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Short communication

Detection of low levels of SARS-CoV-2 RNA from nasopharyngeal swabs using three commercial molecular assays

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ABSTRACT

In response to the COVID-19 pandemic, commercial molecular assays for SARS-CoV-2 testing have been rapidly developed and broadly deployed in laboratories worldwide. Although these assays have been reported to correlate well, we sought to compare the Xpert® Xpress SARS-CoV-2 to the cobas® SARS-CoV-2 or the Lightmix® Modular SARS and Wuhan CoV E-gene assay for nasopharyngeal (NP) swabs with low levels of SARS-CoV-2 RNA. Thirty-seven NP swabs were studied, including 10 samples with a moderate cycle threshold (Ct) between 30–33.9, and 22 with Ct ≥ 34, and 5 negative for SARS-CoV-2. Overall concordance on initial comparison was 86.5 % (32/37), which was 100 % concordance for samples with Ct values ranging between 30 − 33.9. Discordance amongst samples showing a Ct ≥34 was 22.7 % (5/22). Endpoint value analysis on the Xpress SARS-CoV-2 within the discordant samples noted two with an endpoint value > 5, which were detected by the cobas® or Lightmix®. Testing of SARS-CoV-2 on the three commercial assays was comparable for NP swabs with moderate Ct values, while high Ct values were less concordant. Importantly, analysis of Xpert® endpoint values improved interpretation of discrepant results.

1. Introduction

The global COVID-19 pandemic caused by SARS-CoV-2 has challenged healthcare systems throughout the world. Timely diagnosis of COVID-19 cases and subsequent infection control and public health containment measures are essential for healthcare facilities and the community. One of the World Health Organization's key tenets to control the pandemic is to build and maintain public health capacity, including detection of symptomatic cases, testing of suspect cases within 24 h of specimen collection, self-isolation of confirmed cases and contact tracing for confirmed COVID-19 cases [1]. Laboratory testing for SARS-CoV-2 is a crucial component of this process, and as an emerging virus, there has been an ongoing evolution of diagnostic molecular testing including an array of laboratory developed tests and commercial testing platforms with US Food and Drug Administration (FDA) Emergency Use Authorization (EUA) [2].

Starting in February 2020, we implemented clinical testing for SARS-CoV-2 utilizing two different commercial assays: (1) Lightmix® Kit [(LightMix® Modular SARS and Wuhan CoV E-gene (TIB Molbiol, Berlin, Germany) with LightCycler® Multiplex RNA Virus Master (Roche Molecular Diagnostics, Pleasanton, CA)] and (2) cobas® SARS-CoV-2 on the cobas® 6800 (Roche Molecular Diagnostics, Pleasanton, CA). Utilization of these platforms enabled high-volume and automated throughput, as well as the ability to process specimens other than nasopharyngeal (NP) swabs using the Lightmix® Kit. On average, the expected laboratory testing time on these platforms is approximately 3.5 h. A rapid molecular assay, which provides a result in ~45 min, for SARS-CoV-2, Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) also recently received FDA EUA. Comparison of the cobas® and Xpert® showed high overall concordance (> 99 %) [3].

In a public health crisis, rapid molecular assays can have a considerable impact on the ability to make immediate decisions regarding, for example, the isolation of non-adherent patients or the assessment of infection risk to healthcare workers performing invasive procedures on critically ill patients. As the ability of molecular assays to detect SARS-CoV-2 infection can be limited by low amounts of viral RNA (e.g. early or late in COVID-19 disease), we evaluated commercial assays using samples with low levels of viral RNA [Cycle threshold (Ct) ≥30]. This study was performed as part of the clinical validation and verification of the Xpert® and was integrated into the laboratory's quality management system.

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2. Methods

NP swabs previously confirmed positive with a Cycle threshold (Ct) ≥30 for SARS-CoV-2 by the cobas® (targets: Orf-1a and envelope (E) genes; FDA EUA) or the Lightmix® assay (target: E gene; research use only) were selected for comparison with the Xpert® (targets: nucleocapsid (N2) and E genes; FDA EUA). NP swabs negative for SARS-CoV-2 and positive for influenza A, B or RSV were also included. Samples were selected based on their Ct value to identify patients with a low level of detectable SARS-CoV-2 viral RNA. For negative samples, the endpoint values (generally defined as a measure of the total amount of fluorescence detected at the end of the final nucleic acid amplification cycle) for the Xpert® were collected.

3. Results

Of the thirty-seven samples, ten samples had a Ct value between 30–33.9, twenty-two with Ct values ≥34, and five samples were negative for SARS-CoV-2. Overall concordance on initial comparison was 86.5 % (32/37). There was 100 % concordance for samples with Ct values between 30 − 33.9. Results comparing the Xpert® to the cobas® and Lightmix® are shown in Tables 1 and 2, respectively.

Among the samples with a Ct value ≥34, thirteen were initially detected by the Lightmix® assay and nine by the cobas® assay. Discordance within this subgroup was 23 % (5/22). For the five discordant results, two samples had endpoint values > 5, with one positive by the alternate assay (Lightmix®). Of note, sample 1 (Table 1) was a clinical follow up SARS-CoV-2 test. This patient was positive for SARS-CoV-2 twenty-two days prior using the Lightmix® assay (Ct value for E = 28.82), suggesting that the follow-up test represented viral clearance.

4. Discussion

There was complete concordance of the Xpert® Xpress SARS-CoV-2 with the cobas® SARS-CoV-2 or the Lightmix® assay for samples with a Ct value < 34 (100 %). Compared to previously published data indicating > 99 % concordance, overall agreement was lower in our study as we aimed to assess challenging diagnostic samples with low levels of viral RNA [3,4]. Interestingly, on review of the Xpert® results for the discordant signals, endpoint values ≥5 samples were detected in 40 % (2/5) of the samples. Based on our previous study on endpoint values with Xpert® Flu/RSV, our laboratory would test negative samples with endpoint values ≥5 with a second assay due to the concern for a low level viral load [5]. Including those two samples with endpoint values > 5 (Sample 1 and 2, Table 1), concordance between the assays would have been 92 %. Importantly, in the absence of endpoint value review and further testing, a discordance rate of 23 % may result in laboratories that rely exclusively on the Xpert® assay to report negative results in samples harboring low levels of viral RNA. With expansion of testing to identify pre-symptomatic patients, the number of specimens falling into this low level viral RNA range may be expected to increase, and repeat testing of negative Xpert® results may be indicated with a secondary method.

There are clinical, infection prevention and control, and public health implications for the detection of samples with low levels of SARS-CoV-2 viral RNA. For patients presenting early, studies on viral dynamics suggest that they may have a low burden of disease [6]. As a potentially missed diagnosis, these patients can serve as ongoing reservoirs for transmission within the community or healthcare facility [7]. For diagnosed COVID-19 patients, in situations where a test-based strategy is preferred for discontinuation of infection control precautions, identification of these patients with low viral loads is essential to avoid premature discontinuation [8]. Although detection of viral RNA by PCR may not correlate with live transmissible virus [6], a conservative approach to discontinuing transmission-based precautions may be indicated, given this strategy is primarily used for high-risk populations such as inpatients, severely immunocompromised patients, or long-term care facility residents.

There are limitations to this study, including the small sample size which was due to the intended aim of low positive SARS-CoV-2 samples. Inherent in testing these low positives are discordant results, such as in Table 1, which could be the result of sampling bias, specimen storage or assay design/sensitivity. In addition, we were unable to test all samples on the three assays, using the cobas® or Lightmix® assays as the reference Ct in which to test the Xpert®. However, a prospective comparison of 502 nasopharyngeal swabs between the cobas® and Lightmix® reported 99.6 % overall agreement and good correlation ($r^2 = 0.96$) between Ct [4]. This was for pragmatic reasons, as those two assays were already implemented in our laboratory, and due to limited sample volumes and the high demand for patient testing, our laboratory needed to balance the kits used for verification versus clinical testing.

We report on the test characteristics of three commercial assays on specimens with low levels of viral RNA. As laboratories integrate

Table 1
Summary of SARS-CoV-2 results for samples with Ct ≥ 34 on the cobas® SARS-CoV-2 and Xpert® Xpress SARS-CoV-2.

Sample	cobas®		Xpert®				Comment for Discordant Results
	Orf-1a (Ct)	E (Ct)	E (Ct)	E (end point)	N2 (Ct)	N2 (end point)	
1	–	37.29	0	27	0	−1	Negative on Lightmix® (1:2 dilution)
2	34.26	36.46	0	13	0	0	Positive on Lightmix® (E Ct = 36.77)
3	35.56	36.72	40.7	60	41	141	
4	–	36.11	0	34	40.7	134	
5	34.17	35.86	35.3	275	38.7	187	
6	–	39.62	33.2	350	36.4	244	
7	–	36.85	42.3	46	41	118	
8	–	41.47	0	−1	0	−1	Positive on Lightmix® from saliva (E Ct = 25)
9	–	36.43	36.1	98	38.4	189	

Table 2
Summary of SARS-CoV-2 results for samples with Ct ≥ 34 on the Lightmix® and Xpert® Xpress SARS-CoV-2.

Sample	Lightmix®	Xpert®				Comment for Discordant Results
	E (Ct)	E (Ct)	E (endpoint)	N2 (Ct)	N2 (endpoint)	
1	34.62	0	32	39.8	124	
2	36.07	0	2	0	−2	Negative on cobas® (1:2 dilution)
3	34.35	0	21	41.2	118	
4	37.24	0	2	0	−1	Negative on cobas® (1:2 dilution)
5	36.05	39.3	88	40.2	162	
6	35.58	0	30	41.5	109	
7	37.99	37.9	84	39.7	166	
8	36.42	35.8	193	38.5	184	
9	37.67	0	10	41	120	
10	36.7	0	13	44.7	51	
11	34.53	36.5	102	38.1	169	
12	37.42	37.1	143	42.2	107	
13	34.02	35.3	217	39.2	163	

different testing platforms to optimize patient care, such as the cobas® for high-volume testing and the Xpert® for rapid testing, they should consider implementation of appropriate confirmatory algorithms to ensure that the detection of samples with low levels of SARS-CoV-2 viral RNA is not overlooked. Endpoint values reported by the Xpert® assay should be considered when reviewing negative results, and are relevant in the correct clinical context.

Declaration of Competing Interest

The authors have no relevant conflicts of interest to disclose.

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References

[1] World Health Organization, COVID-19 Strategy Update 14 April, (2020) Available athttps://www.who.int/docs/default-source/coronaviruse/covid-strategy-update-14april2020.pdf?sfvrsn=29da3ba0_12&download=true Accessed April 18, 2020.

[2] M.J. Loeffelholz, Y.W. Tang, Laboratory diagnosis of emerging human coronavirus infections–the state of the art, Emerg. Microbes Infect. 9 (2020) 747–756.

[3] A. Moran, K.G. Beavis, S.M. Matushek, C. Ciaglia, V. Tesic, N. Love, The Detection of SARS-CoV-2 using the Cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 assays, 17 April, J. Clin. Microbiol. (2020), https://doi.org/10.1128/JCM.00772-20.

[4] M. Poljak, M. Korva, N. Knap Gašper, K. Fujs Komloš, M. Sagadin, T. Uršič, T. Avšič Županc, M. Petrovec, Clinical evaluation of the cobas SARS-CoV-2 test and a diagnostic platform switch during 48 hours in the midst of the COVID-19 pandemic, 10 April, J. Clin. Microbiol. (2020), https://doi.org/10.1128/JCM.00599-20.

[5] N. Matic, G. Ritchie, T. Lawson, L. Karakas, M.G. Romney, C.F. Lowe, Relevance of reviewing endpoint analysis for negative results on the Xpert® Xpress Flu/RSV, J. Med. Virol. (2020), https://doi.org/10.1002/jmv.25836.

[6] R. Wölfel, V.M. Corman, W. Guggemos, M. Seilmaier, S. Zange, M.A. Müller, D. Niemeyer, T.C. Jones, P. Vollmar, C. Rothe, M. Hoelscher, T. Bleicker, S. Brünink, J. Schneider, R. Ehmann, K. Zwirglmaier, C. Drosten, C. Wendtner, Virological assessment of hospitalized patients with COVID-2019, 1 April, Nature (2020), https://doi.org/10.1038/s41586-020-2196-x.

[7] W.E. Wei, Z. Li, C.J. Chiew, S.E. Yong, M.P. Toh, V.J. Lee, Presymptomatic Transmission of SARS-CoV-2 — Singapore, January 23–March 16, 2020, Morb. Mortal. Rep. Surveill. Summ. 69 (2020) 411–415.

[8] Centers for Disease Control and Prevention. Discontinuation of Transmission-Based Precautions and Disposition of Patients with COVID-19 in Healthcare Settings (Interim Guidance). Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html. Accessed April 18, 2020.