



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Clinical performance and sample freeze-thaw stability of the cobas®6800 SARS-CoV-2 assay for the detection of SARS-CoV-2 in oro-/nasopharyngeal swabs and lower respiratory specimens

Joep J.J.M. Stohr^{a,b,c,*}, Meike Wennekes^d, Martijn van der Ent^d, Bram M.W. Diederens^{b,c,d}, Marjolein F.Q. Kluytmans-van den Bergh^{a,e,f}, Anneke M.C. Bergmans^d, Jan A.J. W. Kluytmans^{a,b,c,e,f}, Suzan D. Pas^{c,d}

^a Department of Infection Control, Amphia Hospital, Breda, the Netherlands

^b Microvida Laboratory for Microbiology, Elisabeth-TweeSteden Hospital, Tilburg, the Netherlands

^c Microvida Laboratory for Microbiology, Amphia Hospital, Breda, the Netherlands

^d Microvida Laboratory for Microbiology, Bravis Hospital, Roosendaal, the Netherlands

^e Amphia Academy Infectious Disease Foundation, Amphia Hospital, Breda, the Netherlands

^f Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

ARTICLE INFO

Keywords:

SARS-CoV-2

COVID-19

Real-time RT-PCR

Viral load

ABSTRACT

Introduction: Studies describing the performance characteristics of the cobas®6800 system for SARS-CoV-2 detection in deep respiratory specimens and freeze-thaw stability are limited. The current study compares the clinical performance of the automated SARS-CoV-2 assay on the cobas®6800 system to a lab-developed assay (LDA) and the cobas impact of freeze-thawing combined with lysis buffer.

Methods: Both retrospective and prospectively selected deep respiratory samples and oro- and nasopharyngeal samples in either E-swab® or GLY- were tested using the SARS-CoV-2 assay on the cobas®6800 System and compared to a lab developed assay. Additionally, SARS-CoV-2 RNA stability was assessed after one freeze-thaw cycle with or without lysis buffer.

Results: In total, 221 (58.3 %) oro- and nasopharyngeal swabs, 131 (34.6 %) deep respiratory specimens, and n = 25 (6.6 %) swabs of unknown origin were included to study clinical performance. Only 4 samples gave discrepant results, all being positive in the LDA and not the cobas®6800 system. For stability testing, 66 samples without and 110 with lysis buffer were included. No clinically significant difference was found in test results after one freeze-thaw cycle and addition of lysis buffer.

Conclusion: Based on our findings, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results as the LDA in oro-/nasopharyngeal swabs and deep respiratory specimens. Moreover, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results before and after a freeze-thaw cycle, with better preservation of low viral loads in lysis buffer.

1. Introduction

In December 2019, severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) was first identified as a causal agent for cases of viral pneumonia in Wuhan, China [1,2]. Since then, SARS-CoV-2 has spread across the globe causing millions of cases of coronavirus disease 2019 (COVID-19). The detection of SARS-CoV-2 RNA with (real-time) reverse transcriptase polymerase chain reaction (RT-PCR) assays is the cornerstone of COVID-19 diagnostics, and a variety of different PCR

assays have been made publicly available since the onset of the pandemic [3,4]. Widespread laboratory testing of potentially infected patients has a central role in attempts to mitigate the spread of SARS-CoV-2 [5], and requires rapid upscaling of the test capacity by automation [6–8]. Several automated systems, like the cobas®6800 assay, have been validated for the detection of SARS-CoV-2 in oro-/nasopharyngeal swabs in virus transport medium [9–11], but performance data for other clinical specimen types are limited. Several studies involving SARS-CoV-2 detection using PCR assays use samples which

* Corresponding author at: Department of Infection Control, Amphia Hospital, Breda, the Netherlands.

E-mail address: j.stohr@etz.nl (J.J.J.M. Stohr).

<https://doi.org/10.1016/j.jcv.2020.104686>

Received 16 September 2020; Received in revised form 30 October 2020; Accepted 1 November 2020

Available online 8 November 2020

1386-6532/© 2020 Elsevier B.V. All rights reserved.

have undergone a freeze-thaw cycle [9,12,13]. Currently, no data is available on the effect of a freeze-thaw cycle, on the performance of the cobas®6800 assay. Although SARS-CoV-2 should be handled as a biosafety level 2 agent for nucleic acid amplification tests according to the World Health Organization, most automated systems do not operate on BSL2 level and should, therefore, include an external lysis step prior to loading [14]. The objective of this study is to compare the clinical performance of the automated SARS-CoV-2 assay on the cobas®6800 assay (Roche Diagnostics, Switzerland) to an ISO15189:2012 lab-developed assay (LDA) for the detection of SARS-CoV-2 RNA in externally lysed (oro-/nasopharyngeal) swabs (E-swab® (Copan, Italy) or swabs in GLY-medium) and lower respiratory tract specimen. The time from receipt of sample by the laboratory until result was compared between the LDA and automated cobas®6800 assay. Furthermore, the Ct-values of both cobas®6800 assay targets in oro-/nasopharyngeal and lower respiratory samples were compared before and after a freeze thaw cycle, with and without additional lysis buffer.

2. Methods

2.1. Setting and study population

All oro-/nasopharyngeal and lower respiratory samples collected from patients submitted to Microvida laboratory for microbiology as part of routine clinical diagnostics were deemed eligible for inclusion in the study. Microvida laboratory for microbiology receives samples from two general, two teaching hospitals, long-term care facilities and general practitioners in the south of the Netherlands.

2.2. SARS-CoV-2 semi-quantitative real-time RT-PCR using cobas®6800 assay and lab-developed assay

Before testing on the cobas®6800 SARS-CoV-2 PCR assay, samples were pre-treated in a BSL3/BSL2 laminar flow cabinet. Oro-/nasopharyngeal swabs (E-swab® in Amies (Copan, Italy) or GLY-medium) were vortexed. Subsequently, 600 µL of E-swab®- or GLY-medium was added to a collection tube containing 600 µL of MagnaPure LC lysis- and binding buffer (LBB) (Roche diagnostics, The Netherlands) and mixed by pipetting. External lysis was allowed for at least 15 min at room temperature. Lower respiratory specimens (sputa, bronchial fluid, and bronchoalveolar lavage fluid) were pre-treated by placing a flake of material in a tube containing 600 µL of LBB and Sentosa SX lysis beads (Vela Diagnostics, Germany) and shaking the suspension in the MagNA lyser (Roche, Switzerland) for 70 s at 5000 rpm. Thereafter, the tubes were centrifuged for 1 min at 6000 g, and external lysis was completed at room temperature for at least 15 min. Following pre-treatment, all samples were analysed on the cobas®6800 with the cobas® SARS-CoV-2 assay according to the manufacturer's instructions. In short, after loading pre-lysed samples, nucleic acid extraction was automatically performed, followed by an internally controlled semi-quantitative real-time reverse transcriptase polymerase chain reaction (sqRT-PCR) on the envelope (E) gene and the RNA dependent RNA polymerase (RdRp) gene and automated analysis of the sqRT-PCR results. The ISO15189:2012 accredited, internally controlled, single-target (Sarbeco-specific E-gene), sqRT-PCR lab-developed assay (LDA) was performed as described previously by Kluytmans-van den Bergh et al. [15]. The cobas®6800 assay was considered positive when both the E-gene and RdRp-gene targets were detected; inconclusive when either E-gene or RdRp-gene target was detected; negative when both targets were undetectable. The cobas®6800 results were considered invalid when either the E-gene or RdRp-gene target did not produce a result, due to internal/external invalid controls.

2.3. Assay comparison

Clinical performance was evaluated both retrospectively and

prospectively. For the retrospective evaluation, stored (−80 °C or +4 °C (for a maximum of 7 days)) LDA confirmed SARS-CoV-2 positive (Ct<35) and LDA SARS-CoV-2 negative oro-/nasopharyngeal, and lower respiratory samples were randomly selected from samples sent to the laboratory for routine testing from the March 1–31, 2020, ensuring the complete dynamic range of Ct-values and variety of sample types. The cobas®6800 assay results were compared to historical data generated using the LDA. For prospective evaluation, samples from patients submitted to Microvida laboratory for microbiology between the April 4–11, 2020, were stored at +4 °C until testing using both the cobas®6800 SARS-CoV-2 PCR assay and LDA. Samples with an inconclusive result in the cobas®6800 assay but not included in the initial comparison (n = 36), were additionally tested in the LDA and included to evaluate samples with viral loads near the lower limit of detection in more detail.

2.4. Time-to-result and invalid results

Additional samples were included to evaluate the time-to-result for the LDA and the cobas®6800 assay and to evaluate the number of invalid results in the cobas®6800 assay. The time-to-result, i.e. the time between sample receipt by the laboratory until generation of the SARS-CoV-2 RNA result, was extracted from the laboratory information system from samples received from March 1–31, 2020, for the LDA and from samples received from April 4–28, 2020 for the cobas®6800 assay. The number of invalid results was calculated for all samples tested on the cobas®6800 assay from April 4–28, 2020.

2.5. Freeze-thawing samples with and without lysis buffer

From April 28- July 1, 2020 lower respiratory and oro-/nasopharyngeal samples, submitted to Microvida laboratory for microbiology for routine clinical diagnostics and that tested positive for SARS-CoV-2 RNA using the cobas®6800 assay, were stored overnight at −80 °C with or without external lysis buffer. Following overnight freezing, the selected samples were thawed at room temperature and re-tested using the cobas®6800 assay. The cobas®6800 SARS-CoV-2 Ct-values were compared before and after this one freeze-thaw cycle for each sample.

2.6. Statistical analysis

A McNemar test for paired samples was performed using Researchpy v0.1.9 and Pandas v1.0.3. Bland-Altman plots comparing the cobas®6800 and LDA SARS-CoV-2 Ct-values of the prospectively collected samples and comparing the cobas®6800 SARS-CoV-2 Ct-values before and after a freeze-thaw cycle, were drawn using Matplotlib v3.2.1. The mean of the differences (and 95 % confidence interval) between the cobas®6800 assay and LDA Ct-values and between the cobas®6800 SARS-CoV-2 Ct-values before and after a freeze-thaw cycle was calculated using NumPy v1.18.4 and SciPy v1.4.1. In the Bland-Altman analysis, a Ct-value of 40 was imputed for a negative result in samples with discrepant results. Samples with imputed values were excluded from calculating the mean difference in Ct-value. A Mann-Whitney *U* test was performed between the median Ct-values of the LDA E-gene, cobas®6800 E-gene and cobas®6800 RdRp of the prospectively collected deep respiratory specimens and oro-/nasopharyngeal swabs using SciPy v1.4.1. The adjusted Wald confidence interval of the proportion of invalid test results per specimen type was calculated using NumPy v1.18.4 and SciPy v1.4.1.

3. Results

3.1. Assay comparison

A total of 377 samples were tested on both the cobas®6800 assay and LDA (retrospectively: n = 172; prospectively: n = 205). In total, 221

(58.6 %) oro-/nasopharyngeal swabs in either E-swab® or GLY-medium, 131 (34.7 %) lower respiratory specimens and n = 25 (6.6 %) swabs of unknown origin were included. In total 118 (31.3 %) samples were positive in both the cobas®6800 assay and LDA, 217 (57.6 %) samples were negative in both assays, 38 (10.1 %) samples had an inconclusive result in the cobas®6800 assay (single RdRp or E gene detectable) and cobas®6800 assay-LDA discrepant results were found in only 4 (1.1 %) samples (all being positive in the LDA and negative cobas®6800 assay) (Table 1). Two of these latter 4 samples were of lower respiratory origin and 2 samples were oro-/nasopharyngeal swabs. The samples with inconclusive results in the cobas®6800 assay were either single E-gene (n = 36) or RdRp-gene target (n = 2) positive. Twenty-two (57.9 %) of these samples with inconclusive results cobas®6800 assay, still had SARS-CoV-2 RNA detectable in the LDA (Table 1). However, the LDA E-gene Ct-values were higher than 35.0 in 16 (72.7 %) of these samples, confirming low viral loads. If all samples with inconclusive results in the cobas®6800 assay were excluded from the analysis, no significant difference was observed between the two assays ($p > 0.05$) (Table 1). Nonetheless, if all inconclusive results in the cobas®6800 assay were considered either positive or negative, the number of discrepant results increased to 20 and 26 respectively resulting in significant difference (all positive: $p = 0.01$; all negative: $p < 0.01$) (Table 1).

The mean difference in Ct-value between the LDA E-gene and cobas®6800 assay E-gene results of the prospectively collected samples was 3.8 (95 % confidence interval (CI): 3.4–4.2). Ct-values being lower in the LDA as compared to the cobas®6800 assay (including all sample types without inconclusive results) (Fig. 1a). The mean difference in Ct-values between the cobas®6800 assay RdRp-gene target and the E-gene LDA of the prospectively collected samples was 3.2 (95 %CI: 2.7–3.7). However, the difference in Ct-value between the LDA E-gene and the cobas®6800 E-gene or RdRp results decreased with increasing mean Ct-value (Fig. 1a and b respectively). The mean difference in E-gene or RdRp Ct-value between the LDA and the cobas®6800 assay was higher in the lower respiratory samples when compared to oro-/nasopharyngeal swabs (Table 2). No statistically significant difference was detected between the median Ct-values of the LDA E-gene, cobas®6800 E-gene and cobas®6800 RdRp gene of the prospectively collected deep respiratory specimens and oro-/nasopharyngeal swabs (Supplementary table S1).

3.2. Time-to-result and invalid results

Additional samples were included to evaluate the time-to-result for the LDA and the cobas®6800 assay and to evaluate the number of invalid results in the cobas®6800 assay. A total of 6609 samples were tested for SARS-CoV-2 RNA using the LDA from the 1st of 31st of March. The median number of hours between the sample arriving in the laboratory until result (time-to-result) during this period, was 20.6 h. From the April 6, 2020, until April 28, 2020, 6746 samples were tested for SARS-CoV-2 RNA presence on the cobas®6800 assay. During this period, the median time to result was 7.2 h. In 48 (0.7 %; 95 %CI: 0.5–0.9%) of these 6746 samples the cobas®6800 SARS-CoV-2 RNA tests yielded invalid results (Table 3) compared to 36 out 6609 samples (0.5 %; 95 %CI: 0.4–0.8%) for the LDA. The percentage of samples with invalid results in the cobas®6800 assay was similar between the lower respiratory samples and the oro-/nasopharyngeal swabs being 1.0 % (95

Table 1

Qualitative SARS-CoV-2 RNA results of all (retrospective and prospective) samples tested in the LDA and cobas®6800 assay.

| Result in LDA | Result in COBAS® 6800 system | | |
|---------------|------------------------------|----------|---------------|
| | Positive | Negative | Inconclusive* |
| Positive | 118 | 4 | 22 |
| Negative | 0 | 217 | 16 |

* Single RdRp or E-gene positive.

%CI: 0.3–2.8 %) and 0.6 % (95 %CI: 0.4–0.8%), respectively (Table 3). However, the percentage of invalid results was higher in the fecal samples tested being 28.9 % (95 %CI: 13.5–46.3 %) as compared to both the oro-/nasopharyngeal swabs and lower respiratory samples (Table 3).

3.3. Effect on ct-value of one freeze-thaw cycle for samples with and without lysis buffer

A total of 176 samples were collected to be frozen (−80 °C) and thawed once, of which 66 samples without and 110 samples with lysis buffer. One sample frozen without lysis buffer was positive (Ct-value: 34.7) in the E-gene target before freezing and negative following freezing. Five samples (n = 3 without lysis buffer, n = 2 with lysis buffer) were positive in the RdRp-gene target before freezing and negative following freezing (mean Ct-value: 31.4 (range 29.4–33.5)). The mean difference in Ct-value before and after a freeze-thaw cycle of the E-gene and RdRp-gene target respectively were: −0.7 (95 %CI: −1.2 to −0.2) and −0.3 (95 %CI: −0.7–0.2) in the samples frozen without lysis buffer; and 0.1 (95 %CI: −0.1–0.3) and 0.1 (95 %CI: −0.1–0.3) in the samples frozen with lysis buffer. In the samples frozen without lysis buffer and with a relatively medium-low load of SARS-CoV-2 RNA (Ct-value cobas®6800 E-gene > 30.0), the difference in Ct-value before and after a freeze-thaw cycle increased for both the E-gene and the RdRp-gene target (Figs. 2a; 3 a). The difference in Ct-values did not increase in the samples containing lysis buffer with a relatively medium-low SARS-CoV-2 RNA load (Ct-value cobas®6800 E-gene > 30.0) (Figs. 2b; 3 b).

4. Discussion

Based on our findings, the cobas®6800 SARS-CoV-2 RNA assay yielded similar qualitative results compared to the LDA in externally lysed (oro-/nasopharyngeal) swabs (E-swab® or swabs in GLY-medium) and pre-treated lower respiratory specimens. However, when cobas®6800 assay inconclusive (single RdRp or E gen positive) results were incorporated in the analysis, the results of both test assays significantly differed. This may be caused by the relatively high number of inconclusive results in the samples included in the study (38/377 (10 %)) with the aim to study clinical sensitivity, as compared to previous studies and our routine clinical samples (92/6698 (0.3 %)) [9,11]. As in previous studies, these inconclusive samples predominantly contained relatively low SARS-CoV-2 RNA loads (Ct-value cobas®6800 E-gene > 35.0) [11]. Most of these samples were only positive in the cobas®6800 E-gene target, which may be explained by the presence of more E gene target RNA due to generation of subgenomic RNA, while this phenomenon is not described for the RdRp gene. A higher sensitivity of the E-gene target as compared to the RdRp target for the detection of SARS-CoV-2 using PCR has already been described in other studies [16–18]. On the other hand, two samples were single positive in the RdRp target. This could be related to a previously described C26340 T mutation in the SARS-CoV-2 E-gene, resulting in a mismatch with primers/probes and thus escape from detection in samples with lower viral loads [19]. This supports the dual-target detection strategy for fast-evolving viruses to limit this risk for false-negative results. Inconclusive results due to low viral loads can be expected during the very beginning or at the end of the course of infection and should therefore be reported as such. A new sample should be requested, which either may confirm rising viral load or undetectable viral RNA.

Analysis of semi-quantitative results showed the mean Ct-value of both cobas®6800 targets were 3.2 and 3.8 higher than the Ct-value of the E gene target in the LDA assay, for RdRp and E gene respectively. Though tempting, this cannot be interpreted as an 8.6–13.0 times less sensitivity based on this Ct-difference alone (calculated with 100 % PCR reaction efficiency), since in samples with a relatively low SARS-CoV-2 RNA load, the difference in Ct-value between the cobas®6800 assay targets and LDA tended to be much lower. This effect confirmed

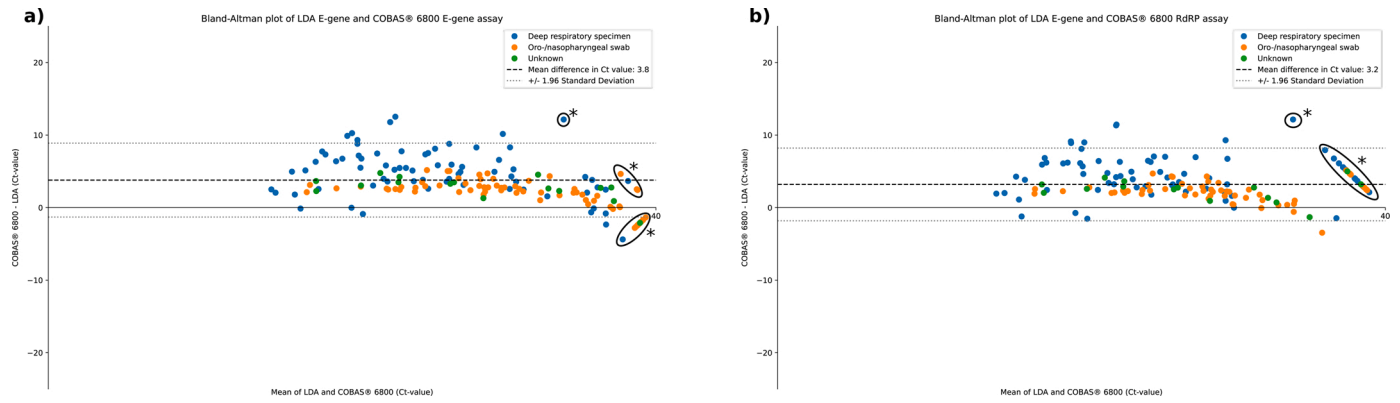


Fig. 1. Bland-Altman plot of the LDA and the cobas®6800 assay semi-quantitative results per specimen type, Ct-value of: **a)** cobas®6800 assay E-gene and LDA E-gene targets **b).** cobas®6800 assay RdRP-gene and LDA E-gene targets. *Samples with imputed results for either the LDA or cobas®6800 assay E-gene assay. Total number of samples: 160 (a), 144 (b).

Table 2

Mean difference in Ct-value between the LDA and cobas®6800 system per specimen type of the prospectively collected samples.

| Specimen source sample | E-gene COBAS® 6800 - E-gene LDA | | RdRP COBAS® 6800 - E-gene LDA | |
|---------------------------|--------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|
| | N of samples positive in both assays | Mean difference in Ct-value* (95% CI) | N of samples positive in both assays | Mean difference in Ct-value* (95% CI) |
| Deep respiratory specimen | 32 | 3.5 (2.7–4.4) | 23 | 3.2 (2.3–4.1) |
| Oro-/nasopharyngeal swabs | 43 | 2.3 (2.0–2.7) | 36 | 1.8 (1.3–2.3) |
| Swabs of unknown origin | 15 | 3.0 (2.4–3.6) | 13 | 2.2 (1.3–3.0) |

* Values above zero indicate a higher Ct value in the COBAS® 6800.

Table 3

Number of samples with a valid or invalid test result per specimen type in the cobas®6800 assay.

| Specimen type | N° of samples (%) | |
|---------------------------|-------------------|----------|
| | valid | invalid |
| Total | 6698 (99.3) | 48 (0.7) |
| Deep respiratory specimen | 379 (99.0) | 4 (1.0) |
| Oro-/nasopharyngeal swab | 5870 (99.3) | 34 (0.7) |
| Swab of unknown origin | 430 (99.3) | 3 (0.7) |
| Feces | 19 (73.1) | 7 (28.9) |

observations in a previous study [10]. The difference in Ct-values between the cobas®6800 and LDA targets was higher in lower respiratory specimens, with no significant effects on qualitative results in this

sample type.

The proportion of invalid results in the cobas®6800 assay, measured in nearly 6700 clinical samples, was similar between the pre-treated lysed lower respiratory samples and lysed swabs, but significantly higher in fecal samples. Therefore, we conclude that stool is not an appropriate sample type for analysis on the cobas®6800 SARS-CoV-2 assay in our hands. The median time-to-result was far lower using cobas®6800 assay, which can partly be due to the cobas® efficient workflow. With the most efficient LDA workflow, time to result can be as fast as 4.5–6 hours compared to 2.5–3 hours for the cobas®6800. However, due to a limited and delayed supply of extraction reagents and disposables for the MagnaPure96 system at the time, samples were tested in full run batches to optimize test capacity and efficacy, prolonging the LDA time-to-result. Since there were no difficulties regarding the supply of reagents for the cobas®6800 assay during this

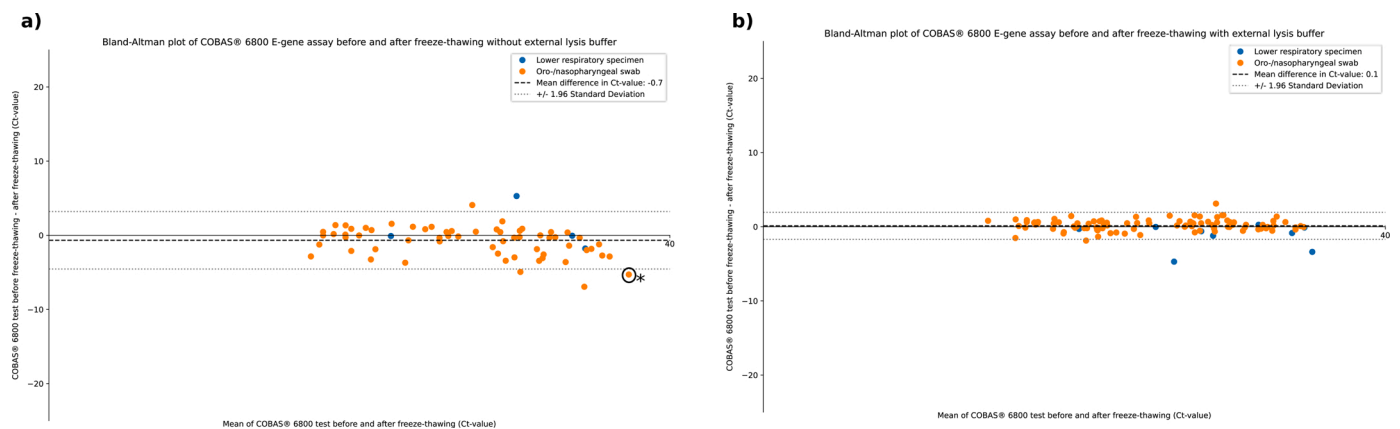


Fig. 2. Bland-Altman plot of the cobas®6800 assay E-gene target semi-quantitative results before and after freeze-thaw cycle per specimen type **a)** without lysis buffer; **b)** with lysis buffer. * Samples with imputed results for either the cobas®6800 assay E-gene or RdRP-gene assay before or after freeze thawing. Total number of samples is 66.

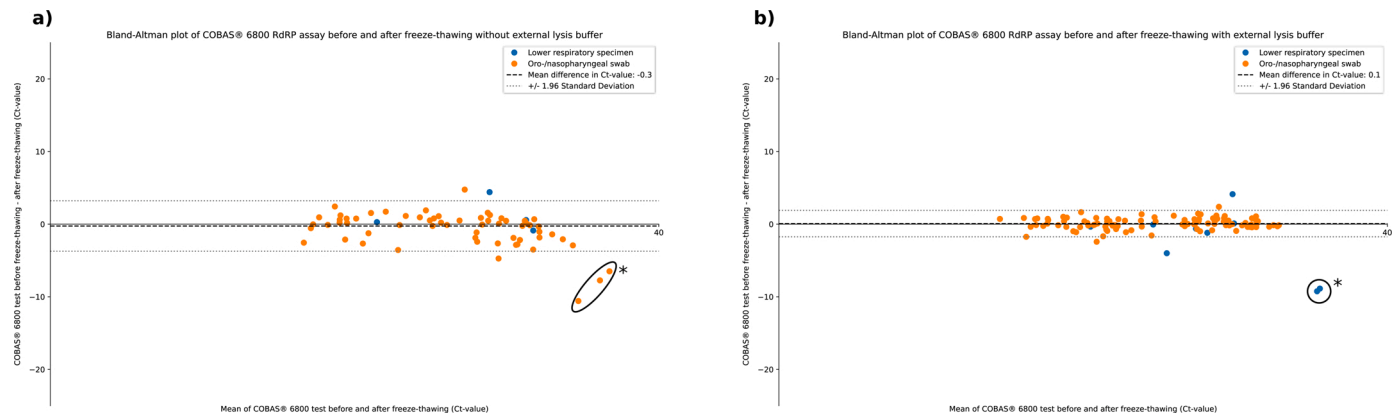


Fig. 3. Bland-Altman plot of the cobas®6800 assay semi-quantitative results before and after freeze-thawing with lysis buffer per specimen type: a) E-gene target; b) RdRP-gene target. * Samples with imputed results for either the cobas®6800 assay E-gene or RdRP-gene assay before or after freeze thawing. Total number of samples is 110.

study period, samples were tested as soon as they entered the laboratory, which reduced the time-to-result.

Analysis of the semi-quantitative results of the Ct-values generated by the cobas®6800 assay revealed similar results in samples before and after freeze-thawing, with and without external lysis buffer. Despite this, 6 samples converted from positive to inconclusive result after a single freeze-thaw cycle. Moreover, in the absence of external lysis buffer the difference in Ct-values of both targets before and after a freeze-thaw cycle increased in samples with a relatively high SARS-CoV-2 load. The current study only evaluated the performance of the cobas®6800 assay before and after freeze thawing. Future studies should evaluate the effect of freeze-thawing on the performance of other assays for SARS-CoV-2 detection.

Concluding, based on our findings, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results as the LDA in lysed (oro-/nasopharyngeal) swabs and lower respiratory specimens. Moreover, the cobas®6800 SARS-CoV-2 RNA assay yielded similar results before and after a freeze-thaw cycle, with better preservation of low viral loads in lysis buffer.

5. Credit author statement

JS analysed and interpreted the data, and drafted the manuscript. MW performed the experiments. ME performed the experiments. BD analysed and interpreted the data, and revised the manuscript. MK analysed and interpreted the data, and revised the manuscript. AB analysed and interpreted the data, and revised the manuscript. JK analysed and interpreted the data, and revised the manuscript. SP performed the experiments, analysed and interpreted the data, and revised the manuscript. All authors read and approved the final manuscript.

Funding

None to report.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We would like to thank all laboratory staff of Microvida Laboratory for Microbiology, location Bravis, Zorgsaam and Amphia for their efforts in processing the samples used in this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jcv.2020.104686>.

References

- [1] N. Zhu, D. Zhang, W. Wang, et al., A novel coronavirus from patients with pneumonia in China, 2019, *N. Engl. J. Med.* 382 (8) (2020) 727–733, <https://doi.org/10.1056/NEJMoa2001017>.
- [2] C. Huang, Y. Wang, X. Li, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, *Lancet* 395 (10223) (2020) 497–506, [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5).
- [3] V.M. Corman, O. Landt, M. Kaiser, et al., Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, *Eurosurveillance* 25 (3) (2020) 1–8, <https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045>.
- [4] Chan JF-W, Yip CC-Y, To KK-W, et al., Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens, *McAdam AJ, ed. J. Clin. Microbiol.* 58 (5) (2020) 1–10, <https://doi.org/10.1128/JCM.00310-20>.
- [5] M.P. Cheng, J. Papenburg, M. Desjardins, et al., Diagnostic testing for severe acute respiratory syndrome-related coronavirus-2, *Ann. Intern. Med.* 13 (2020) M20–1301, <https://doi.org/10.7326/M20-1301>. Published online April.
- [6] J.J.C. Voermans, S. Seven-Deniz, P.L.A. Fraaij, A.A. van der Eijk, M.P.G. Koopmans, S.D. Pas, Performance evaluation of a rapid molecular diagnostic, multicode based, sample-to-answer assay for the simultaneous detection of Influenza A, B and respiratory syncytial viruses, *J. Clin. Virol.* 85 (2016) 65–70, <https://doi.org/10.1016/j.jcv.2016.10.019>.
- [7] J.J.C. Voermans, D.G.J.C. Mulders, S.D. Pas, M.P.G. Koopmans, A.A. van der Eijk, R. Molenkamp, Performance evaluation of the panther fusion® respiratory tract panel, *J. Clin. Virol.* 123 (2020), 104232, <https://doi.org/10.1016/j.jcv.2019.104232> (December 2019).
- [8] D. Nörz, N. Fischer, A. Schultze, et al., Clinical evaluation of a SARS-CoV-2 RT-PCR assay on a fully automated system for rapid on-demand testing in the hospital setting, *J. Clin. Virol.* 128 (1) (2020), 104390, <https://doi.org/10.1016/j.jcv.2020.104390>.
- [9] E. Pujadas, N. Ibeh, M.M. Hernandez, et al., 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) 1–4, <https://doi.org/10.1002/jmv.25988> (April).
- [10] C.F. Lowe, N. Matic, G. Ritchie, et al., Detection of low levels of SARS-CoV-2 RNA from nasopharyngeal swabs using three commercial molecular assays, *J. Clin. Virol.* 128 (2020), 104387, <https://doi.org/10.1016/j.jcv.2020.104387> (April).
- [11] M. Poljak, M. Korva, N. Knap Gasper, et al., 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.* 58 (6) (2020) 1–7, <https://doi.org/10.1128/jcm.00599-20>.
- [12] F.M. Liotti, G. Menchinelli, S. Marchetti, et al., Evaluation of three commercial assays for SARS-CoV-2 molecular detection in upper respiratory tract samples, *Eur. J. Clin. Microbiol. Infect. Dis.* (2020), <https://doi.org/10.1007/s10096-020-04025-0>. Published online.
- [13] H.H. Mostafa, D.M. Lamson, K. Uhteg, et al., Multicenter evaluation of the NeuMoDx™ SARS-CoV-2 test, *J. Clin. Virol.* 130 (2020), 104583, <https://doi.org/10.1016/j.jcv.2020.104583> (July).
- [14] World Health Organization, Laboratory Biosafety Guidance Related to Coronavirus Disease 2019 (COVID-19): Interim Guidance, 12 February 2020, 2020. <https://apps.who.int/iris/bitstream/handle/10665/331138/WHO-WPE-GIH-2020.1-eng.pdf?sequence=1&isAllowed=y>.

- [15] Bergh M.F.Q.K.-Van Den, SARS-- CoV-- 2 Infection in 86 Healthcare Workers in Two Dutch Hospitals in March, 2020, pp. 1–16, <https://doi.org/10.1101/2020.03.23.20041913> (March).
- [16] C.B.F. Vogels, A.F. Brito, A.L. Wyllie, et al., Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets, *Nat. Microbiol.* 5 (10) (2020) 1299–1305, <https://doi.org/10.1038/s41564-020-0761-6>.
- [17] T. Pilonel, V. Scherz, K. Jaton, G. Greub, C. Bertelli, Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR, *Eurosurveillance* 25 (21) (2020) 1–2, <https://doi.org/10.2807/1560-7917.ES.2020.25.21.2000880>.
- [18] A.K. Nalla, A.M. Casto, M.-L.W. Huang, et al., Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit, McAdam AJ, ed. *J. Clin. Microbiol.* 58 (6) (2020) 1–6, <https://doi.org/10.1128/JCM.00557-20>.
- [19] M. Artesi, S. Bontems, P. Göbbels, et al., 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, Caliendo AM, ed. *J. Clin. Microbiol.* 58 (10) (2020) 1–8, <https://doi.org/10.1128/JCM.01598-20>.