Evaluation of a fully closed real time PCR platform for the detection of SARS-CoV-2 in nasopharyngeal swabs: a pilot study

Caterina De Luca,[1] Gianluca Gragnano,[1] Floriana Conticelli,[1] Michele Cennamo,[2] Pasquale Pisapia ,[1] Daniela Terracciano,[2] Umberto Malapelle ,[1] Emma Montella,[1] Maria Triassi,[1] Giancarlo Troncone ,[1] Giuseppe Portella[2]

► Supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jclinpath-2021-207516).

[1]Public Health, University of Naples Federico II, Naples, Italy
[2]Translational Medical Sciences, University of Naples Federico II, Naples, Italy

Correspondence to
Professor Giancarlo Troncone, Public Health, University of Naples Federico II, 80131 Naples, Italy; giancarlo.troncone@unina.it

Received 1 March 2021
Revised 21 March 2021
Accepted 24 March 2021

To cite: De Luca C, Gragnano G, Conticelli F, *et al*. *J Clin Pathol* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jclinpath-2021-207516

ABSTRACT

Aims To date, reverse transcriptase PCR (RT-PCR) on nasopharyngeal swabs is the 'gold standard' approach for the diagnosis of COVID-19. The need to develop easy to use, rapid, robust and with minimal hands-on time approaches are warranted. In this setting, the Idylla SARS-CoV-2 Test may be a valuable option. The aim of our study is to evaluate the analytical and clinical performance of this assay on previously tested SARS-CoV-2 people by conventional RT-PCR based approach in different settings, including initial diagnosis and clinical follow-up.

Methods To evaluate the sensitivity and specificity of the Idylla SARS-CoV-2 Test, we retrieved 55 nasopharyngeal swabs, previously analysed by a fully validated assay, from symptomatic patients or from people who have been in close contact with COVID-19 positive cases. Discordant or high discrepant cases were further analysed by a third technique. In addition, a second subset of 14 nasopharyngeal swab samples with uncertain results (cycle threshold between 37 and 40), by using the fully validated assay, from patients with viral infection beyond day 21, were retrieved.

Results Overall, Idylla showed a sensitivity of 93.9% and a specificity of 100.0%. In addition, in the additional 14 nasopharyngeal swab samples, only five (35.7%) featured a positive result by the Idylla SARS-CoV-2 Test.

Conclusions We demonstrated that the Idylla SARS-CoV-2 Test may represent a valid, fast, highly sensitive and specific RT-PCR test for the identification of SARS-CoV-2 infection.

INTRODUCTION

The COVID-19 caused by the SARS-CoV-2 was first identified in Wuhan (Hubei region, China) at the end of 2019.[1 2] The infection has rapidly spread all over the world, and the pandemic status was determined by the WHO on 11 March 2020.[3] At the time of writing (12 February 2021), more than 107 million people have been infected by SARS-CoV-2 with more than 2 million deaths, worldwide.[4] Overall, SARS-CoV-2 is a betacoronavirus, enveloped, positive-sense, single stranded RNA virus from the Coronaviridae family.[5 6] The viral genome, composed by about 30 000 nucleotides, contains genes encoding for nucleocapsid (N), envelop (E), membrane (M), internal (I) and spike (S) structural proteins and two open reading frame genes (*ORF1a* and *ORF1b*) encoding for 16 non-structural proteins including the RNA-dependent RNA polymerase.[7] The 'gold standard' for the diagnosis of COVID-19 is reverse transcriptase PCR (RT-PCR) on nasopharyngeal swabs.[8 9] However, despite the high sensitivity and specificity, several limitations affected the routinely performance of this diagnostic tool. In particular, highly trained personnel is required. In addition, a complex infrastructure is mandatory to manage hundreds or thousands of daily testing while contemporary reducing personnel biological risks exposure. Finally, it is fundamental to mediate between the necessities to batch a minimum number of testing samples while giving the results as quickly as possible (within 24 hours). In order to overcome these limitations, several efforts have been spent to develop novel, easy to use, rapid, robust and with minimal hands-on time requirements diagnostic tools.[10] In this setting, the fully automated RT-PCR Idylla platform (Biocartis NV, Mechelen, Belgium) may be a valuable solution. This technology enables a rapid, robust, sensitive and specific approach useful to reduce the risk of sample cross-contamination and personnel exposure to high risk specimens.[11] As previously reported, the Idylla platform has been successfully adopted by our molecular predictive laboratory at the Department of Public Health of the University of Naples Federico II for predictive purposes in advanced stage solid tumour patients during the healthcare emergency.[12] Besides the predictive role, a novel cartridge (see the Materials and methods section) to assess the infectious SARS-CoV-2 status has been developed by Biocartis NV.[13]

In the present study, our aim is to evaluate the analytical and clinical performance of this novel disposable cartridge on previously tested SARS-CoV-2 people by conventional RT-PCR based approach in different settings, including initial diagnosis and clinical follow-up.

MATERIALS AND METHODS

Study design

This study was designed to evaluate the efficiency of Idylla SARS-CoV-2 Test to identify SARS-CoV-2 viral RNA. To this end, we have retrieved from the archive of the Clinical Pathology Laboratory at the Department of Translational Medical Sciences,

University of Naples Federico II, 55 nasopharyngeal swabs that had been collected and preserved in sterile viral medium Universal Transport Medium (UTM, Copan Diagnostic, Brescia, Italy) from symptomatic patients or from people who have been in close contact with COVID-19 positive cases. These latter, had previously been tested by a fully validated assay (Real-Time SARS-CoV-2 kit, #09N77-095, Abbott Laboratories, Chicago, Illinois, USA) which detect the *RdRP* and the *N* gene of the SARS-CoV-2, that had been Food and Drug Administration authorised for the emergency use, featuring a complete analysis run of 6 hours on average. To assess sensitivity and specificity of the Idylla SARS-CoV-2 Test against the Abbott Real-Time SARS-CoV-2 kit, samples, including positive (n=35) and negative (n=20) nasopharyngeal swabs, were retested by the Idylla SARS-CoV-2 Test. Cases showing discrepant results and lack of agreement between Idylla SARS-CoV-2 Test and Abbott Real-Time SARS-CoV-2 kit, were further analysed by a third technique (RealStar SARS-CoV-2 RT-PCR Kit, Altona Diagnostics GmbH, Hamburg, Germany). In addition, with the aim to evaluate the adoption of the Idylla SARS-CoV-2 Test to end the quarantine, a second subset of 14 nasopharyngeal swab samples with uncertain results (cycle threshold (Ct) between 37 and 40), by using Abbott Real-Time SARS-CoV-2 assay, from patients with viral infection beyond day 21, were retrieved. In all these instances, a third methodology (RealStar SARS-CoV-2 RT-PCR Kit) was also adopted.

Abbott Real-Time SARS-CoV-2 kit

At the Clinical Pathology Laboratory at the Department of Translational Medical Sciences, University of Naples Federico II, nasopharyngeal swabs are routinely analysed by using Abbott Real-Time SARS-CoV-2 kit, as described in the instructions of the manufacturer, on the Alinity platform. Briefly, 800 µL of UTM were required. Results were considered as positive when the Ct value on *N* and/or on *ORF1b* genes was equal or less than 37.[14]

Idylla SARS-CoV-2 Test

The Idylla SARS-CoV-2 Test is a fully automated RT-PCR system that adopts disposable cartridges. Briefly, 200 µL of UTM were directly pipetted into the SARS-CoV-2 Test cartridge. Via microfluidic channels, RNA was extracted after cell lysis performed by a combination of HIFU, enzymatic/chemical digestion, and heat. The extracted RNA was converted in complementary DNA (cDNA) by the RT enzyme and it was then transported into five PCR chambers for amplification. Here, there are dried primers and probes designed to detect two *N* and three *ORFb1* target regions. This is a fluorescent-based assay allows to detect two SARS-CoV-2 RNA targets such as *N* gene (nucleocapsid phosphoprotein gene) and *ORF1b* region, they are covered by five PCR targets (two *N* and three *ORF1b* targets). In addition, each amplification chamber the amplification of MS2 Bacteriophage is adopted as an internal control, to monitor that RNA extraction and amplification steps have been correctly carried out into the cartridge.

After 90 min run, the final report is displayed on the Idylla console as 'positive', 'negative' or 'not valid'. As a general rule, a positive result requires at least two *N* amplified targets (with a Ct value ≤41.9) and/or at least one or more *ORF1b* amplified targets (in this case a Ct value cut-off is not required, due to the very high specificity of this gene amplification). Negative results indicate the absence of SARS-CoV-2 target amplification.

Table 1 Comparison between the results of the Idylla SARS-CoV-2 Test and the results of routine Abbott Real-Time SARS-CoV-2 assay

		Idylla SARS-CoV-2 Test		
		Positive	Negative	Total
Abbott Real-Time SARS-CoV-2	Positive	31	2	33
	Negative	0	20	20
	Total	31	22	53

RealStar SARS-CoV-2 RT-PCR kit

RealStar SARS-CoV-2 RT-PCR Kit is a RT-PCR based technology that enabling the qualitative detection of target regions in SARS-Cov-2 *E* and *S* genes, has been adopted following manufacturer instructions.

RESULTS

Overall, Idylla assay generated valid results in almost all analysed samples (96.4%, 53/55). Interestingly, among successfully analysed cases, a 96.2% concordance rate (51/53), between the Idylla SARS-CoV-2 Test and the Real-Time SARS-CoV-2 kit, has been obtained. Of note no false positive results have been reported by the Idylla SARS-CoV-2 Test (specificity 100.0%) (table 1). Conversely, in only two (6.1%, 2/33) cases Idylla did not detect SARS-CoV-2 (Ct 34.73 and 36.11 with the gold standard technology) suggesting a possibility of a false negative result (figure 1) (online supplemental table 1). In only one case this possibility was suggested by the detection of the virus by the Altona assay. In addition, in five cases (#1, #16, #25, #30 and #31) with a high discrepancy between the standard technology and the Idylla SARS-CoV-2 Test in terms of Ct and quantification cycle (Cq), respectively, the third methodology (Altona assay) confirmed in all instances a positive result (online supplemental table 2). Of note, in the supplementary 14 nasopharyngeal swab samples taken after 21 days from the first positive nasopharyngeal swab showing an uncertain result with Abbott Real-Time SARS-CoV-2 assay, only five (35.7%) featured a positive result by the Idylla SARS-CoV-2 Test (online supplemental table 3). Interestingly, in the vast majority of analysed samples (11/14, 78.6%) the adoption of a third methodology (RealStar SARS-CoV-2 RT-PCR Kit) confirmed the Idylla SARS-CoV-2 Test results (online supplemental table 3). Conversely, three positive Idylla SARS-CoV-2 Test cases were classified as 'uncertain' (n=2) or 'negative' (n=1) with the third methodology (online supplemental table 3).

DISCUSSION

Our study demonstrated that the Idylla Test is a sensitive, specific, easy to use and rapid assay for SARS-CoV-2 detection in nasopharyngeal swabs. In particular, in our experience the Idylla SARS-CoV-2 Test was able to reach a specificity of 100.0% and a sensitivity of 93.9% by comparing the results with a reliable reference technology. Only two cases showed discrepancies with Idylla assay being negative. These cases may represent true false negative cases although is not possible to rule-out technical issues related to the thawing of archival frozen nasopharyngeal swabs.

In the difficult time of COVID-19 pandemic, there is an urgent need of rapid and automated tests for the detection of SARS-CoV-2 infection. In fact, RT-PCR requires several dozen or even hundreds of samples to be grouped in batches to be tested in parallel, with a delay in results (6–24 hours). In this setting, it is key the employment of rapid assays for urgent cases, such as the characterisation of the COVID-19 status before an emergency surgery or in a vaginal delivery. The possibility to

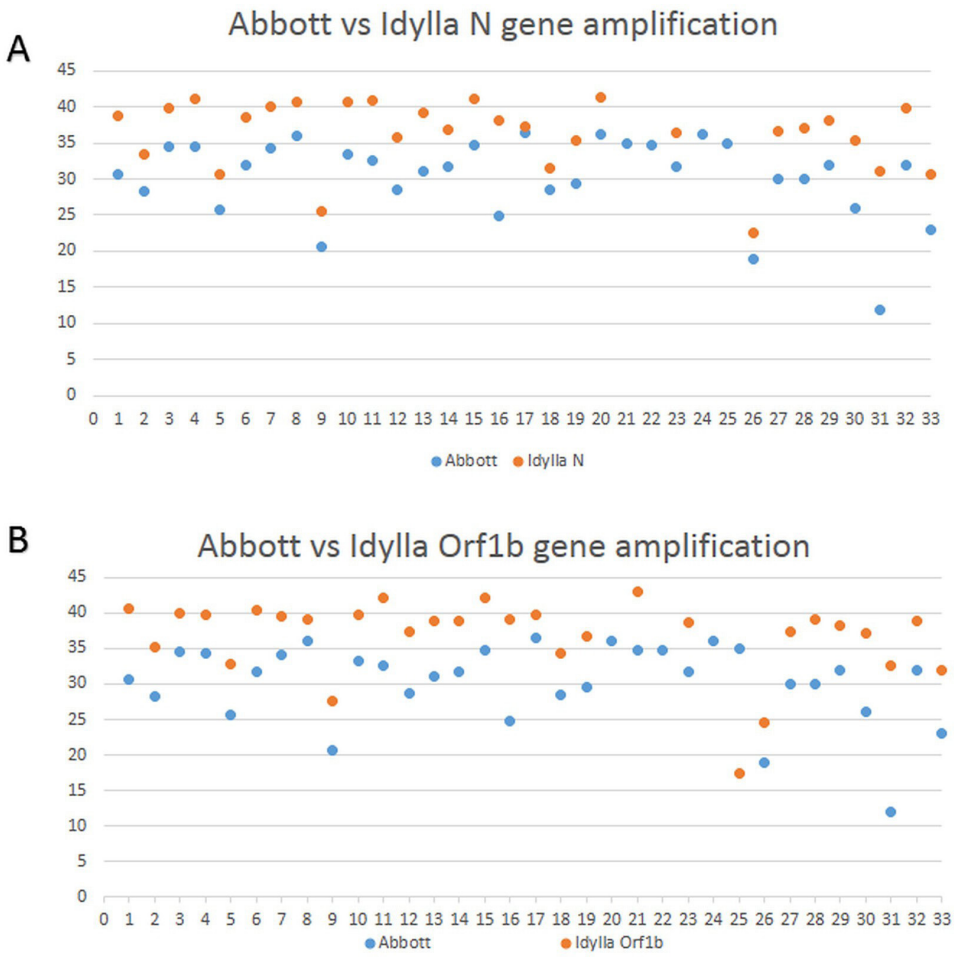

Figure 1 Graphical comparison between cycle threshold (Ct) (Abbott) versus quantification cycle (Cq) (Idylla) for N and *ORF1b* gene amplification.

quickly process selected cases represents an advantage in clinical practice that has led the Idylla technology to be widespread for mutational testing in oncological patients. Indeed, this automated molecular technology is often present in molecular laboratories dealing with oncological procedures which represents also an opportunity to apply rapid assay to select the patients with COVID-19.

To date, several tests and technologies have been developed for the diagnosis of SARS-CoV-2 infection.[15–18] However, in particular to reduce hands-on time, potential exposure to SARS-CoV-2 infected samples and to reduce the risk of sample cross-contamination, it is mandatory to draw the attention to fully automated technologies. In this setting, the fully automated Idylla platform may represent a valid diagnostic tool. During the COVID-19 pandemic, a technological shift towards fully automated platform due to laboratory personnel reduction to cope the spread of virus has been reported. In a single and a multicentre experience coordinated by the Molecular Predictive Pathology Laboratory at the Department of Public Health of the University of Naples Federico II, fully automated platforms, such as Idylla, may significant simplify predictive molecular testing, while contemporary ensuring safety and cost-effectively management of laboratory staff.[12 19] Recently, the novel Idylla SARS-CoV-2 Test has been developed.[13] Overall, the Idylla system adopted a console computer that can be associated with up to eight separately operating instruments. Each separately operating instruments can process disposable cartridges able to perform viral RNA extraction, retrotranscription, amplification and data analysis in about 90 min.[13] Interestingly, this assay has recently obtained the CE-IVD mark with a lower limit of detection for viral genomic of 500 copies/mL.[13] In our experience, among 53 successfully analysed cases, only two 'false negative' results have been reported by the Idylla SARS-CoV-2 Test. Overall, these cases were further analysed by another RT-PCR assay (Altona RealStar SARS-CoV-2 RT-PCR Kit).[20] This latter was able to analyse target regions in *E* and *S* genes. Interestingly, with this RT-PCR approach a negative and undetermined results have been reported (online supplemental table 1). In addition, in five cases (#1, #16, #25, #30 and #31) with a high discrepancy between the standard technology and the Idylla SARS-CoV-2 Test in terms of Ct and Cq, respectively, the third methodology (Altona assay) confirmed a positive result (online supplemental table 2). However, it should be borne in mind that these discrepancies may related to adoption a of a fluorescence threshold by using Abbott technology, whereas the Idylla SARS-CoV-2 calculates the Cq based on normalised amplification curves. In this study, we have selected only positive cases within a Ct equal or less than 37. Interestingly, it has been reported that positive amplification with a Ct value >37 in patients with a viral infection beyond day 21, may be not able to spread the virus.[21] To this end, in a selection of 14 nasopharingeal swabs belonging to patients with viral infection beyond day 21 and tested as uncertain by Abbott Real-Time SARS-CoV-2 kit, only 5 (35.7%) out of 14 nasopharyngeal swabs featured a positive result by the Idylla SARS-CoV-2 Test. These data may arise interest for the adoption of the Idylla SARS-CoV-2 Test to end the quarantine.

Of note, in the vast majority of these samples (11/14, 78.6%) the adoption of a third methodology (RealStar SARS-CoV-2 RT-PCR Kit) confirmed the Idylla SARS-CoV-2 Test results (online supplemental table 3). Conversely, only three positive Idylla SARS-CoV-2 Test cases were classified as 'uncertain' (n=2) or 'negative' (n=1) with the third methodology (online supplemental table 3).

However, despite the encouraging results, several limitations affected this pilot study. In particular, the number of analysed samples may be extremely low to better assess the sensitivity and specificity of this novel assay. Thus, further investigation is required to assess whether the Idylla SARS-CoV-2 Test may be useful to further analyse cases with undetermined and unclear results (Ct between 37 and 40) that requires a rapid evaluation. In these cases, the Idylla SARS-CoV-2 Test may be considered a component of a diagnostic algorithm that exploits both conventional and automated RT-PCR platforms.

In conclusion, we demonstrated that the Idylla SARS-CoV-2 Test may represent a valid, fast, highly sensitive and specific RT-PCR test for the identification of SARS-CoV-2 infection.

Take home messages

- To date, reverse transcriptase PCR (RT-PCR) on nasopharyngeal swabs is the 'gold standard' approach for the diagnosis of COVID-19.
- There is a urgent need to develop easy to use, rapid, robust and with minimal hands-on time approaches.
- In this setting, the Idylla SARS-CoV-2 Test may be a valuable option.
- We demonstrated that the Idylla SARS-CoV-2 Test may represent a valid, fast, highly sensitive and specific RT-PCR test for the identification of SARS-CoV-2 infection.

Handling editor Runjan Chetty.

Twitter Pasquale Pisapia @PasqualePisapia and Umberto Malapelle @UmbertoMalapel1

Contributors Conceptualisation: CDL, GT and GP. Methodology: all authors. Software: all authors. Validation: all authors. Formal analysis: all authors. Investigation: all authors. Resources: all authors. Data curation: all authors. Writing – original draft preparation: CDL, FC, PP, GT, GP. Writing – review and editing: all authors. Visualisation: all authors. Supervision: GT and GP. Project administration: GT and GP. Funding acquisition: GT.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests UM has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientifics, Diaceutics, GSK, Merck and AstraZeneca, unrelated to the current work. GT reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer and Bayer, unrelated to the current work.

Patient consent for publication Not required.

Ethics approval All information regarding human material was managed using anonymous numerical codes, and all samples were handled in compliance with the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplemental information.

ORCID iDs
Pasquale Pisapia http://orcid.org/0000-0002-6429-0620
Umberto Malapelle http://orcid.org/0000-0003-3211-9957
Giancarlo Troncone http://orcid.org/0000-0003-1630-5805

REFERENCES

1 Zhu N, Zhang D, Wang W, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
2 Lai C-C, Shih T-P, Ko W-C, *et al*. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. *Int J Antimicrob Agents* 2020;55:105924.
3 World Health Organization (WHO). Coronavirus disease (COVID-19). Available: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-11-march-2020
4 Gisand Data. COVID-19 Dashboard by the center for systems science and engineering (CSSE) at Johns Hopkins University (JHU). Available: https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6 [Accessed 11 Feb 2021].
5 Paules CI, Marston HD, Fauci AS. Coronavirus Infections-More than just the common cold. *JAMA* 2020;323:707–8.
6 Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020;5:536–44.
7 Kim D, Lee J-Y, Yang J-S, *et al*. The architecture of SARS-CoV-2 transcriptome. *Cell* 2020;181:914–21.
8 Benzigar MR, Bhattacharjee R, Baharfar M, *et al*. Current methods for diagnosis of human coronaviruses: pros and cons. *Anal Bioanal Chem* 2021;413:2311–30.
9 Böger B, Fachi MM, Vilhena RO, *et al*. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. *Am J Infect Control* 2021;49:21–9.
10 Allam M, Cai S, Ganesh S, *et al*. COVID-19 diagnostics, tools, and prevention. *Diagnostics* 2020;10:409.
11 Uguen A, Troncone G. A review on the Idylla platform: towards the assessment of actionable genomic alterations in one day. *J Clin Pathol* 2018;71:757–62.
12 Malapelle U, Pisapia P, Iaccarino A, *et al*. Predictive molecular pathology in the time of coronavirus disease (COVID-19) in Europe. *J Clin Pathol* 2020. doi:10.1136/jclinpath-2020-206957. [Epub ahead of print: 31 Jul 2020].
13 Biocartis. IdyllaTM SARS-CoV-2 test. Available: https://www.biocartis.com/en/meet-idylla/idylla-infectious-disease-assays/idylla-sars-cov-2-test
14 Wang J, Cai K, Zhang R, *et al*. Novel one-step single-tube nested quantitative real-time PCR assay for highly sensitive detection of SARS-CoV-2. *Anal Chem* 2020;92:9399–404.
15 Li C, Zhao C, Bao J, *et al*. Laboratory diagnosis of coronavirus disease-2019 (COVID-19). *Clin Chim Acta* 2020;510:35–46.
16 Lippi G, Horvath AR, Adeli K, Editorial AK. Editorial and executive summary: IFCC interim guidelines on clinical laboratory testing during the COVID-19 pandemic. *Clin Chem Lab Med* 2020;58:1965–9.
17 Thompson S, Bohn MK, Mancini N, *et al*. IFCC interim guidelines on Biochemical/Hematological monitoring of COVID-19 patients. *Clin Chem Lab Med* 2020;58:2009–16.
18 Vandenberg O, Martiny D, Rochas O, *et al*. Considerations for diagnostic COVID-19 tests. *Nat Rev Microbiol* 2021;19:171–83.
19 Malapelle U, De Luca C, Iaccarino A, *et al*. Predictive molecular pathology in the time of COVID-19. *J Clin Pathol* 2021;74:234–7.
20 RealStar® SARS-CoV-2 RT-PCR Kit RUO. Altona diagnostics GmbH. Available: https://altona-diagnostics.com/en/products/reagents-140/reagents/realstar-real-time-pcr-reagents/realstar-sars-cov-2-rt-pcr-kit-ruo.html
21 Cevik M, Tate M, Lloyd O, *et al*. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* 2021;2:e13–22.