabs, placed in viral transport medium (VTM) from various manufacturers, collected from symptomatic and asymptomatic individuals, with no bias towards age or gender.

The detailed methodology for the current evaluation was conducted as follows (Fig. S1). VTM aliquots from selected fresh specimens were manually transferred into sterile tubes containing lysis buffer in a 1:1 proportion. If available, surplus specimen material was stored at −80 for subsequent analysis. SARS-CoV-2 molecular testing was first performed with the TMA Procleix SARS-CoV-2 assay. Using the same inactivated aliquot, nucleic acid extraction was performed with automated systems recommended by the manufacturer and SARS-CoV-2 detection was sequentially executed using the RT-PCR assay Allplex™ SARS-CoV-2.

In case of discrepant results, stored samples were thawed, inactivated and processed with an additional RT-PCR technique: the Alinity m SARS-CoV-2 assay (Abbott Molecular, US) or the RT-PCR cobas® SARS-CoV-2 test (Roche Molecular Systems, US). Also, discrepant cases were further analyzed through an exhaustive review of the medical record.

All technical procedures were performed as described by the manufacturers and results were interpreted according to the manufacturer's criteria.

Description of the TMA Procleix SARS-CoV-2 assay

The TMA Procleix SARS-CoV-2 assay is a novel multiplex NAAT based on TMA methodology for the qualitative molecular detection of SARS-CoV-2 virus.[4] The assay, EUA granted by the FDA, incorporates an exogenous internal control, and specifically targets the viral N gene. The test is ready to be run on the fully automated all-in-one Procleix Panther system (Grifols International, S.A.) using 750 μL material from upper-respiratory swabs. In this regard, the assay provides a ratio value that corresponds to an analytical parameter based on chemiluminescense with a qualitative interpretation. Values ≥1.00 are considered reactive/positive, according to the manufacturer's criteria.

Data analyses

Statistical analyses were performed using GraphPad Prism version 8.0.1 (GraphPad Software Inc., USA). The Cohen's kappa statistic (κ) was used to evaluate the agreement between both assays, and 95% confidence intervals (CI) were calculated by exact methods.

Minor disagreement was defined as a positive test with one assay and an inconclusive test with the other. In the current

Table 1
Comparison between TMA and RT-PCR assays ($n = 610$).

TMA Procleix SARS-CoV-2	RT-PCR Allplex™ SARS-CoV-2			Level of concordance% (95% CI)	Kappa value(95% CI)
	Positive	Inconclusive[b]	Negative		
Positive[a]	106	11	36	92.0	0.772
Negative	2	0	455	(89.5–94.0)	(0.715–0.830)

[a] The instrument provides a ratio value that corresponds to an analytical parameter based on chemiluminescense with a qualitative interpretation. Values ≥1.00 are considered reactive/positive, according to the manufacturer's criteria. In this regard, all positive results yielded ratio values ≥2.00.
[b] Inconclusive cases are considered those testing positive for the E target, but negative for both the N and the RdRP/S targets with the RT-PCR Allplex™ SARS-CoV-2 assay, according to the manufacturer's criteria.
Abbreviations: TMA: transcription-mediated amplification; RT-PCR: real-time reverse-transcriptase PCR; CI: confidence interval.

comparison, only the RT-PCR assay Allplex™ SARS-CoV-2 could yield inconclusive results, defined by the manufacturer as those cases testing positive for the E target, but negative for both the N and the RdRP/S targets. Major disagreement was considered that reverting from positive to negative depending on the study assay. If available, discrepant specimens were retested with an additional RT-PCR method and managed as follows. A confirmed case of SARS-CoV-2 was considered that discrepancy testing positive with the additional RT-PCR assay. A probable case was considered that testing negative with the additional RT-PCR technique, but having epidemiological or clinical data suggestive and compatible with recent history of SARS-CoV-2 infection. Unresolved cases were reported as inconclusive.

Institutional Review Board approval (PR(AG)259/2020) was obtained from the HUVH Clinical Research Ethics Committee.

Results

The qualitative comparison of the 610 study samples is displayed in Table 1. Overall, the level of concordance between both assays was 92.0% (95% CI, 89.5–94.0%), with a κ value of 0.772 (0.715–0.830). Of the 38 major discrepancies, 36 (94.7%) corresponded to samples testing positive with the TMA Procleix SARS-CoV-2 assay and negative with the RT-PCR assay Allplex™ SARS-CoV-2.

A comprehensive analysis of discrepant results is detailed in Tables 2A and 2B. While Table 2A focuses on the analytical approach of this evaluation, Table 2B provides an in-depth analysis of discrepant cases by incorporating some key epidemiological features from the medical record. The main conclusions of this assessment are summarized in Table 3. Overall, from the 36 discrepancies mentioned above, 28 (77.8%) were finally classified as confirmed (11/36) or probable (17/36) SARS-CoV-2 cases. On the other hand, both major discrepancies testing positive with the RT-PCR assay Allplex™ SARS-CoV-2 but negative with the TMA Procleix SARS-CoV-2 assay remained inconclusive. Finally, eight of the 11 minor disagreements (72.7%), were at last reported as confirmed (6/11) or probable (2/11) SARS-CoV-2 cases.

Discussion

The current wide availability of commercial assays to address the critical need for large-scale SARS-CoV-2 testing also requires comprehensive analytical, clinical and real-life experiences to better understand the strengths and limitations of each diagnostic technique. The present multicenter study compares the clinical performance of the TMA Procleix SARS-CoV-2 assay to a commercial RT-PCR test for the qualitative detection of SARS-CoV-2 RNA.

Overall, the TMA Procleix SARS-CoV-2 assay performed well for the detection of SARS-CoV-2 compared to the RT-PCR assay AllplexTM SARS-CoV-2, with a substantial agreement between assays (κ, 0.772). Most major discrepancies (95%) corresponded to samples testing positive with the TMA Procleix SARS-CoV-2 assay, but reverting to negative results with the RT-PCR test. When pondering the discrepant analyses, many of these cases (31%) were analytically confirmed to be positive for SARS-CoV-2, while many others (47%) were classified as probable positive according to the epidemiological and clinical history. These results suggest that the TMA Procleix SARS-CoV-2 assay may be more sensitive for the detection of SARS-CoV-2 RNA compared to RT-PCR methods, similar to what has been reported for this and other TMA tests such as the Aptima SARS-CoV-2 assay (Hologic, Inc., US).[4–7] On the other hand, most minor discrepancies, these were specimens testing positive with the TMA test but inconclusive with the RT-PCR assay Allplex™ SARS-CoV-2, were also reported as confirmed or probable SARS-CoV-2 cases in accordance with the hypothesis of an inherent higher sensitivity of the TMA assay. In this regard, findings from an *in vitro* supplemental experiment, using 10-fold dilutions in five SARS-CoV-2 positive samples, analytically reinforce this conclusion (Fig. S2).

Our study evidences that the TMA Procleix SARS-CoV-2 assay is highly sensitive. The methodology used strongly suggests that this feature, compared to RT-PCR methods, is not a matter of pre-analytical variability rather the intrinsic chemistry behind TMA technology.[8,9] Despite the presumptively high specificity of this technique, the increased sensitivity and the lack of quantitative/semi-quantitative parameters as a rough measure of SARS-CoV-2 RNA load (such as the cycle-threshold [Ct] value in RT-PCR methods) may hinder the clinical interpretation of some positive results, particularly among asymptomatic individuals, thus overestimating the true prevalence/incidence of active and transmissible infections. In low-prevalence settings, over-reporting positive cases may lead to unnecessary community isolation and contact tracing.[10] Nevertheless, in this scenario the low incidence of COVID-19 may also allow confirmation of positive TMA results with RT-PCR methods to limit this inherent deviation and optimize the clinical specificity of SARS-CoV-2 testing.[10] Moreover, pooling strategies, nowadays common for SARS-CoV-2 testing in clinical laboratories,[11,12] may clearly benefit from this highly sensitive TMA method.[4]

The study does require some considerations. First, information is missing in some discrepant cases due to the retrospective nature of the epidemiological and clinical data collection, and some specimens were unavailable for discrepant analytical testing. Also, at some point of the study, the TMA Procleix SARS-CoV-2 assay was used to actively select positive results, thus biasing the true positivity rate during that period. Furthermore, we did not strictly included acute SARS-CoV-2 infections and, as a consequence, some cases may correspond to the final stages of COVID-19 disease with uncertain clinical relevance. Finally, although chemiluminescense ratios ≥1.00 with the TMA Procleix SARS-CoV-2 assay are considered reactive/positive for SARS-CoV-2 according to the manufacturer's criteria, all positive results in this evaluation

Table 2A
Analytical evaluation of discrepant results ($n = 49$).

Case	TMA Procleix		RT-PCR Allplex™				Type of disagreement	RT-PCR Alinity m		Analytical result
		Ratio[a]		Ct				Result	Ct[c]	
	Result	*N*	Result	*E*	RdRP	*N*			RdRP & N	
1	P	4.18	N	NA	NA	NA	Major	N	NA	N
2	N	NA	P	NA	NA	37.59	Major	ND[d]	–	ND
3	P	3.92	N	NA	NA	NA	Major	P	40.99	P
4	P	3.81	N	NA	NA	NA	Major	P	39.53	P
5	P	3.81	I[b]	37.96	NA	NA	Minor	P	40.94	P
6	P	4.00	N	NA	NA	NA	Major	N	NA	N
7	P	4.24	N	NA	NA	NA	Major	N	NA	N
8	P	4.24	I[b]	38.02	NA	NA	Minor	P	40.03	P
9	P	4.18	N	NA	NA	NA	Major	ND[d]	–	ND
10	P	4.20	I[b]	36.91	NA	NA	Minor	ND[d]	–	ND
11	P	3.73	N	NA	NA	NA	Major	N	NA	N
12	P	4.15	N	NA	NA	NA	Major	P	40.90	P
13	P	4.36	N	NA	NA	NA	Major	P	39.60	P
14	P	4.21	N	NA	NA	NA	Major	P	37.21	P
15	P	4.16	N	NA	NA	NA	Major	P	40.90	P
16	P	4.05	I[b]	37.88	NA	NA	Minor	ND[d]	–	ND
17	N	NA	P	NA	NA	37.63	Major	ND[d]	–	ND
18	P	4.21	N	NA	NA	NA	Major	N	NA	N
19	P	4.23	I[b]	38.06	NA	NA	Minor	P	40.99	P
20	P	4.50	N	NA	NA	NA	Major	P	40.99	P
21	P	4.46	N	NA	NA	NA	Major	N	NA	N
22	P	3.95	N	NA	NA	NA	Major	N	NA	N
23	P	3.97	N	NA	NA	NA	Major	N	NA	N
24	P	4.00	N	NA	NA	NA	Major	N	NA	N
25	P	4.12	I	38.23	NA	NA	Minor	P	40.99	P

Case	TMA Procleix SARS-CoV-2		RT-PCR Allplex™ SARS-CoV-2				Type of disagreement	RT-PCR cobas® SARS-CoV-2			Analytical result
	Result	Ratio[a]	Result	Ct				Result	Ct		
		N		*E*	RdRP	*N*			ORF-1ab	*E*	
26	P	4.17	N	NA	NA	NA	Major	N	NA	NA	N
27	P	3.78	N	NA	NA	NA	Major	I[b]	NA	35.63	I[b]
28	P	3.84	N	NA	NA	NA	Major	I[b]	NA	37.73	I[b]
29	P	4.16	N	NA	NA	NA	Major	N	NA	NA	N
30	P	3.93	N	NA	NA	NA	Major	P	32.4	34.98	P
31	P	3.90	N	NA	NA	NA	Major	N	NA	NA	N
32	P	4.22	N	NA	NA	NA	Major	I[b]	NA	38.47	I[b]
33	P	4.02	N	NA	NA	NA	Major	N	NA	NA	N
34	P	3.95	N	NA	NA	NA	Major	N	NA	NA	N
35	P	4.28	N	NA	NA	NA	Major	P	36.19	37.22	P
36	P	3.92	I[b]	36.58	NA	NA	Minor	P	33.15	35.01	P
37	P	4.05	N	NA	NA	NA	Major	N	NA	NA	N
38	P	4.59	I[b]	38.15	NA	NA	Minor	P	33.35	34.51	P
39	P	4.03	N	NA	NA	NA	Major	N	NA	NA	N
40	P	4.25	N	NA	NA	NA	Major	I[b]	NA	38.01	I[b]
41	P	4.22	N	NA	NA	NA	Major	P	35.58	35.87	P
42	P	3.99	N	NA	NA	NA	Major	N	NA	NA	N
43	P	3.84	N	NA	NA	NA	Major	N	NA	NA	N
44	P	4.04	I[b]	38.18	NA	NA	Minor	N	NA	NA	I[b]
45	P	4.46	I[b]	38.26	NA	NA	Minor	N	NA	NA	I[b]
46	P	4.04	N	NA	NA	NA	Major	I[b]	NA	37.94	I[b]
47	P	4.35	N	NA	NA	NA	Major	P	30.80	31.60	P
48	P	4.15	I[b]	38.14	NA	NA	Minor	N	NA	NA	I[b]
49	P	4.33	N	NA	NA	NA	Major	N	NA	NA	N

[a] The ratio provided by the instrument corresponds to an analytical parameter based on chemiluminescense with a qualitative interpretation. Values ≥1.00 are considered reactive/positive, according to the manufacturer's criteria.

[b] Inconclusive cases are considered: (i) those testing positive for the E target, but negative for both the N and the RdRP/S targets with the RT-PCR Allplex™ SARS-CoV-2 assay, according to the manufacturer's criteria, (ii) those testing positive for the E target, but negative for the ORF-1ab target with the RT-PCR cobas® SARS-CoV-2 assay, according to the manufacturer's criteria, and (iii) those unresolved after the discrepant analytical testing.

[c] The two SARS-CoV-2-specific probes are labelled with the same fluorophore, so the test provides a unique Ct value for both RdRP and N targets.

[d] Specimens unavailable for discrepant analytical testing.

A minor disagreement is considered that discrepancy testing positive with one assay but inconclusive with the other study technique. Contrary, a major disagreement is considered that reverting from positive to negative depending on the study assay.

Abbreviations: TMA: transcription-mediated amplification; RT-PCR: real-time reverse-transcriptase PCR; Ct: cycle-threshold; P: positive; N: negative; I: inconclusive; NA: not amplified; ND: not determined.

Table 2B
Comprehensive analysis of discrepant results ($n = 49$).

Case	Age	Gender	Centre	Analytical result	SC2 serostatus[a]	SC2 NAAT last month	SC2 NAAT subsequent week	Relevant clinical history	Conclusion
1	39.7	F	HUB	N	ND	P	ND	History of SC2 infection	Probable
2	41.2	F	HUB	ND	ND	N	ND	Unknown	I
3	65.5	F	HUB	P	P	P	ND	History of SC2 infection	Confirmed
4	55.1	M	HUB	P	N	N	ND	Pneumonia	Confirmed
5	23.5	F	HUB	P	ND	ND	P	Contact of SC2 infected individual	Confirmed
6	47.9	F	HUB	N	ND	P	ND	History of SC2 infection	Probable
7	21.1	F	HUB	N	ND	P	ND	History of SC2 infection	Probable
8	68.9	M	HUB	P	ND	P	P	History of SC2 infection	Confirmed
9	56.7	M	HUB	ND	N[b]	P	ND	History of SC2 infection	Probable
10	20.0	M	HUB	ND	P	P	P	History of SC2 infection	Probable
11	8.0	F	HUB	N	ND	ND	ND	Unknown	I
12	85.2	M	HUB	P	ND	ND	P	Contact of SC2 infected individual	Confirmed
13	56.8	M	HUB	P	P	ND	ND	History of SC2 infection[c]	Confirmed
14	25.4	M	HUB	P	ND	ND	ND	Unknown	Confirmed
15	45.2	F	HUB	P	P	P	ND	History of SC2 infection	Confirmed
16	39.2	F	HUB	ND	ND	P	ND	History of SC2 infection	Probable
17	66.3	M	HUB	ND	ND	ND	ND	Symptomatic contact of SC2 infected individual	I
18	34.1	F	HUB	N	P	P	ND	History of SC2 infection	Probable
19	70.4	F	HUB	P	ND	ND	ND	History of SC2 infection[c]	Confirmed
20	46.8	F	HUB	P	P	P	ND	History of SC2 infection	Confirmed
21	45.9	M	HUB	N	N[b]	P	ND	History of SC2 infection	Probable
22	31.8	M	HUB	N	ND	ND	ND	History of SC2 infection[c]	Probable
23	5.0	M	HUB	N	ND	ND	ND	Unknown	I
24	61.0	F	HUB	N	ND	N	ND	Unknown	I
25	2.0	M	HUB	P	ND	ND	P	Unknown	Confirmed
26	36.1	M	HUVH	N	ND	ND	ND	History of SC2 infection[d]	Probable
27	1.8	M	HUVH	I	ND	ND	ND	Asymptomatic screening	Probable
28	31.6	F	HUVH	I	P	P	ND	History of SC2 infection	Probable
29	57.7	F	HUVH	N	ND	ND	ND	History of SC2 infection[d]	Probable
30	14.9	M	HUVH	P	ND	ND	ND	Symptomatic contact of SC2 infected individual	Confirmed
31	37.8	M	HUVH	N	ND	ND	ND	Contact of SC2 infected individual	I
32	37.4	F	HUVH	I	ND	ND	ND	Symptomatic contact of SC2 infected individual	Probable
33	26.5	F	HUVH	N	ND	ND	ND	Mild symptoms compatible with COVID-19	I
34	54.1	F	HUVH	N	P	P	ND	History of SC2 infection	Probable
35	94.8	F	HUVH	P	P	ND	ND	History of SC2 infection	Confirmed
36	59.1	M	HUVH	P	ND	ND	ND	Mild symptoms compatible with COVID-19	Confirmed
37	44.5	F	HUVH	N	P	P	ND	History of SC2 infection	Probable
38	42.7	M	HUVH	P	ND	ND	ND	Mild symptoms compatible with COVID-19	Confirmed
39	19.4	F	HUVH	N	ND	N	ND	History of SC2 infection[c]	Probable
40	57.1	F	HUVH	I	ND	ND	ND	Asymptomatic screening	I
41	18.1	F	HUVH	P	P	N	ND	History of SC2 infection[c]	Confirmed
42	57.5	F	HUVH	N	P	ND	ND	History of SC2 infection[c]	Probable
43	56.3	M	HUVH	N	ND	ND	ND	Contact of SC2 infected individual	I
44	11.9	M	HUVH	I	ND	ND	ND	Asymptomatic screening	I
45	50.6	M	HUVH	I	ND	ND	ND	Contact of SC2 infected individual	I
46	58.4	F	HUVH	I	P	P	ND	History of SC2 infection	Probable
47	63.6	F	HUVH	P	P	ND	ND	History of SC2 infection[d]	Confirmed
48	30.0	F	HUVH	I	ND	ND	ND	Mild symptoms compatible with COVID-19	I
49	16.4	M	HUVH	N	ND	ND	ND	Contact of SC2 infected individual	I

[a] SC2 serostatus refers to the presence or absence of antibodies suggestive and compatible with history of SARS-CoV-2 infection, disregarding vaccination.

[b] These cases correspond to non-reactive results near the positivity threshold. Of note, this phenomenon may suggest the initial immune response against SARS-CoV-2 with the incipient production of specific antibodies.

[c] History of positive SARS-CoV-2 NAAT before the last month.

[d] History of positive SARS-CoV-2 antigen test in the last month.

Abbreviations: SC2: SARS-CoV-2; NAAT: nucleic acid amplification test; M: male; F: female; P: positive; HUB: Hospital Universitari Bellvitge; HUVH: Hospital Universitari Vall d'Hebron; N: negative; ND: not determined; I: inconclusive.

Table 3
Summary of the discrepant analysis described in Tables 2A and 2B (*n* = 49).

Type of disagreement		Analytical result[b] *N.* (%)				Conclusion[c] *N.* (%)		
		Positive	Negative	Inconclusive[d]	Not determined[e]	Confirmed	Probable	Inconclusive
TMA Procleix Positive/RT-PCR Allplex™ Negative (n = 36)	Major	11 (30.6)	19 (52.8)	5 (13.9)	1 (2.8)	11 (30.6)	17 (47.2)	8 (22.2)
TMA Procleix Negative/RT-PCR Allplex™ Positive (n = 2)	Major	–	–	–	2 (100.0)	–	–	2 (100.0)
TMA Procleix Positive/RT-PCR Allplex™ Inconclusive[a] (n = 11)	Minor	6 (54.6)	–	3 (27.3)	2 (18.2)	6 (54.6)	2 (18.2)	3 (27.3)

[a] Inconclusive cases are considered those testing positive for the *E* target, but negative for both the *N* and the RdRP/S targets with the RT-PCR Allplex™ SARS-CoV-2 assay, according to the manufacturer's criteria.

[b] The statements correspond to the analytical result of discrepant of cases, presented in Table 2A. In this assessment, discrepancies were further studied with an additional RT-PCR technique for SARS-CoV-2 testing.

[c] The statements correspond to the comprehensive analysis of discrepant results, presented in Table 2B. In this assessment, discrepant cases were further studied through a comprehensive clinical and epidemiological review of the medical record.

[d] Unresolved cases after the discrepant analytical testing (identified as I-inconclusive in Tables 2A and 2B).

[e] Specimens unavailable for discrepant analytical testing (identified as ND-not determined in Tables 2A and 2B).

A minor disagreement is considered that discrepancy testing positive with one assay but inconclusive with the other study technique. Contrary, a major disagreement is considered that reverting from positive to negative depending on the study assay.

yielded ratio values ≥2.00. Of note, in our experience, positive ratios between 1.00 and 2.00 are rarely confirmed with RT-PCR methods.

In conclusion, the TMA Procleix SARS-CoV-2 assay performed well for the qualitative detection of SARS-CoV-2 RNA in a multisite clinical setting. Furthermore, this novel TMA assay demonstrated greater sensitivity in comparison to RT-PCR tests for the molecular detection of SARS-CoV-2, and this performance characteristic should be considered when making testing algorithm decisions. Nevertheless, additional experiences are required to fully refine the applicability of this methodology to the volatile dynamics of COVID-19 pandemic.

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Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eimc.2022.01.014.

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