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Real-life head-to-head comparison of performance of two high-throughput automated assays for detection of SARS-CoV-2 RNA in nasopharyngeal swabs: the Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2 assays

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Abstract (219/220 words)

Abbott’s Alinity m is a recently launched fully integrated, automated molecular analyzer allowing continuous loading of samples and sample-to-result molecular detection of several microorganisms. Manufacturer-independent clinical evaluation of Alinity m SARS-CoV-2 (Alinity) against cobas 6800 SARS-CoV-2 (cobas) as a standard comparator was performed on 2,157 consecutive nasopharyngeal swabs. Valid initial results of Alinity and cobas were obtained from 2,129/2,157 (98.7%) and 2,157/2,157 (100%) samples, respectively. Overall percent agreement of 98.3% (2,092/2,129; 95%CI:97.6–98.7%), positive percent agreement of 100.0% (961/961; 95%CI:99.6–100.0%), negative percent agreement of 96.8% (1,131/1,168; 95%CI:95.7–97.7%), and a high kappa value of 0.965 (95%CI:0.954–0.976) were observed on 2,129 samples with valid results for both assays. There were 37 discordant results and based on discordant analyses, including previous and/or follow-up PCR results, 22 could be considered Alinity analytically true positive with high probability. Due to the lack of additional information and inability of repeated/further testing, the status of the remaining 15 discordant samples remains unresolved. Comparative real-life throughput of both analyzers assessed while testing 564 samples in parallel across two 8-hour shifts and comparative turnaround time assessment measured while processing in parallel the first 94 routine samples received in the laboratory each working day for 5 consecutive days showed similar real-life performance of both analyzers with certain differences, which has potential importance in some laboratory settings.
INTRODUCTION

Coronavirus disease 2019 (COVID-19) pandemic has affected over 135 million people, with over 2.9 million COVID-19-related deaths as of 10 April 2021. Highly reliable laboratory diagnostics for COVID-19 are essential for case identification, patient management, and contact tracing. Detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in nasopharyngeal swabs is still considered the COVID-19 reference laboratory diagnostic standard. 1,2 Although several commercial SARS-CoV-2 RNA assays have received U.S. Food and Drug Administration (FDA) emergency-use authorization (EUA), only a few have been designed for analyzers with high sample throughput to cope with the unprecedented demand for SARS-CoV-2 RNA testing and allow significant scaling up due to the fully automated sample-to-result solution. 3 The cobas 6800 System and cobas 8800 System (Roche Molecular Systems, Branchburg, NJ, USA) are fully integrated and automated analyzers allowing sample-to-result qualitative and quantitative molecular detection of several microorganisms. The U.S. FDA recently approved a range of cobas 6800/8800 System molecular assays (https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests, last accessed April 10, 2021), including assays for SARS-CoV-2, which received FDA EUA on 12 March 2020. Several studies have evaluated the performance of this highly reliable and robust SARS-CoV-2 assay, 4–11 and it has become a primary comparator in many performance evaluations of novel SARS-CoV-2 RNA assays.

Alinity m (Abbott Molecular, Des Plaines, IL, USA) is another recently launched fully integrated, automated sample-to-result molecular analyzer allowing continuous loading of samples and random-access testing. Seven molecular assays have been developed for use with Alinity m, 12–23 including an assay for SARS-CoV-2, which received U.S. FDA EUA on 11 May
Unlike the cobas SARS-CoV-2 assay with much-published performance data, peer-reviewed literature contains only limited verification and validation data for the Alinity m SARS-CoV-2 assay.\textsuperscript{20}

Here the results of the large manufacturer-independent clinical evaluation of the Alinity m SARS-CoV-2 assay against the cobas 6800 SARS-CoV-2 assay as a standard comparator are presented. The head-to-head study evaluated the clinical performance of two high-throughput automated assays on 2,157 consecutive nasopharyngeal swabs in routine diagnostic settings. In addition, comparative real-life throughput assessment of two analyzers on 564 samples across two 8-hour shifts as well as comparative turnaround time assessment on the first 94 samples received in the laboratory each working day for 5 consecutive days was performed.

\textbf{MATERIALS AND METHODS}

\textbf{Samples used for head-to-head clinical comparison.} Clinical performances of the Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2 assays were compared on a total of 2,157 unselected nasopharyngeal swabs routinely collected during a 10-day period in mid-January 2021. All samples were obtained from patients treated in the University Clinical Center Ljubljana, the largest tertiary hospital in the country with 2,138 beds which is also the principal Slovenian COVID-19 hospital with the largest COVID-19 intensive care unit. Samples were collected in a 3 ml commercial transport medium VTM (Liofilchem, Roseto degli Abruzzi, Italy) from 2,157 individuals referred for COVID-19 testing during the study period. The median transport time of samples from the collection site to the laboratory was 1 hour 51 minutes. Upon arrival at the laboratory, swabs were vortexed for 1 minute at maximum speed and two VTM
aliquots were prepared: 800 µl for Alinity m SARS-CoV-2 testing and 700 µl for cobas 6800 SARS-CoV-2 testing.

**Alinity m SARS-CoV-2 testing.** The Alinity m SARS-CoV-2 assay is a real-time reverse transcriptase (RT) PCR-based assay for the qualitative detection of SARS-CoV-2 RNA in nasal, nasopharyngeal, and oropharyngeal swabs. 20 By using target-specific fluorescent-labeled oligonucleotide probes, the assay allows simultaneous detection and amplification of two SARS-CoV-2–specific sequences targeting the RdRp gene and N gene reported as a combined signal in one channel and individually an internal control (IC) target sequence for evaluation of sample extraction and amplification efficiency reported separately in another channel. The assay is performed on Alinity m—a fully integrated, automated molecular analyzer that allows continuous loading of samples and performs sample preparation, RT-PCR assembly, amplification, detection, calculation, and reporting of the results. Alinity m provides two different reports: “Result” (each signal is reported either as “Not Detected” if a specimen cycle number (CN) is not generated or “CN value” if CN is less than 42) and “Interpretation” (either Negative or Positive). Prior to transferring the results into the laboratory information system (LIS), the results can be reviewed directly in the system or as a printed report.

For this study, 800 µl sample aliquots were transferred into Alinity m Aliquot Tubes, loaded on Alinity m, and tested following the manufacturer’s instructions.

**Cobas 6800 SARS-CoV-2 testing.** The cobas SARS-CoV-2 assay is a two-target RT-PCR for qualitative detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swab samples. 4 One target is viral ORF1, a region unique to SARS-CoV-2 (target 1), and the second a conserved region in the E gene for pan-Sarbecovirus detection (target 2). The assay utilizes RNA internal control as sample preparation and PCR amplification processing controls and the Uracil-
N-glycosylase system for prevention of PCR contamination. The assay is performed on either the cobas 6800 System or cobas 8800 System instrument, which consists of the sample supply module, transfer module, processing module, and analytic module. Automated data management is performed by the manufacturer’s software, which assigns test results. The results can be reviewed directly on the system screen, printed as a report, or transferred to an LIS. According to the manufacturer’s instructions, a tested sample was considered SARS-CoV-2 positive if cobas showed positive results either for both the ORF1 (target 1) and E (target 2) genes or for the ORF1 gene only. In the case of positivity for the E gene only (target 2), the result was reported as SARS-CoV-2 presumptive positive.

For this study, 700 µl sample aliquots were transferred into barcoded secondary tubes, loaded on the cobas 6800 System, and tested following the manufacturer’s instructions. Testing was performed in 94-sample batches plus one negative and positive control each.

**Data analysis.** A contingency table was constructed to assess percent overall, positive, and negative agreements with 95% confidence intervals (CIs). The level of agreement between tests was assessed using Cohen’s kappa statistics. All statistical analyses were performed using Excel 2016 version 16.0.5134.1000 (Microsoft, Redmond, WA, USA) and R software version 3.2.5 (Free Software Foundation, Boston, MA, USA). The study conforms with the World Medical Association Declaration of Helsinki and was approved by the Medical Ethics Committee of the Republic of Slovenia (No 0120-211/2020/7).

**Comparative throughput assessment.** To comparatively assess real-life throughput of Alinity m and the cobas 6800 System across two 8-hour shifts, 564 samples were processed in parallel on both systems by experienced operators starting on the morning of 19 January 2021. Both analyzers were initialized at the same time and run in parallel until all samples were processed.
and all final results obtained. Sample handling time, instrument handling time, and total hands-on time were meticulously measured by two independent observers for each analyzer. To visualize the release dynamics of the final results across two 8-hour shifts, a time curve for each analyzer was generated and the time curves obtained were comparatively evaluated.

**Comparative turnaround time assessment.** To determine turnaround time (TAT) for both SARS-CoV-2 assays in a real-life setting, the first 94 routine samples received in the laboratory each working day for 5 consecutive days were processed in parallel on both analyzers by experienced operators. The entire testing procedure was meticulously monitored by two independent observers for each analyzer, and the following timepoints were recorded for each of the 470 (94 × 5) samples processed: sample admission time, starting point of sample handling, starting point of instrument processing, and time of result release. From the collected data, total pre-analytical time, instrument-on-board time, and total TAT were calculated for each of the 470 samples processed.

**RESULTS**

**Head-to-head clinical comparison.** Out of 2,157 nasopharyngeal swabs tested, valid initial results of Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2 were obtained from 2,129/2,157 (98.7%) and 2,157/2,157 (100%) samples, respectively. Total of 28 samples were excluded from further analysis due to initial invalid Alinity m SARS-CoV-2 results; all invalid results were due to no amplification of the target and internal control failure. Table 1 summarizes the results of comparative clinical evaluation of Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2 on 2,129 samples with initial valid results of both assays. The diagnostic approaches showed an overall percent agreement of 98.3% (2,092/2,129; 95%CI: 97.6–98.7%), positive percent
agreement of 100.0% (961/961; 95%CI: 99.6–100.0%), negative percent agreement of 96.8% (1,131/1,168; 95%CI: 95.7–97.7%), and a high kappa value of 0.965 (95%CI: 0.954–0.976) on 2,129 samples with valid results for both assays.

Thirty-seven discordant results were obtained: four samples were positive using Alinity m SARS-CoV-2 and presumptive positive by cobas 6800 SARS-CoV-2, and 33 were positive using Alinity m SARS-CoV-2 and negative by cobas 6800 SARS-CoV-2 (Table 1). Mean and median Alinity m SARS-CoV-2 CN values among 961 concordantly positive samples were 20.6 and 18.8 (range 8.9–39.1), respectively, and among 37 samples with discordant results 37.8 and 37.9 (range 31.1–41.9), respectively.

An insufficient volume of leftover specimens prevented retesting of samples with discrepant Alinity m SARS-CoV-2 / cobas 6800 SARS-CoV-2 results. To resolve the status of samples with discrepant results, all previous and follow-up testing results for the 37 individuals with discrepant results recorded in our LIS were reviewed. In 18/37 individuals with Alinity m SARS-CoV-2 / cobas 6800 SARS-CoV-2 discrepant results, previous and/or follow-up SARS-CoV-2 PCR result(s) were identified: 10/18 had a recorded previous SARS-CoV-2 PCR positive result in samples collected 0, 6, 8, 9, 9, 10, 11, 27, 59, 90 days (mean: 22.9 days; median: 9.5 days; range 0–90 days) before the sample in this study was collected (for multiple previous samples identified, the first previously positive sample was considered for each individual) and 8/18 had a recorded follow-up SARS-CoV-2 PCR positive result in samples collected 0, 0, 0, 2, 2, 5, 6, 7 days (mean: 2.8 days; median: 2 days; range 0–7 days) after the sample in this study was collected (for multiple follow-up samples identified, the first positive follow-up sample was considered for each individual). For 19 individuals with Alinity m SARS-CoV-2 / cobas 6800 SARS-CoV-2 discrepant results, no previous and/or follow-up SARS-CoV-2 PCR results were
identified in either database. Of these 19 individuals, four were Alinity m SARS-CoV-2 positive / cobas 6800 SARS-CoV-2 presumptive positive (positive for target 2 only) and 15 were Alinity m SARS-CoV-2 positive / cobas 6800 SARS-CoV-2 negative. Based on the discordant analyses described, of 37 samples with Alinity m SARS-CoV-2 positive / cobas 6800 SARS-CoV-2 negative discrepant results, 22 (18+4) samples could be considered Alinity m SARS-CoV-2 analytically true positive with high probability. Due to the lack of additional information and the inability of repeated/further testing, the status of the remaining 15 discordant samples remains unresolved.

**Comparative throughput assessment.** Fig. 1 shows Alinity m and cobas 6800 System time curves visualizing release dynamics of final results while testing 564 samples in parallel across two 8-hour shifts. Alinity m produced the first results sooner than the cobas 6800 System; the first 12 results were released 2:35h after initialization. Afterward Alinity m released results consistently every 16 minutes in batches of 12, releasing 96, 192, 288, 384, 480, and 564 results 4:25, 6:50, 8:55, 11:05, 13:10, and 15:30 hours after initialization, respectively (Fig. 1). On the other hand, the cobas 6800 System required 3:40h from initialization to release the first batch of 94 results. Afterward the cobas 6800 System released results in 96 batches, releasing 188, 282, 376, 470, and 564 results 5:15, 6:50, 8:25, 10:00, and 11:35 hours after initialization, respectively. The total hands-on time for testing 564 samples was similar for both instruments: 305 min for Alinity m and 315 min for the cobas 6800 System. However, the cobas 6800 System required slightly less total instrument handling time than Alinity m (35 vs. 50 min), but slightly more total sample handling time (280 vs. 255 min). In addition, the cobas 6800 System required laboratory staff presence during seven similar time slots (40–50 min each) whereas Alinity m required presence during 12 varying time slots (5–90 min; Fig. 1).
Comparative TAT assessment. Parallel routine processing of the first 94 samples received in the laboratory each working day for 5 consecutive days on both analyzers showed that TATs for almost all samples were shorter for Alinity m (143–257 min) in comparison to the cobas 6800 System (243–269 min). Although the instrument-on-board time range was similar between the two analyzers (Alinity m 130–215 min, cobas 6800 System 168–174 min), the total pre-analytical time range differs for most samples (Alinity m 12–86 min, cobas 6800 System 72–91 min).

DISCUSSION

Unprecedented demand for SARS-CoV-2 diagnostics led to development of a range of molecular assays designed for analyzers with high sample throughput allowing significant scaling up due to the fully automated sample-to-result solution. At present at least six high-throughput SARS-CoV-2 RNA assays are available: cobas SARS-CoV-2 4-11, Alinity m SARS-CoV-2 20, Panther Fusion SARS-CoV-2 and Aptima SARS-CoV-2 (Hologic, San Diego, CA, USA) 5, 8, 24-27, NeuMoDx SARS-CoV-2 (NeuMoDx Molecular, Ann Arbor, MI, USA) 28, 29, and GeneXpert Infinity SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA). Although several evaluations of these assays were published, in addition to present study only a single head-to-head comparison of two SARS-CoV-2 high-throughput assays was identified. In this particular study, clinical performance of cobas SARS-CoV-2 and Panther Fusion SARS-CoV-2 was compared on 389 nasopharyngeal swabs and assays showed high overall percent agreement of 96.4% and a high kappa value of 0.922. 8

Among fully automated analyzers enabling SARS-CoV-2 RNA testing, Alinity m was the most recently launched (in 2019), and as of April 2021, seven molecular assays were developed and
launched for use with Alinity m. They are intended for: (i) quantitative detection of hepatitis B virus DNA; \textsuperscript{13, 17, 19, 21} (ii) quantitative detection of hepatitis C virus RNA; \textsuperscript{12, 17, 18, 21} (iii) quantitative detection of HIV-1 RNA; \textsuperscript{15–17, 21} (iv) qualitative detection of SARS-CoV-2 RNA; \textsuperscript{20} (v) qualitative detection of 14 high-risk human papillomaviruses coupled with extended genotyping; \textsuperscript{14, 22} (vi) qualitative detection and differentiation of \textit{Chlamydia trachomatis}, \textit{Trichomonas vaginalis}, \textit{Mycoplasma genitalium}, and \textit{Neisseria gonorrhoeae} \textsuperscript{23} and (vii) qualitative detection and differentiation of SARS-CoV-2, influenza A and B viruses, and respiratory syncytial virus. Twelve evaluations of Alinity m assays are available in peer-reviewed literature, \textsuperscript{12–23} but only a single analytical and clinical evaluation of Alinity m SARS-CoV-2 assay on a limited number of samples is known to have been published to date. \textsuperscript{20} In that study, analytical verification of Alinity m SARS-CoV-2 confirmed the manufacturer’s limit of detection claim of 100 copies/mL, with clinical evaluation performed on 203 residual nasopharyngeal swabs from symptomatic and asymptomatic individuals with suspected SARS-CoV-2 infection. Samples were comparatively tested using Alinity m SARS-CoV-2 and RealTime SARS-CoV-2 assay (Abbott), and positive and negative percent agreement between both assays of 92.2\% (95\%CI: 85.3–96.6\%) and 92.0\% (95\%CI: 84.8–96.5\%), respectively, were observed. CN values of 95 concordantly positive samples exhibited high correlation ($R^2$ value=0.95); however, on average for 14.1 higher CN values were reported on Alinity m SARS-CoV-2 than on RealTime SARS-CoV-2, probably due to the first 10 unread cycles with RealTime SARS-CoV-2. \textsuperscript{20} Some limited performance data are also provided by the manufacturer, which compared Alinity m SARS-CoV-2 with an undeclared RT-PCR assay holding FDA EUA on 104 clinical samples with a positive and negative percent agreements between both assays of 100.0\% (95\%CI: 95.5–100.0\%) and 96.5\% (95\%CI: 87.9–99.6\%),
respectively, with two more samples found positive by Alinity m SARS-CoV-2, both with CN>40. Performance of the assays was also assessed on 144 asymptomatic individuals, of whom 19 were concordantly positive, yielding percent positive and negative agreement of 100.0% (95%CI: 82.4–100.0%) and 100.0% (95%CI: 97.1–100.0%), respectively. In addition, no *in silico* impact on detecting different SARS-CoV-2 strains/variants or cross-reactivity with similar viruses has been observed. A manufacturer’s Technical Brief of 18 January 2021 reported that Alinity m SARS-CoV-2 performance is unaffected by three emerging variants of concern: VOC 202012/01-B.1.1.7, VOC 501Y.V2-B.1.351, and IC-0561-B.1.1248. In this study, Alinity m SARS-CoV-2 showed excellent overall, positive, and negative agreement with the comparator cobas 6800 SARS-CoV-2 on 2,129 samples with valid results for both. Thirty-seven discordant results were identified. Discordance between the assays was completely unidirectional (Table 1), with significantly higher mean and median Alinity m SARS-CoV-2 CN values among discordant samples versus concordant positive samples, suggesting slightly higher analytical sensitivity of Alinity m SARS-CoV-2 over cobas 6800 SARS-CoV-2. These results agree with recent data on assay performance on an U.S. FDA SARS-CoV-2 reference panel allowing comparative performance among different FDA EUA assays. These data indicate Alinity m SARS-CoV-2 has a relative sensitivity of 600 NAAT detectable units (NDU) per ml (Package insert, EUA version R7, December 2020) compared to cobas 6800 SARS-CoV-2, with a relative sensitivity of 1,800 NDU/mL ([https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-reference-panel-comparative-data, last accessed April 10, 2021](https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-reference-panel-comparative-data)). Higher sample volume input of Alinity m SARS-CoV-2 (800 vs. 600 µl) might contribute to higher analytical sensitivity over cobas 6800 SARS-CoV-2. Since Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2 are targeting different SARS-CoV-2 genes, there is also a
possibility that discordant performance is a consequence of different assay design (ORF1 and E genes versus N and RdRp genes). Namely, it has been reported recently that certain deletions/mutations in SARS-CoV-2 genome can affect commercial PCRs. However such a scenario is less likely for Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2, since both are designed as a two-target assay making them less prone to false negative results. Nevertheless, constant monitoring of emergence of polymorphisms that might adversely affect PCRs used in diagnostics of SARS-CoV-2 is needed.

Higher analytical sensitivity of a particular SARS-CoV-2 PCR test does not necessarily indicate better clinical value of an analytically more sensitive test. Namely, great majority of clinically relevant SARS-CoV-2 RNA positive samples contain high viral load (and have relatively low CN/Ct values) and are consequently concomitantly positive by different PCR-based assays. Clinical benefit of PCR-based assays with higher analytical sensitivity over standard PCRs is usually evident only in limited fraction of positive samples having low SARS-CoV-2 RNA viral load (and having high CN/Ct values) mainly due to suboptimal sample collection or being obtained in very early phase of COVID-19 infection. Discordant analysis of samples weakly positive by one assay and negative by another is usually challenging. Due to the high sample volume requirement for both assays evaluated in the present study and consequent insufficient volume of the leftover specimen, retesting samples with discrepant Alinity m SARS-CoV-2 / cobas 6800 SARS-CoV-2 results was not possible. Thus, resolving the true SARS-CoV-2 RNA status in samples with discrepant results relied on previous and follow-up testing results for particular individuals recorded in our LIS. Based on detailed discordant analyses, out of 37 individuals with a sample yielding discordant results, 22 individuals (59.5%) could be considered Alinity m SARS-CoV-2 analytically true positive with high probability. Of these 22 individuals,
for at least eight a higher analytical sensitivity of Alinity m SARS-CoV-2 over cobas 6800 SARS-CoV-2 was clearly clinically beneficial because they tested cobas 6800 SARS-CoV-2 positive in one or more follow-up samples after the initial cobas 6800 SARS-CoV-2 negative result in the present study. This clinical benefit potentially also extends to four individuals with cobas 6800 SARS-CoV-2 presumptive positive / Alinity m SARS-CoV-2 positive results in the study sample, but without previous or follow-up results available. The clinical benefit of the higher analytical sensitivity of Alinity m SARS-CoV-2 in the remaining 10 individuals with a sample yielding an Alinity m SARS-CoV-2 analytically true positive / cobas 6800 SARS-CoV-2 negative result, but cobas 6800 SARS-CoV-2 positive in one or more previous sample(s), is less evident because the clinical value of extremely high-sensitivity molecular tests in follow-up testing of patients with clear laboratory-confirmed infection remains controversial. Unfortunately, due to the lack of additional information and the inability of repeated/further testing for 15/37 individuals with discordant Alinity m SARS-CoV-2 / cobas 6800 SARS-CoV-2 results, the status of their samples yielding discordant results remains unresolved.

Alinity m SARS-CoV-2 internal control is a noninfectious Armored RNA sequence unrelated to the SARS-CoV-2 sequence and is introduced into each specimen at the beginning of the sample preparation. Internal control is simultaneously amplified by PCR to demonstrate that the entire testing procedure has proceeded correctly for each sample. In this study, 28/2,157 (1.3%) samples showed initial invalid Alinity m SARS-CoV-2 results, all due to no amplification of target and internal control failure. Insufficient leftover volume allowed retesting of only 10/28 study samples with initial invalid Alinity m SARS-CoV-2 results, and after retesting a valid Alinity m SARS-CoV-2 result was obtained in 9/10 (90%) of initially failed samples. Currently, the cobas 6800 System has been installed in our laboratory for 2 years and Alinity m was
installed a week before the study. Although SARS-CoV-2 RNA invalid results were also initially an issue with the cobas 6800 System and were mainly caused by clots, mucus, or physical contamination detected by the instrument during sample aspiration or insufficient sample volume identified in sample tubes or processing plates, the instrument soon “stabilized,” and no significant problems with invalid results on the cobas 6800 System have been recorded in 10 months while routinely testing 120,000+ samples using the cobas 6800 SARS-CoV-2. After closing the head-to-head study, we continued monitoring the rate of invalid results on Alinity m for 8 more weeks. Interestingly, the rate of initially invalid results of Alinity m SARS-CoV-2 in the first 4 weeks remained similar to that recorded during the head-to-head study; specifically, 41/1,562 (2.6%), 44/2,375 (1.9%), 42/2,819 (1.5%), and 42/3059 (1.4%) in weeks 1–4, respectively. However, in the following 4 weeks a significant decrease in the rate of initially invalid results was observed; specifically, 10/3,949 (0.3%), 9/3,830 (0.2%), 11/4,070 (0.3%) and 6/2,761 (0.2%) in weeks 5–8, respectively. The most probable reason for the improvement might be a combination of an increased analyzer’s stability and accumulated operators’ experiences. Due to the insufficient leftover volume in most routine samples, in our laboratory all samples with initially invalid results of Alinity m SARS-CoV-2 are currently being repeated with another testing protocol requiring a smaller sample volume (200 µl), as described previously. However, for this study all routinely tested samples with initially invalid results that had 800 µl leftover volume available were repeated with Alinity m SARS-CoV-2, and a valid result was obtained in 77/78 (98.7%) of initially failed samples. Thus, if required sample volume is available, simple repeated testing (without further sample manipulation) is recommended for samples with initially invalid results of Alinity m SARS-CoV-2.
When selecting a SARS-CoV-2 RNA assay, virologists must consider not only sensitivity and specificity, but also sample throughput, time-to-result, test complexity, reagent and instrument availability, and cost per reportable result. Assay throughput is especially a crucial parameter for large-scale testing. Alinity m and the cobas 6800 System are both fully integrated and automated analyzers allowing sample-to-result detection of SARS-CoV-2 RNA, but this comparative throughput assessment showed potentially important differences (Fig. 1). Alinity m produced the first reportable results much sooner than the cobas 6800 System (2:35h vs. 3:40h), but the cobas 6800 System finished testing 564 samples in parallel almost 2 hours earlier than Alinity m (11:35h vs. 15:30h). Similarly, although the total hands-on time for testing 564 samples was almost equal for both instruments, the cobas 6800 System required slightly less total instrument handling time than Alinity m, but slightly more total sample handling time. In addition, during testing of 564 samples in parallel across two 8-hour shifts, the cobas 6800 System required the presence of laboratory staff during seven similar time slots whereas Alinity m required staff presence during 12 varying time slots (Fig. 1). Similar potentially important differences between the analyzers were also recorded during parallel routine processing of the first 94 samples received at the laboratory each working day for 5 consecutive days. This test on 470 samples showed that TATs for most samples were somewhat shorter for Alinity m in comparison to the cobas 6800 System. This discrepancy was mainly due to differences observed in total pre-analytical time between the analyzers for most samples and because our laboratory routinely (and most economically) processed 12-sample batches for Alinity m and 94-sample batches for the cobas 6800 System. Thus in different laboratory settings the differences between the analyzers observed in this study could be less evident and less important.
When asked to qualitatively compare their experience with both analyzers for routine detection of SARS-CoV-2 RNA, laboratory staff agreed that the main comparative advantages of Alinity m are rapid TAT for smaller batches and flexible STAT prioritization, and the main disadvantages are the need to pre-thaw and centrifuge reagents, more frequent instrument interactions and sample loading/unloading, and limited (48-hour) onboard stability of positive and negative controls. The main comparative advantages of the cobas 6800 System were higher 24-hour throughput, ready-to-use reagents not requiring thawing or mixing, and less frequent instrument interactions and sample loading/unloading, and the main disadvantage (in our laboratory setting) was needing sample centrifugation to avoid problems during sample aspiration due to clots, mucus, or physical contamination.

In conclusion, the results of the manufacturer-independent evaluation of Alinity m SARS-CoV-2 on 2,157 samples in routine diagnostic settings against cobas 6800 SARS-CoV-2 showed that Alinity m SARS-CoV-2 is a reliable assay for the qualitative detection of SARS-CoV-2 in nasopharyngeal swab samples. Assays showed excellent overall, positive, and negative percent agreements with a high kappa value. Slightly higher analytical sensitivity of Alinity m SARS-CoV-2 was clinically beneficial in a limited number of samples. Comparative real-life throughput and TAT assessments showed similar performance of both assays with performance differences, which could be potentially important in some laboratory settings.

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REFERENCES

1. Ravi N, Cortade DL, Ng E, Wang SX: Diagnostics for SARS-CoV-2 detection: a comprehensive review of the FDA-EUA COVID-19 testing landscape. Biosens Bioelectron 2020, 165:112454.

2. Loeffelholz MJ, Tang YW: Laboratory diagnosis of emerging human coronavirus infections—the state of the art. Emerg Microbes Infect 2020, 20:1–26.

3. Arena F, Pollini S, Rossolini GM, Margaglione M: Summary of the available molecular methods for detection of SARS-CoV-2 during the ongoing pandemic. Int J Mol Sci 2021, 22:1298.

4. Poljak M, Korva M, Knap Gašper N, Fujs Komloš K, Sagadin M, Uršič T, Avšič Županc T, Petrovec M: 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. J Clin Microbiol 2020, 58:e00599-20.

5. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL: Comparison of commercially available and laboratory-developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. J Clin Microbiol 2020, 58:e00821-20.

6. Cradic K, Lockhart M, Ozbolt P, Fatica L, Landon L, Lieber M, Yang D, Swickard J, Wongchaowart N, Fuhrman S, Antonara S: Clinical evaluation and utilization of multiple molecular in vitro diagnostic assays for the detection of SARS-CoV-2. Am J Clin Pathol 2020, 154:201–207.
7. Pujadas E, Ibeh N, Hernandez MM, Waluszko A, Sidorenko T, Flores V, Shiffrin B, Chiu N, Young-Francois A, Nowak MD, Paniz-Mondolfi AE, Sordillo EM, Cordon-Cardo C, Houldsworth J, Gitman MR: Comparison of SARS-CoV-2 detection from nasopharyngeal swab samples by the Roche cobas 6800 SARS-CoV-2 test and a laboratory-developed real-time RT-PCR test. J Med Virol 2020, 92:1695–1698.

8. Craney AR, Velu PD, Satlin MJ, Fauntleroy KA, Callan K, Robertson A, La Spina M, Lei B, Chen A, Alston T, Rozman A, Loda M, Rennert H, Cushing M, Westblade LF: Comparison of two high-throughput reverse transcription-PCR systems for the detection of severe acute respiratory syndrome coronavirus 2. J Clin Microbiol 2020, 58:e00890-20.

9. Smithgall MC, Scherberkova I, Whittier S, Green DA: Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the rapid detection of SARS-CoV-2. J Clin Virol 2020, 128:104428.

10. Moran A, Beavis KG, Matushek SM, Ciaglia C, Francois N, Tesic V, Love N: Detection of SARS-CoV-2 by use of the Cepheid Xpert Xpress SARS-CoV-2 and Roche cobas SARS-CoV-2 assays. J Clin Microbiol 2020, 58:e00772-20.

11. Wirden M, Feghouli L, Bertine M, Nere ML, Le Hingrat Q, Abdi B, Boutolleau D, Ferre VM, Jary A, Delaunay C, Marcelin AG, Descamps D, Legoff J, Visscaux B, Chaix ML: Multicenter comparison of the cobas 6800 System with the RealStar RT-PCR kit for the detection of SARS-CoV-2. J Clin Virol 2020, 130:104573.

12. Chevaliez S, Onelia F, Pacenti M, Goldstein E, Galán JC, Martínéz-García L, Vilas A, Glass A, Maree L, Krügel M, Ehret R, Knechten H, Braun P, Naeth G, Bonanzainga S, Jackson K, Abravaya K, Dhein J, Huang S, Joseph AM, Lucic D, Marlowe N, Palm MJ, Pféifer K,
Toolsie D, Reinhardt B, Obermeier M, Gunson R: Multicenter clinical evaluation of Alinity m HCV assay performance. J Clin Virol 2020, 129:104531.

13. Bonanzinga S, Onelia F, Jackson K, Glass A, Maree L, Krügel M, Pacenti M, Gunson R, Goldstein E, García LM, Galán JC, Vilas A, Ehret R, Knechтен H, Naeth G, Braun P, Obermeier M, Marlowe N, Palm MJ, Pfeifer K, Joseph AM, Dhein J, Reinhardt B, Lucic D, Chevaliez S: Multicenter clinical evaluation of Alinity m HBV assay performance. J Clin Virol 2020, 129:104514.

14. Oštrbenk Valenčak A, Šterbenc A, Seme K, Poljak M: Alinity m HR HPV assay fulfills criteria for human papillomavirus test requirements in cervical cancer screening settings. J Clin Microbiol 2019, 58:e01120-19.

15. Braun P, Glass A, Maree L, Krügel M, Pacenti M, Onelia F, Gunson R, Goldstein E, Martínez-García L, Galán JC, Vilas A, D’costa J, Sameer R, Ehret R, Knechтен H, Naeth G, Bouvier-Alias M, Marlowe N, Palm MJ, Joseph AM, Dhein J, Reinhardt B, Pfeifer K, Lucic D, Obermeier M: Multicenter clinical comparative evaluation of Alinity m HIV-1 assay performance. J Clin Virol 2020, 129:104530.

16. Maree L, Krügel M, Reinhardt B, Glass AJ: Evaluation of the Alinity m HIV-1 assay for the quantification of HIV-1 RNA plasma viral load in a high-throughput molecular laboratory in South Africa. J Clin Virol 2020, 132:104644.17.

17. Mouna L, Pallier C, Proust S, Prégermain C, Roque-Afonso AM: Comparison of the Abbott Alinity m and m2000 assays for the quantification of HIV-1, HCV and HBV in clinical samples. J Clin Virol 2020, 126:104331.
18. Goldstein EJ, Shepherd SJ, Gunson RN: Investigating utilising the Alinity m platform to detect hepatitis C virus RNA in dried blood spot samples. J Clin Virol 2020, 132:104647.

19. Jackson K, Tekoaua R, Li X, Locarnini S: Real-world application of the Xpert HBV viral load assay on serum and dried blood spots. J Med Virol 2021: Online ahead of print. https://doi.org/10.1002/jmv.26662

20. Hirschhorn JW, Kegl A, Dickerson T, Glen WB Jr, Xu G, Alden J, Nolte FS: Verification and validation of SARS-CoV-2 assay performance on the Abbott m2000 and Alinity m systems. J Clin Microbiol 2021, Epub ahead of print. https://doi.org/10.1128/jcm.03119-20

21. Galindo LT, Domingues Hristov A, Girotto Gentil L, Scarpelli L, Santiago J, Levia JE: Performance evaluation of the fully automated molecular system Alinity m in a high-throughput central laboratory. J Clin Virol 2021: In press. https://doi.org/10.1016/j.jcv.2021.104786

22. Dhillon SK, Valenčak AO, Xu L, Poljak M, Arbyn M: Clinical and analytical evaluation of the Alinity m HR HPV assay within the VALGENT-3 framework. J Clin Microbiol 2021, Epub ahead of print. https://doi.org/10.1128/jcm.00286-21

23. Herrmann B, Malm K: Comparison between Abbott m2000 RealTime and Alinity m STI systems for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium*. Eur J Clin Microbiol Infect Dis 2021, Epub ahead of print. https://doi.org/10.1007/s10096-020-04135-9
24. Zhen W, Manji R, Smith E, Berry GJ: Comparison of four molecular in vitro diagnostic assays for the detection of SARS-CoV-2 in nasopharyngeal specimens. J Clin Microbiol 2020, 58:e00743-20.

25. Trémeaux P, Lhomme S, Abravanel F, Raymond S, Mengelle C, Mansuy JM, Izopet J: Evaluation of the Aptima™ transcription-mediated amplification assay (Hologic®) for detecting SARS-CoV-2 in clinical specimens. J Clin Virol 2020, 129:104541.

26. Smith E, Zhen W, Manji R, Schron D, Duong S, Berry GJ: Analytical and clinical comparison of three nucleic acid amplification tests for SARS-CoV-2 detection. J Clin Microbiol 2020, 58:e01134-20.

27. Hogan CA, Sahoo MK, Huang C, Garamani N, Stevens B, Zehnder J, Pinsky BA: Comparison of the Panther Fusion and a laboratory-developed test targeting the envelope gene for detection of SARS-CoV-2. J Clin Virol 2020, 127:104383.

28. Mostafa HH, Lamson DM, Uhteg K, Geahr M, Gluck L, de Cárdenas JNB, Morehead E, Forman M, Carroll KC, Hayden RT, George KS: Multicenter evaluation of the NeuMoDx SARS-CoV-2 test. J Clin Virol 2020, 130:104583.

29. Lima A, Healer V, Vendrone E, Silbert S: Validation of a modified CDC assay and performance comparison with the NeuMoDx™ and DiaSorin® automated assays for rapid detection of SARS-CoV-2 in respiratory specimens. J Clin Virol 2020, 133:104688.

30. Artesi M, Bontems S, Göbbels P, Franckh M, Maes P, Boreux R, Meex C, Melin P, Hayette MP, Bours V, Durkin K: A recurrent mutation at position 26340 of SARS-CoV-2 is
associated with failure of the E gene quantitative reverse transcription-PCR utilized in a commercial dual-target diagnostic assay. J Clin Microbiol 2020, 58:e01598-20.

31. Ramírez JD, Muñoz M, Patiño LH, Ballesteros N, Paniz-Mondolfi A: Will the emergent SARS-CoV2 B.1.1.7 lineage affect molecular diagnosis of COVID-19? J Med Virol 2021, 93:2566-8.
FIGURE 1 Time curves visualizing release dynamics of final SARS-CoV-2 RNA results of Alinity m (red curve) and the cobas 6800 System (blue curve) while testing 564 nasopharyngeal swab samples in parallel across two 8-hour shifts. Sample handling times, instrument handling times, and total hands-on times measured by two independent observers for each analyzer are presented with red (Alinity m) and blue (the cobas 6800 System) time boxes; the dark part of each box represents sample handling time, and the lighter part of each box instrument handling time.
TABLE 1 Results of comparative clinical evaluation of the Alinity m SARS-CoV-2 assay against the cobas 6800 SARS-CoV-2 assay on 2,129 nasopharyngeal swab samples with valid initial results of both assays. A total of 28 samples were excluded from further analysis due to initial invalid Alinity m SARS-CoV-2 results. Interpretation of 37 discordant results, including four Alinity m SARS-CoV-2 positive / cobas 6800 SARS-CoV-2 presumptive positive results, is provided in detail in Results and Discussion.

| Alinity m SARS-CoV-2 | cobas 6800 SARS-CoV-2 | Overall agreement (95% CI) | Kappa value (95% CI) |
|----------------------|------------------------|---------------------------|---------------------|
| Positive             | 961                    | 98.3% (97.6–98.7%)        | 0.965 (0.954–0.976) |
| Negative             | 0                      | 1,131                     |                     |
| Total                | 961                    | 1,168                     | 2,129               |
Number of final results released

Time (hh:mm)

cobas
Alinity

total hands-on time: 315 min
total hands-on time: 305 min

total hands-on time: 315 min
total hands-on time: 305 min