Shortening turnaround time for high-priority patients during the COVID-19 epidemic: evaluation of the Xpert Xpress SARS-CoV-2 test

Rok Kogoj, Katarina Resman Rus, Tina Uršič

Abstract

Background: Although several molecular tests are now available for detecting SARS-CoV-2 RNA in nasopharyngeal swab samples, the number of requested tests exceeds the capacity of many diagnostic laboratories. Unfortunately, the available high-throughput platforms exhibit longer turnaround times than those required for management of high-priority patients.

Methods: The aim of this study was to evaluate the performance and possible benefits of the Cepheid Xpert Xpress SARS-CoV-2 test, focusing mainly on turnaround time when applied to high-priority patients. We evaluated the Xpert Xpress SARS-CoV-2 test in comparison to the Roche’s cobas 6800 SARS-CoV-2 test by monitoring turnaround times and by retrospectively testing 20 nasopharyngeal swabs from COVID-19 patients with various viral loads. In addition, 50 patients were tested by both methods prospectively.

Results: We observed a lower limit of detection of one SARS-CoV-2 genome equivalent/µL and 100% (95% CI, 92.6−100%) specificity and 95.5% (95% CI, 77.2−99.9%) sensitivity in comparison to the cobas SARS-CoV-2 test. When applying the Xpert Xpress SARS-CoV-2 test for high-priority patients the turnaround time could be greatly reduced, i.e. from 3 - 5 hours that take our routine diagnostics methods to about 1 hour.

Conclusion: The novel Xpert Xpress SARS-CoV-2 test is a useful, easy to perform tool, valuable for rapid and reliable diagnosis of COVID-19, especially in high-priority patients when a short turnaround time is of key importance for further patient management.
1 Introduction

The novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a recently emerged member of the coronavirus family (1) firstly detected in December 2019 in the city of Wuhan in China’s, Hubei Province (2). Although most patients have a good prognosis, in some cases, usually when patients have underlying accompanying diseases, death may occur (3,4). Fortunately, the virus does not seem to be highly pathogenic, but high human-to-human transmission ability (5) allowed it to spread all over the world in less than 3 months after its emergence, therefore making it a serious global concern and exerting an enormous burden on healthcare systems.

The rapid development and wide implementation of reliable diagnostic tests plays a key role in controlling the pandemic, better understanding the epidemiology of the disease, and allowing countries to implement adequate emergency measures (6). To meet diagnostic needs as the pandemic grows, the U.S. FDA expanded enforcement discretion to speed up COVID-19 test access, resulting in granting EUA to 81 different SARS-CoV-2 commercial assays and 32 laboratory developed tests as of 28 May 2020 (7).

From March 2020 onward, Slovenian diagnostics laboratories were faced with unforeseen demand for fast and reliable COVID-19 testing. Although we were able to partially meet these demands by quickly switching the diagnostic protocol from automatic nucleic acid extraction using the MagNA Pure Compact system (Roche Applied Science, Mannheim, Germany) and manual rtRT-PCR preparation (8) to the high-throughput cobas 6800 SARS-CoV-2 test (Roche Molecular Diagnostics, Pleasanton, CA, USA) – cobas SARS-2 test (9), the number of tests requested per day exceeded all expectations. A demand for faster results arose when dealing with high-priority patients, from intensive care units (ICU), surgical units, transplantation wards, oncology and haematology departments, and intensive paediatric care units (PICU).
The aim of this study was to evaluate the performance of the Xpert Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA) – Xpert SARS-2 test focusing mainly on turnaround time before its implementation as a method of choice for those high-priority patients who require a COVID-19 result sooner than our usual turnaround time of 3 to 5 hours.

2 Material and Methods

2.1 Analytical PCR efficiency and lower limit of detection

A serial dilution of inactivated SARS-CoV-2 virus was prepared in fresh RPMI–1640 (Sigma-Aldrich, St. Louis, MI, USA) medium from $1.0 \times 10^4$ copies/µL ($100$ pfu/mL) to one copy/µL ($0.01$ pfu/mL). Each dilution was tested in triplicate and, from the results obtained, mean Ct values and the standard deviation (SD) were calculated. Calibration curves were plotted, and PCR efficiency for both target genes was calculated.

2.2 Validation on patient samples’ panel

In the first validation part, we retrospectively tested 20 nasopharyngeal swabs stored at $-30^\circ$C in Universal Transport Medium; UTM (Copan, Brescia, Italy), from patients diagnosed with COVID-19 during routine diagnostics by using a cobas SARS-2 test performed on a cobas 6800 system, which detects two genes, ORFb1 as Target 1 and E as Target 2, as previously described (9). The samples were selected according to the cycle threshold value (Ct) with values between 12 and 40 in order to cover the entire analytical range of a real-time RT-PCR method. In the second part, we prospectively tested 50 nasopharyngeal swab samples from high-priority patients that were processed in parallel on both the Xpert SARS-2 test and cobas SARS-2 test. After comparing the results to the cobas SARS-2 test as a reference method, we calculated the specificity, sensitivity, positive predictive value, negative predictive value, and Cohen’s kappa agreement coefficient, and we performed a Bland-Altman analysis. In addition, we also calculated turnaround and hands-on times.

2.3 Xpert Xpress SARS-CoV-2 test

The Xpert SARS-2 test detects two genes: the E gene and N2 gene. The test was performed according to the manufacturer's instructions. Briefly, nasopharyngeal swabs inserted in UTM were thoroughly mixed for 30 seconds and transferred directly to the test cartridge with the accompanying pipette. The prepared cartridges were loaded into a GX-XVI instrument (Cepheid). Analysis of the results was performed automatically by using GeneXpert Dx software version 4.8 (Cepheid).

2.4 Laboratory testing requirements and turnaround times

The number of total and high-priority tests ordered during the government-declared COVID-19 epidemic (calendar weeks 10 through 20) was exported from the laboratory information system (LIS) and used to analyze the turnaround times per day for SARS-CoV-2 RNA detection by LightMix Modular Wuhan CoV E-, RdRp- and N-gene Kits
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(TIB Molbiol, Berlin, Germany) – LMM or cobas SARS-2 test at the Institute of Microbiology and Immunology (IMI), Faculty of Medicine, University of Ljubljana. The turnaround time's results were compared to those that would have been achieved by using the Xpert Xpress SARS-CoV-2 test.

2.5 Ethical compliance

In line with the principles expressed in the Declaration of Helsinki, the Oviedo Convention on Human Rights and Biomedicine, and the Slovenian Code of Medical Deontology, all human samples were anonymized, and data on patient sex and age were linked only to randomized numerical codes. Because no additional samples or data were collected, the study was deemed low risk and the need for additional ethical approval from the National Medical Ethics Committee was waived.

3 Results

3.1 Limit of detection and PCR efficiency

Our results show that the Xpert SARS-2 test has a 98.0% (slope: −3.3700) and 94.0% (slope: −3.4757) PCR efficiency for the E gene and N2 gene, respectively. We observed the same limit of detection (LoD) as stated by the manufacturer; namely, 0.01 pfu/ml (one copy of SARS-CoV-2 RNA/µL) (Figure 1).

3.2 Retrospective testing

The results of the retrospective testing show that the Xpert SARS-2 test correctly identified 85.0% (17/20) of samples which tested positive by cobas SARS-2 and 95.0% (19/20) when Xpert SARS-2 test presumptive positive results were calculated as positive (Table 1). After comparing Ct values, a strong posi-

![Figure 1: Xpert SARS-2 test results of inactivated SARS-CoV-2 virus serial dilutions in RPMI-1640 from $1 \times 10^4$ copies/µL (100 pfu/mL) down to 1 copy/µL (0.01pfu/mL).]
tive correlation was observed for the E gene / Target 2 (R = 0.89; p < 0.00001) and a moderate correlation for the N2 gene / Target 1 (R = 0.69; p = 0.0008). From both Ct comparison plots (Figures 2 and 3), it can also be observed that the decrease in correlation strength is due to greater dispersion of high Ct values (> 34.0). We did not observe any failed results due to internal control status for

Table 1: Detailed results of the Xpert SARS-2 test in comparison with the reference method (cobas SARS-2 test) with respective Ct values for both targets, internal control status, and automatic interpretation of the overall result.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cobas 6800 SARS-CoV-2 test</th>
<th>Xpert Xpress SARS-CoV-2 test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target 1 (ORF1ab)</td>
<td>Target 2 (E gene)</td>
</tr>
<tr>
<td>P1</td>
<td>12.6</td>
<td>16.3</td>
</tr>
<tr>
<td>P2</td>
<td>14.2</td>
<td>17.3</td>
</tr>
<tr>
<td>P3</td>
<td>16.1</td>
<td>18.1</td>
</tr>
<tr>
<td>P4</td>
<td>18.2</td>
<td>19.3</td>
</tr>
<tr>
<td>P5</td>
<td>20.2</td>
<td>20.6</td>
</tr>
<tr>
<td>P6</td>
<td>22.3</td>
<td>22.8</td>
</tr>
<tr>
<td>P7</td>
<td>24.3</td>
<td>25.1</td>
</tr>
<tr>
<td>P8</td>
<td>26.9</td>
<td>26.1</td>
</tr>
<tr>
<td>P9</td>
<td>27.4</td>
<td>27.1</td>
</tr>
<tr>
<td>P10</td>
<td>28.3</td>
<td>28.7</td>
</tr>
<tr>
<td>P11</td>
<td>29.4</td>
<td>30.2</td>
</tr>
<tr>
<td>P12</td>
<td>30.3</td>
<td>31.7</td>
</tr>
<tr>
<td>P13</td>
<td>31.3</td>
<td>31.8</td>
</tr>
<tr>
<td>P14</td>
<td>38.0</td>
<td>32.8</td>
</tr>
<tr>
<td>P15</td>
<td>35.2</td>
<td>33.2</td>
</tr>
<tr>
<td>P16</td>
<td>37.8</td>
<td>34.3</td>
</tr>
<tr>
<td>P17</td>
<td>37.7</td>
<td>35.2</td>
</tr>
<tr>
<td>P18</td>
<td>39.9</td>
<td>35.2</td>
</tr>
<tr>
<td>P19</td>
<td>36.3</td>
<td>36.5</td>
</tr>
<tr>
<td>P20</td>
<td>35.2</td>
<td>37.6</td>
</tr>
</tbody>
</table>

IC – internal control; Neg – negative
all samples tested. The Bland-Altman analysis revealed that the mean difference in Ct values for both targets was in favour of the Xpert SARS-2 test for −0.5 and −3.5 (at 95% CI) for E versus Target 2 and N2 versus Target 1 genes, respectively; however, the difference did not reach statistical significance ($p = 0.59$ and $p = 0.096$).

**Figure 2:** Ct value correlation (a) and Bland-Altman comparison plot (b) for the N2 gene (Xpert SARS-2) against Target 1 – ORF1ab gene (cobas SARS-2).

**Figure 3:** Ct value correlation (a) and Bland-Altman comparison plot (b) for the E gene (Xpert SARS-2) against Target 2 – E gene (cobas SARS-2).
Table 2: Detailed results of prospective testing.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert Xpress SARS-CoV-2</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>Cobas 6800 SARS-CoV-2</td>
<td>2</td>
<td>48</td>
</tr>
</tbody>
</table>

3.3 Prospective testing

For the prospective part of the study, we tested 50 consecutive symptomatic patients. The study population was composed of 42.0% (21/50) males and 58.0% (29/50) females, with a mean age of 50 years (< 1 to 97). Twenty-eight percent (14/50) were children under 18 years of age, 42.0% (21/50) were working-age adults, and 30.0% (15/50) were people over the age of 65. Four percent of the patients (2/50) were positive and 96.0% (48/50) were negative (Table 2). For one sample, we were unable to obtain the result with the Xpert SARS-2 test due to internal control amplification failure. However, after retesting the same sample, the result was negative. In conclusion, no discrepancies were observed between the Xpert SARS-2 and cobas SARS-2 tests in the prospective part of the study.

3.4 Specificity, sensitivity and method agreement with the cobas 6800 SARS-2 test

After combining the results from the retrospective and prospective testing, we calculated the Xpert SARS-2 test specificity (including presumptive positive results) in comparison to the cobas SARS-2 test to be 100% (92.6–100% at 95% CI), sensitivity 95.5% (77.2–99.9% at 95% CI). Cohen’s kappa agreement coefficient was 98.6% (86.8–99.9% at 95% CI). If the presumptive positive samples were omitted from the calculation, the specificity value remains the same, whereas the sensitivity lowers to 95% (75.1%-99.9% at 95% CI).

3.5 High-priority testing requirements and turnaround times during the epidemic

The analysis of data from our LIS shows that after the detection of the first COVID-19 case in Slovenia on 4 March 2020 (in calendar week 10), the number of tests ordered per day has increased rapidly (Table 3). Despite our efforts, turnaround times started to increase mainly due to infrastructure and human resources limitations. Nevertheless, we were able to provide results for 89.6% (31997/35723) of tested samples in time-frame of 6 hours. Similarly, the number of high-priority tests ordered from ICUs, surgical units, transplantation wards, oncology and haematology departments and PICUs, followed the same trend. A more detailed analysis reveals that in weeks 10 and 11 only 1 ± 1 and 5 ± 3 tests on average per day were requested respectively by the units mentioned above. In the following weeks, a decisive increase in the number of high-priority tests requested was observed (Table 3, Figure 4). Average weekly turnaround times after an initial increase from 3:13 (in week 10) to 3:44 (in week 11) actually started to decrease in week 13, when we switched our diagnostics approach to a combination of LMM and cobas SARS-2 test. Consequently, in week 17, for the high-priority samples the shortest average turnaround time was about 3 hours (2:59). However, such turnaround time was not sustained until the end of the study period mainly due to a complete testing switch to the cobas SARS-2 test as the primary COVID-19 diagnostic method.
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requirements and turnaround

3.5 High-priority testing

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(week 10) to 3:44 (in week 11) actually

Figure 4). Average weekly turnaround
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during respective week, and total weekly percentage of high-priority tests from the announcement of the COVID-19
epidemic until it was declared over in Slovenia.

Table 3: Average number of total tests ordered per day during respective weeks, high-priority tests ordered per day
during respective week, and total weekly percentage of high-priority tests from the announcement of the COVID-19

<table>
<thead>
<tr>
<th>Calendar week</th>
<th>W10</th>
<th>W11</th>
<th>W12</th>
<th>W13</th>
<th>W14</th>
<th>W15</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
<th>W19</th>
<th>W20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests per day, n (SD)</td>
<td>92 (59)</td>
<td>92 (110)</td>
<td>495 (94)</td>
<td>571 (146)</td>
<td>487 (136)</td>
<td>558 (223)</td>
<td>460 (145)</td>
<td>578 (167)</td>
<td>449 (168)</td>
<td>533 (173)</td>
<td>488 (133)</td>
</tr>
<tr>
<td>Priority tests per day, n (SD)</td>
<td>1 (1)</td>
<td>5 (3)</td>
<td>22 (8)</td>
<td>29 (10)</td>
<td>41 (5)</td>
<td>50 (11)</td>
<td>50 (9)</td>
<td>53 (11)</td>
<td>34 (27)</td>
<td>41 (16)</td>
<td>41 (8)</td>
</tr>
<tr>
<td>% of priority tests per week</td>
<td>1.1%</td>
<td>1.3%</td>
<td>4.4%</td>
<td>5.0%</td>
<td>8.3%</td>
<td>9.0%</td>
<td>10.8%</td>
<td>9.1%</td>
<td>8.3%</td>
<td>7.8%</td>
<td>8.5%</td>
</tr>
</tbody>
</table>

W – Week, SD – Standard deviation

Figure 4: Number of SARS-CoV-2 RNA detection tests requested by ICUs, surgical units, transplantation wards, oncology
and haematology departments and PICUs per day with the weekly average of tests requested and weekly average
turnaround times. Red: first confirmed COVID-19 case in Slovenia. Yellow: platform switch for low-priority samples from
LightMix to cobas 6800. Green: switch to cobas 6800 as the sole platform for SARS-CoV-2 RNA detection.
4 Discussion

More than a month before the first COVID-19 case in Slovenia, our institution already implemented an in-house rtRT-PCR method, proposed by the WHO, as described previously (8). The first positive case was detected on 4 March. The government declared a SARS-CoV-2 epidemic on 12 March. Slovenian authorities decided to almost completely shut down public life: public gatherings were forbidden, and preschools, schools, restaurants, hotels, and almost all stores except grocery stores were closed. No public transport was available, and travel between municipalities was prohibited. Because the numbers of tests ordered per day were growing and due to human resource limitations, in calendar week 13 we switched our diagnostics approach to the high-throughput cobas 6800 system, as described previously (9). The switch resulted in extended turnaround times from approximately 3 hours to 5 hours, but it significantly decreased the labour required for testing samples. At the same time, the demand for shorter turnaround time for high-priority patients arose due to need for COVID-19 testing before hospitalization or elective surgery. Consequently, a steady increase in the number of high-priority tests requested was observed; from an average of 1 ± 1 to 22 ± 8 tests per day in weeks 10 through 12 and later constantly over 40 ± SD with the exception of week 18, when an average of 34 ± 27 high-priority tests were ordered per day. Based on the fact that the number rose again to an average of over 40 ± SD in the following weeks, the most probable reason is the lower number of patients and medical doctors on duty. This observation is also supported by a similar drop in the number of overall tests ordered, for an average of approximately 100 tests per day during week 18.

Thus the need for faster diagnostic approach for high-priority patients like the Xpert SARS-2 test was urgent and inevitable. In the retrospective part of the study, the Xpert SARS-2 test correctly identified 95% (19/20) of samples previously found positive by the cobas SARS-2 test, and a strong positive correlation was observed for the E gene Ct values / Target 2 Ct values and a moderate correlation for the N2 gene Ct values / Target 1 Ct values. In the prospective part of the study, 50 consecutive symptomatic patients were tested and no discrepancies were observed. Four percent of patients (2/50) were positive and 96.0% (48/50) were negative. When comparing the results of the Xpert SARS-2 test to cobas SARS-2 test, which has previously been shown to have a 100% analytical specificity and sensitivity (9,10), the overall Xpert SARS-2 test specificity and sensitivity (including presumptive positive results) were 100% (92.6–100% at 95% CI) and 95.5% (77.2–99.9% at 95% CI), respectively. We have chosen to determine the presumptive positive results as positive and incorporate them in the calculation because we already knew the samples were positive by our reference method. However, we must stress that in a routine setting such results should be interpreted with caution. We advise that presumptive positive results should be confirmed by another (possibly reference) method before reporting the result. If these samples were omitted from the calculation, the sensitivity value dropped to 95% (75.1%-99.9% at 95% CI). Additionally, we must point out, that our study sample selection does not reflect the true sensitivity and specificity of the Xpert SARS-2 test as the study was designed to compare the Xpert SARS-2 test...
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to the cobas SARS-2 test and not to clinically confirmed COVID-19 cases. Since data on clinically confirmed COVID-19 cases were not available to us, such calculation was not possible. Therefore, our results reflect only the performance of the Xpers SARS-2 test in comparison to the cobas SARS-2 test. The CI interval of the sensitivity calculation is rather wide, since a relatively small number of positive samples were included in the study. We were unable to include more positive samples from high-priority patients since, at that time, more such patients have not been identified. Therefore, this limitation must be taken into account when looking at our results. The lower sensitivity with the wider CI interval implies that false negative results occasionally occur. Nevertheless, based on our results, it would appear that the false negative results occur for samples with a very low concentration of SARS-CoV-2. At this point in time, it is still questionable whether such low concentrations of SARS-CoV-2 in samples are clinically significant, as such patients might not be infectious at all. Specialized studies to determine the infectivity of such cases might be considered in the future to shed more light on this matter. Finally, during our validation we did not observe Xpert SARS-2 test false positive results.

Similar performance results of the Xpert SARS-2 test were observed in a previous study (11), which showed a 100% agreement with a Centers for Disease Control and Prevention (CDC) laboratory-developed test. In contrast to our results, that study was able to detect a positive sample with an Xpert SARS-2 test Ct result of 42.6 and 42.7 for the E and N2 genes, respectively; however, this occurred in two separate runs after repeating the test. In our case, we observed a Ct value of $40.0 \pm 1.1$ (E gene) and $41.4 \pm 1.0$ (N2 gene) for one SARS-CoV-2 genome copy per µL of sample. Judging from the previously published results (11) it would seem that the Xpert SARS-2 test can occasionally detect even lower amounts of target RNA per µL of sample. Another performance evaluation of the Xpert SARS-2 test was recently published (12) where the discrepancies between the cobas SARS-CoV-2 test and Panther Fusion (Hologic, USA) were described. After testing 14 discrepant low-viral-burden samples (Ct > 35), more of them agreed with the cobas SARS-2 test (nine samples) than with Panther Fusion (five samples), thus indirectly showing a very low detection limit of the Xpert SARS-2 test. Finally, in a study dedicated completely to the evaluation of the Xpert SARS-2 test (13) the results are similar to ours. The study also showed high agreement between the Xpert SARS-2 and the cobas SARS-2 test (99%) and a similar lower mean Ct value for both target genes ($−1.57$ and $−5.34$ at 95% CI). Moreover, an excellent agreement with the cobas SARS-2 test was also demonstrated before (14), with a total of 98.9% (92.9-99.9%) agreement which was mainly due to one low positive sample wrongly identified as negative by the Xpert SARS-2 test.

When comparing the cobas SARS-2 test to the Xpert SARS-2 test, the hands-on time per sample is merely a few minutes for both methods. On the other hand, a greater difference can be observed in turnaround time: 3 hours for the cobas SARS-2 and less than an hour for the Xpert SARS-2 test. The cobas 6800 system can process up to 94 samples in 3 hours (and up to 1,400 samples in 24 hours) whereas for the Xpert SARS-2 test the number depends on the size of the GeneXpert Dx system. It must be pointed out, that such system de-
5 Conclusion

In conclusion, due to the Gene Xpert system design, the throughput of the Xpert SARS-2 test does not compare to that of the high-throughput instruments like the cobas 6800 system and therefore in epidemic settings, when the demand for testing reaches unusually high volumes, its use is somewhat limited. However, based on our results, if used alongside a high-throughput system (e.g. cobas 6800), it can be very beneficial especially for SARS-CoV-2 testing in high-priority patients since it provides the result in as little as 1 hour. Finally, caution should be exerted when a presumptive positive result is obtained and the result confirmed by a reference method before reporting, also in the case of clinically evident infection that is highly suspicious of COVID-19 and a negative Xpert SARS-2 test result, re-testing from a new respiratory sample would be highly recommended.

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