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Short communication

Evaluation of the Aptima™ transcription-mediated amplification assay (Hologic®) for detecting SARS-CoV-2 in clinical specimens

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ABSTRACT

Background: The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which appeared in late 2019, has been limited by isolating infected individuals. However, identifying such individuals requires accurate diagnostic tools.
Objective: This study evaluates the capacity of the Aptima™ Transcription-Mediated Amplification (TMA) assay (Hologic® Panther System) to detect the virus in clinical samples.
Study design: We compared the Aptima™ assay to two in-house real-time RT-PCR techniques, one running on the Panther Fusion™ module and the other on the MagNA Pure 96 and Light-Cycler 480 instruments. We included a total of 200 respiratory specimens: 100 tested prospectively and 100 retrospectively (25 -ve/75 +ve).
Results: The final Cohen's kappa coefficients were: $\kappa = 0.978$ between the Aptima™ and Panther Fusion™ assays, $\kappa = 0.945$ between the Aptima™ and MagNA/LC480 assays and $\kappa = 0.956$ between the MagNA/LC480 and Panther Fusion™ assays.
Conclusion: These findings indicate that the Aptima™ SARS-CoV-2 TMA assay data agree well with those obtained with our routine methods and that this assay can be used to diagnose coronavirus disease 2019 (COVID-19).

1. Background

The emergence of a new severe acute respiratory syndrome coronavirus (SARS-CoV-2) in late 2019 and its rapid spread worldwide led to over 6 million cases of coronavirus disease 2019 (COVID-19) by 1 June 2020 [1,2]. As the rapid identification of infected individuals and their isolation is essential to prevent the spread of the disease, it became necessary to develop accurate, easy-to-use molecular diagnostic assays. Virology laboratories first developed and published in-house techniques [3,4], which were soon followed by commercial assays. These must be validated on clinical specimens in addition to preliminary tests on culture lysates/supernatants or serially diluted RNA transcripts. Nasopharyngeal swabs are generally used for diagnosing viral respiratory tract infections, while deeper samples, such as tracheal aspirations or broncho-alveolar lavage fluids are used only in severe cases [5].

2. Objectives

This study evaluates the performance of the automated commercial Aptima™ SARS-CoV-2 assay (Hologic® Panther System) on clinical samples. This test, which uses transcription-mediated amplification (TMA) to detect SARS-CoV-2, was compared to two in-house reverse transcriptase – polymerase chain reaction (RT-PCR) assays we presently use to diagnose COVID-19.

3. Study design

3.1. Samples

We tested 200 respiratory samples (198 nasopharyngeal swabs and 2 tracheal aspirations) from patients suspected of having COVID-19 in April and May 2020 (54.5 % men; median [IQR] age: 61 [38–83] years). Half of the samples were tested with all three assays in parallel. These were the prospective samples (98 nasopharyngeal swabs + 2

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tracheal aspirations). The remaining 100 nasopharyngeal swabs were selected based on the results obtained with the Panther Fusion™ module in open access (see below). They included 25 samples that were negative, 25 that were positive with a cycle threshold (Ct) value > 35, 25 that were positive with a Ct of 25–35, and 25 that were positive with a Ct < 25. These retrospective samples had been stored at −20 °C for up to 43 days.

3.2. Methods

The Aptima™ SARS-CoV-2 assay was performed following the manufacturer's instructions. Virus transport medium (500 µL) was manually placed in the Panther™ tube containing 710 µL of lysis buffer. The instrument used 360 µL of this mix for the lysis and capture of nucleic acids. The Aptima™ assay targets two virus sequences located on the ORF1ab gene. An internal control was included.

Our two in-house assays that both target virus sequences on the SARS-CoV-2 RNA-dependent RNA-polymerase (RdRp) gene, use primers and probes (IP-2 and IP-4) and amplification programs from the Institut Pasteur, Paris [4]. The first assay uses the MagNA Pure 96 instrument (Roche Diagnostics) to extract nucleic acids from 200 µL of virus transport medium eluted in 100 µL. RT-PCR is then performed on the Light-Cycler 480 instrument (LC480) (Roche Diagnostics) using the AccuStart™ Taq DNA polymerase (QuantaBio) and 2 µL of nucleic acids (total reaction volume: 10 µL). The second assay uses the same primers and probes on the Panther Fusion™ module (Hologic®) in open access. Samples were prepared as described above for the Aptima™ assay.

3.3. Data analysis

Because there is no reference standard method for the detection of SARS-CoV-2, we calculated the concordance between assays by using Cohen's kappa coefficient (κ), the positive percent agreement, the negative percent agreement and overall percent agreement, all with 95 % confidence intervals [6,7].

4. Results and discussion

4.1. Prospective study

The prospective study provided 2 positive and 98 negative samples, and there was perfect concordance between results of the Aptima™ assay and those of the Panther Fusion™ assay and very good concordance between the Aptima™ assay and the MagNA/LC480 assay. One sample was faintly positive with the MagNA/LC480 assay (virus IP-4 target, Ct = 39.97 and IP-2 -ve) and negative with the other two assays (sample A2 0141 0134, *cf*. Table S1 in Supplementary materials).

4.2. Retrospective study

All positive samples with Ct values < 35 (n = 50) with the Panther Fusion™ assay tested positive with the other two assays. The MagNA/LC480 assay detected only one virus target (IP-4) in 6 of these samples (initial Ct: 32–35) (*cf*. Table S2 in Supplementary materials).

The 25 (Ct > 35) positive samples detected by the Panther Fusion™ assay included 20 (80 %) samples that were positive with The Aptima™ assay and 17 (68 %) that were positive with the MagNA/LC480 assay. These latter included 13 (65 %) in which only one virus target (IP-4) was detected. Five (20 %) samples were negative with the Aptima™ assay and eight (32 %) with the MagNA/LC480 assay, four of these were negative with both assays. These nine samples were tested again with the Panther Fusion™ assay since sample storage can result in the partial degradation of virus RNA, and thus to reduced assay sensitivity. Four were negative and five were positive or inconclusive (three with only IP-2, one with only IP-4 and one with both targets) (*cf*. Table S2). These discrepancies could also be due to selection bias - samples were chosen based on the Panther Fusion™ assay results.

All the Panther Fusion™ assay negative samples were also negative with the Aptima™ assay, except for one invalid sample. One sample was positive with the MagNA/LC480 assay, but displayed atypical PCR curves. A second RT-PCR on the same nucleic acid extract was negative (sample A2 0139 3655, *cf*. Table S2).

4.3. Concordance between assays

The invalid rate, all results included, with the Aptima™ assay was 0.5 % (1/200). The Aptima™ and Panther Fusion™ assays gave a Cohen's coefficient κ = 0.978 [0.949–1.000], a positive percent agreement = 97.3 % [95.0–99.6 %], a negative percent agreement = 100 % [100 − 100%] and an overall percent agreement = 99.0 % [97.6–100 %] after re-testing of discrepancies. The Aptima™ and MagNA/LC480 assays had a Cohen's coefficient κ = 0.945 [0.898 − 0.993], a positive percent agreement = 98.6 % [96.9–100 %], a negative percent agreement = 96.9 % [94.5–99.3 %] and an overall percent agreement = 97.5 % [95.3–99.7 %]. The MagNA/LC480 and Panther Fusion™ assays had a Cohen's coefficient κ = 0.956 [0.914 − 0.999], a positive percent agreement = 94.5 % [91.4–97.7 %], a negative percent agreement = 100 % [100 − 100%] and an overall percent agreement = 98.0 % [96.1–99.9 %] (*cf*. Table 1).

The Aptima™ SARS-CoV-2 TMA assay was given emergency use authorization by the United States Food and Drug Administration [8] and the CE mark for *in vitro* diagnostic use in Europe.

We conclude that the results of the commercial Aptima™ SARS-CoV-2 TMA assay agreed very closely with those obtained with our in-house assays. It includes an internal control to detect inhibition of amplification. The Panther™ instrument also provides random access, which allows urgent samples prioritization to obtain results within 3.5 h with the Aptima™ assay, and can deliver up to 60 results per hour. This can help laboratories provide rapid, high-throughput diagnosis during the COVID-19 pandemic crisis.

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Table 1
Concordance of qualitative results.

A. Aptima™ and Panther Fusion™ assays

Aptima™\Panther Fusion™	Positive	Negative	Total
Positive	72	0	72
Negative	2	125	127
Total	74	125	199[a]

B. Aptima™ and MagNA/LC480 assays

Aptima™\MagNA/LC480	Positive	Negative	Total
Positive	68	4	72
Negative	1	126	127
Total	69	130	199*

C. MagNA/LC480 and Panther Fusion™ assays

MagNA/LC480\Panther Fusion™	Positive	Negative	Total
Positive	69	0	69
Negative	4	127	131
Total	73	127	200

[a] One of the 200 samples was invalid with the Aptima™ assay.

Declaration of Competing Interest

None declared.

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.2020.104541.

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