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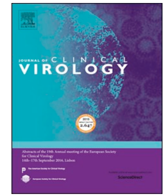

High-Throughput Transcription-mediated amplification on the Hologic Panther is a highly sensitive method of detection for SARS-CoV-2

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ABSTRACT

Background: As the demand for laboratory testing for SARS-CoV-2 increases, additional varieties of testing methodologies are being considered. While real time polymerase chain reaction (RT-PCR) has performed as the main method for virus detection, other methods are becoming available, including transcription mediated amplification (TMA). The Hologic Aptima SARS-CoV-2 Assay utilizes TMA as a target amplification mechanism, and it has only recently received Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA).

Objectives: We sought to compare the sensitivity and specificity of the Aptima SARS-CoV-2 Assay to RTPCR as a means of SARS-CoV-2 detection in a diagnostic setting.

Study design: We performed a limit-of-detection study (LoD) to assess the analytical sensitivity of TMA and RT-PCR. This preceded a comparison of the methods using previously evaluated clinical specimens (nasopharyngeal swabs) using 116 human specimens tested by both methodologies. Specimens included sixty-one (61) specimens found reactive by real-time PCR, fifty-one (51) found non-reactive, and four (4) deemed inconclusive.

Results: The Aptima SARS-CoV-2 Assay showed a markedly higher analytical sensitivity than RT-PCR by LoD study. Evaluation of clinical specimens resulted in fewer inconclusive results by the SARS-CoV-2 assay, leading to potentially higher clinical sensitivity.

Conclusions: Higher analytical sensitivity may explain TMA's ability to ascertain for the presence of SARS-CoV-2 genome in human specimens deemed inconclusive by real-time PCR. TMA provides an effective, highly sensitive means of detection of SARS-CoV-2 in nasopharyngeal specimens.

1. Introduction

The foundation of COVID-19 diagnostic testing has included direct detection of viral genomes through nucleic acid amplification methods. For tests that have been utilized under an Emergency Use Authorization by the Food and Drug Administration (FDA), the sole mechanism of amplification used in such tests to date of this manuscript has been real-time PCR (RT-PCR). Assessments of the sensitivities of these tests have been generated and provided to the scientific community, including comparisons of CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel, the Hologic Panther Fusion and the Diasorin Simplexa, among others [1–4]. Tests that utilize mechanisms of amplification other than the polymerase chain reaction are now becoming available. Among them is the Hologic Panther (non-Fusion) Aptima platform which utilizes transcription mediated amplification (TMA) to amplify the number of target genomes. The Hologic Panther is not uncommonly located in public health and clinical laboratories. This is primarily due to its ease-of-use and high-throughput capability. At the time of preparation of this manuscript, the Hologic Panther is located in 54 % of public health laboratories, making it a strong candidate for contributing to the high-throughput testing needs of states and counties seeking to reopen their businesses and institutes [5]. Additionally, while throughput of testing is a key feature that is sought in detection assays for SARS-CoV-2, sensitivity is likely also a very significant trait. Reports of false negative results from real time PCR testing indicate that even the high analytical sensitivity of RT-PCR may be challenged by the pathology of COVID-19

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[6,7]. For this reason, any new tool in the diagnostic arsenal should be assessed for its ability to detect sensitively this virus. Herein, we present data from a comparison study between RT-PCR and TMA as employed by the Hologic Aptima SARS-CoV-2 Assay. This study includes nasopharyngeal swabs taken from individuals tested for SARS-CoV-2 either in a medical or public health screening setting. Additionally, an assessment of analytical sensitivity and limit-of-detection was performed using a quantitated solution of viral genome diluted in transport matrices.

2. Materials and methods

Specimens (116) were collected throughout the state of Nevada (April 1 – May 8, 2020) and included symptomatic individuals (self-reported presence of fever, cough or shortness of breath) or individuals associated with an outbreak at a facility, regardless of symptomology. Specimens were taken by nasopharyngeal swab and transported to the Nevada State Public Health Laboratory in viral transport medium (VTM). Specimens were transported on cold packs and stored by refrigeration (4 − 8 °C) for 72 h or less prior to being subject to nucleic acid extraction and subsequent real time PCR. Extraction was performed by Omega Biotek MagBind Viral DNA/RNA 96 Kit following manufacturer's instructions with an elution volume of 100 μl. Eluted RNA (5 μl for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (111 specimens), 10 μl for the Taqpath COVID-19 (EUA) Multiplex assay (Thermo Scientific, Waltham, MA) (5 specimens) was subjected to real time PCR either by the CDC EUA Real Time PCR for SARS-CoV-2, or the Taqpath COVID-19 Multiplex assay. Both assays were performed according to their respective Emergency Use Authorized procedures. Comparison of the Taqpath COVID-19 Multiplex relative to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel showed 100 % sensitivity and specificity relative to one another, with 100 % concordance through 20 specimens. Real-time specimens may be "reactive", "non-reactive" and "inconclusive". Inconclusive results by the utilized RT-PCR assays are caused when only one of two or three genomic targets shows amplification for a particular specimen. The cause of this is due either to the presence of very low numbers of viral genomic targets for detection [2], or false, low-level reactivity for a singular primer/probe set.

Patient specimens were stored at −80 °C for 1–5 weeks prior to assessment by transcription-mediated amplification. Transcription-mediated amplification (TMA) was performed on the Hologic Panther using RUO versions of the SARS-CoV-2 detection assay and included the following steps: 500 μl of VTM was added to 710 μl of Hologic lysis tube solution. Such specimens were loaded onto the Hologic Panther and tested by programmed protocol, which includes the analysis of 360 μl of lysed specimen. Specimens resulted by the Panther are recorded as "positive", "negative" or "Invalid" ("invalid" did not occur during the course of this study). With consideration of inconclusive specimens: true positivity is ascertained by reactivity by two orthogonal molecular tests (in this case, TMA and RT-PCR). As a result, two of the evaluated inconclusive specimens in this study are deemed true positives and two are considered true negatives.

Analytical sensitivity / limit-of-detection analysis was performed using quantitated genomic RNA from SARS-CoV-2, Isolate USA-WA1/2020 (BEI Resources, Manassas, VA). The stock solution of 5.5 × 10e7 genome equivalents/mL was diluted in 10-fold series in viral transport media or Aptima Multitest Collection fluid. Dilutions were tested in replicates of five.

3. Results

To assess differences in analytical sensitivity between real-time PCR and TMA, we performed a limit-of-detection (LoD) study by creating a dilution series of purified / quantified SARS-CoV-2 genomic material either in VTM or APTIMA collection matrix. Specimens at each dilution were tested in replicates of five each by either detection method. As shown in Table 1, the Taqpath COVID-19 Multiplex assay demonstrated detection capability of 5/5 (100 %) samples at 5.5 × 10e5 copies per milliliter and at least one SARS-CoV-2 gene in 5/5 (100 %) of samples at a concentration of 5.5 × 10e4 copies per milliliter. CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel detected 100 % of specimens at 5.5 × 10e5 copies per milliliter and 2/5 (40 %) of specimens at 5.5 × 10e4 copies per milliliter. The Hologic Panther Aptima SARS-CoV-2 Assay detected SARS-CoV-2 genomes in 5/5 (100 %) of samples at a minimal concentration of 5.5 × 10e3 copies per milliliter and detected SARS-CoV-2 genome at 5.5 × 10e2 copies in 1 out of 5 specimens (20 %).

Noting the sensitivity difference demonstrated by the LoD study, we sought to assess the performance of TMA on specimens previously tested by RT-PCR for SARS-CoV-2. One hundred and sixteen (116) specimens which were nasopharyngeal swabs, and for which sufficient volume existed were selected for testing. This included specimens that were tested by real-time PCR that were: reactive (51), non-reactive (61) and inconclusive [4]. Reactive specimens, as evaluated by RT-PCR for the N1 gene had a Ct mean and standard deviation of 26.20 ± 6.67, a median of 25.50 and an interquartile range of 10.55. For the N2 gene they possessed a mean Ct and standard deviation of 26.86 ± 7.53, a median of 26.44 and an interquartile range of 13.98. Notable results from performance of TMA include one specimen that was positive by RT-PCR but negative by TMA (CDC EUA real time PCR Ct Value: 35.73). Four specimens found inconclusive (reactive in duplicate for one of two genes detectable by the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel) by real time PCR resulted conclusively as two positive and two negative by TMA. Omitting the inconclusive specimens from analyses, the Panther demonstrated a 98 % sensitivity (50/51 detected) and generated zero false positive results (100 % specificity). Including inconclusive specimens in the analysis (according to definition above which shows them to be two positive and two negative specimens) results in a sensitivity of 98.1 % (52/53) for TMA and 96.2 % (51/53) for real-time PCR. Percent agreement for specimens found non-reactive ("negative" by TMA) by real-time PCR was 100 % and was 98 % for those found reactive ("positive" by TMA) (Table 2).

4. Discussion

The Hologic Panther SARS-CoV-2 transcription mediated amplification test showed higher analytical sensitivity when compared to real time PCR for the detection of SARS-CoV-2. It additionally provided fewer inconclusive results when evaluating human specimens - perhaps as a result of enhanced sensitivity. In the assessment of clinical specimens, there was one specimen found to be reactive by RT-PCR and non-reactive by TMA. This result is surprising considering the observed higher analytical sensitivity of TMA. We hypothesize that the freezing and thawing, and perhaps storage of specimens may have had a detrimental effect on the specimen, prior to TMA testing. It is notable as well that specimens (collected in VTM) were subject to some dilution prior

Table 1
Analytical Sensitivity Comparison.

copies/mL*	TMA** reactivity	Taqpath RT-PCR reactivity†	CDC RT-PCR††
5.5 × 10e5	5/5 (100 %)	5/5 (100 %)	5/5 (100 %)
5.5 × 10e4	5/5 (100 %)	5/5*** (100%)	2/5 (40 %)
5.5 × 10e3	5/5 (100 %)	0/5 (0%)	0/5 (0%)
5.5 × 10e2	1/5 (20 %)	0/5 (0%)	0/5 (0%)
5.5 × 10e1	0/5 (0%)	0/5 (0%)	0/5 (0%)
5.5 × 10e0	0/5 (0%)	0/5 (0%)	0/5 (0%)

* Concentration of SARS-CoV-2 genomic RNA, BEI Resources, NR-52285.
** Hologic Panther SARS-CoV-2 Assay.
† ThermoFisher Taqpath COVID-19 Multiplex Assay.
†† CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.
*** Specimens were reactive for only one of three genes in assay.

Table 2
Performance of TMA and RT-PCR on nasopharyngeal specimens.

	TMA	RT-PCR*
NEGATIVE	64	61
POSITIVE	52	51

* RT-PCR generated 4 inconclusive results; two inconclusive results were positive by TMA, two were negative.

to testing on the Hologic Aptima system. Using manufacturer's recommendations, 500 µl of specimen material was first added to 710 µl of lysis buffer prior to testing. This means that the sensitivity of the Aptima test may have been underestimated in the testing of human specimens. The finding of two specimens deemed inconclusive by RT-PCR but positive by TMA match the findings of higher analytical sensitivity.

A differential in sensitivities between the two mechanisms is not surprising. Previous studies have shown higher sensitivities associated with TMA based assays compared to RT-PCR for viral detection [8,9]. Specimens subjected to TMA include a process where 360 µl of collected specimen (transport medium) enter the detection process and is tested. For real time PCR, while 200 µl enters the testing process (extraction), only 5 or 10 µl are subjected to PCR. Moreover, the mechanisms of amplification are very different. PCR involves a doubling of target nucleic acid in each of 40 cycles while transcription-mediated amplification includes the generation of potentially thousands of transcribed copies of target which can each be subsequently turned into transcriptional templates. An additional benefit of the Panther (TMA) platform, not quantified in this study, was its ease-of-use relative to RT-PCR. Samples need only be loaded by random access to the Panther device, which extracts and amplifies/detects in a fully automated fashion. RT-PCR can only be effectively utilized in a batch testing system, which includes extensive extraction prior to detection. This ability to perform high-throughput testing, on a sensitive molecular detection platform has potential to make a significant contribution to the COVID-19 pandemic, where such high demands exist for testing. The Hologic Panther SARS-CoV-2 assay appears to have these traits.

Declaration of Competing Interest

The authors of "High-Throughput Transcription-mediated amplification on the Hologic Panther is a highly sensitive method of detection for SARS-CoV-2" have no conflicts of interest, including no personal relationships or financial relationships that could appear to influence the work in this manuscript.

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References

[1] L. Bordi, A. Piralla, E. Lalle, F. Giardina, F. Colavita, M. Tallarita, G. Sberna, F. Novazzi, S. Meschi, C. Castilletti, A. Brisci, G. Minnucci, V. Tettamanzi, F. Baldanti, M.R. Capobianchi, Rapid and sensitive detection of SARS-CoV-2 RNA using the Simplexa^{TM} COVID-19 direct assay, J. Clin. Virol. 128 (2020) 104416, https://doi.org/10.1016/j.jcv.2020.104416 [Epub ahead of print] PubMed PMID: 32388470; PubMed Central PMCID: PMC7198196.

[2] X. Lu, L. Wang, S.K. Sakthivel, B. Whitaker, J. Murray, S. Kamili, et al., US CDC real-time reverse transcription PCR panel for detection of severe acute respiratory syndrome coronavirus 2, Emerg. Infect. Dis. (2020), https://doi.org/10.3201/eid2608.201246 [date cited].

[3] K. Okamaoto, K. Shirato, N. Nao, S. Saito, T. Kageyama, H. Hasegawa, T. Suzuki, S. Matsuyama, M. Takeda, An assessment of real-time RT-PCR kits for SARS-CoV-2 detection, Jpn. J. Infect. Dis. (2020), https://doi.org/10.7883/yoken.JJID.2020.108 [Epub ahead of print] PubMed PMID: 32350226.

[4] W. Zhen, R. Manji, E. Smith, G.J. Berry, Comparison of four molecular in vitro diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens, J. Clin. Microbiol. (2020), https://doi.org/10.1128/JCM.00743-20 pii: JCM.00743-20, [Epub ahead of print] PubMed PMID: 32341143.

[5] COVID-19 Public Health Laboratory Capacity and Capabilities Weekly Data Report | Week 4, (2020) (05.04.2020).

[6] A.T. Xiao, Y.X. Tong, S. Zhang, False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: rather than recurrence, J. Med. Virol. (2020), https://doi.org/10.1002/jmv.25855 [Epub ahead of print] PubMed PMID: 32270882.

[7] B.E. Young, S.W.X. Ong, S. Kalimuddin, J.G. Low, S.Y. Tan, J. Loh, O.T. Ng, K. Marimuthu, L.W. Ang, T.M. Mak, S.K. Lau, D.E. Anderson, K.S. Chan, T.Y. Tan, O.T.Y. Ng, L. Cui, Z. Said, L. Kurupatham, M.I. Chen, M. Chan, S. Vasoo, L.W.F. Wang, B.H. Tan, R.T.P. Lin, V.J.M. Lee, Y.S. Leo, D.C. Lye, Singapore 2019 Novel Coronavirus Outbreak Research Team, Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore, JAMA (2020), https://doi.org/10.1001/jama.2020.3204 [Epub ahead of print] PubMed PMID: 32125362;PubMed Central PMCID: PMC7054855.

[8] A. Hatzakis, H. Papachristou, S.J. Nair, J. Fortunko, T. Foote, H. Kim, T.L. Peling, A.J. Worlock, Analytical characteristics and comparative evaluation of Aptima HIV-1 Quant Dx assay with Ampliprep/COBAS TaqMan HIV-1 test v2.0, Virol. J. 13 (1) (2016) 176 PubMed PMID: 27769309; PubMed Central PMCID: PMC5073876.

[9] W.P. Hofmann, V. Dries, E. Herrmann, B. Gärtner, S. Zeuzem, C. Sarrazin, Comparison of transcription mediated amplification (TMA) and reverse transcription polymerase chain reaction (RT-PCR) for detection of hepatitis C virus RNA in liver tissue, J. Clin. Virol. 32 (4) (2005) 289–293 PubMed PMID: 15780807.