The Journal of Molecular Diagnostics, Vol. 22, No. 7, July 2020

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Is Comparable in Clinical Samples Preserved in Saline or Viral Transport Medium

Jared Radbel,[*†] Sugeet Jagpal,[*] Jason Roy,[‡] Andrew Brooks,[†§] Jay Tischfield,[§¶] Michael Sheldon,[§] Christian Bixby,[§] Dana Witt,[§] Maria L. Gennaro,[‖] Daniel B. Horton,[‡¶**] Emily S. Barrett,[††] Jeffrey L. Carson,[*] Reynold A. Panettieri, Jr,[*††] and Martin J. Blaser[*‡‡]

From the Departments of Medicine and Pediatrics,[¶] Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey; the Environmental and Occupational Health Sciences Institute,[†] the Rutgers University Cell and DNA Repository Infinite Biologics and Human Genetics Institute of New Jersey and Department of Genetics,[§] and the Center for Advanced Biotechnology and Medicine,[‡‡] Rutgers University, Piscataway, New Jersey; the Department of Biostatistics and Epidemiology,[‡] Rutgers School of Public Health, Piscataway, New Jersey; the Public Health Research Institute,[‖] Department of Medicine, New Jersey Medical School, Rutgers University, Newark, New Jersey; the Rutgers Center for Pharmacoepidemiology and Treatment Science,** Institute for Health, Health Care Policy and Aging Research, New Brunswick, New Jersey; and the Rutgers Institute for Translational Medicine and Science,[††] New Brunswick, New Jersey*

Accepted for publication April 28, 2020.

Address correspondence to Martin J. Blaser, M.D., Center for Advanced Biotechnology and Medicine, Rutgers University, 679 Hoes Ln. W., Room 106A, Piscataway, NJ 08854. E-mail: martin.blaser@cabm.rutgers.edu.

As the coronavirus disease 2019 (COVID-19) pandemic sweeps across the world, the availability of viral transport medium (VTM) has become severely limited, contributing to delays in diagnosis and rationing of diagnostic testing. Given that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral RNA has demonstrated stability, we posited that phosphate-buffered saline (PBS) may be a viable transport medium, as an alternative to VTM, for clinical real-time quantitative PCR (qPCR) testing. The intra-individual reliability and interindividual reliability of SARS-CoV-2 qPCR were assessed in clinical endotracheal secretion samples transported in VTM or PBS to evaluate the stability of the qPCR signal for three viral targets (*N* gene, *ORF1ab*, and *S* gene) when samples were stored in these media at room temperature for up to 18 hours. We report that the use of PBS as a transport medium allows high intra-individual and interindividual reliability, maintains viral stability, and compares with VTM in the detection of the three SARS-CoV-2 genes through 18 hours of storage. This study establishes PBS as a clinically useful medium that can be readily deployed for transporting and short-term preservation of specimens containing SARS-CoV-2. Use of PBS as a transport medium has the potential to increase testing capacity for SARS-CoV-2, aiding more widespread screening and early diagnosis of COVID-19. *(J Mol Diagn 2020, 22: 871—875; https://doi.org/10.1016/j.jmoldx.2020.04.209)*

In December 2019, a novel coronavirus was recognized as causing a cluster of pneumonia cases in Wuhan, China.[1] The infectious agent, an RNA virus, was termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and was identified as the cause of coronavirus disease 2019 (COVID-19), a clinical syndrome manifested by an influenza-like illness that can progress to acute lung injury or acute respiratory distress syndrome with substantial mortality.[2] COVID-19 has affected >3.1 million individuals, causing >220,000 deaths worldwide (*https://coronavirus.jhu.edu/map.html*, last accessed April 29, 2020).

SARS-CoV-2 detection using standard testing of upper airway secretions requires a nasopharyngeal or oropharyngeal swab that is transported to a clinical laboratory using

Supported by NIH grants U01AI22285 (M.J.B.), U01HL133817 (J.L.C.), UL1TR003017 NCATS (R.A.P.), UH3AI122309 (M.L.G.), P30ES005022 (J.Ra., E.S.B., A.B., J.T., R.A.P.), R01HL149450 (M.L.G.), and K23AR070286 (D.B.H.). In-kind logistics support was provided by Marken/UPS and dfYoung.

Disclosures: None declared.

J.Ra., S.J., and J.Ro. contributed equally to this work.

J.L.C., R.A.P., and M.J.B. contributed equally to this work as senior authors.

https://doi.org/10.1016/j.jmoldx.2020.04.209

viral transport medium (VTM; *https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2*, last accessed April 29, 2020). Recently, oropharyngeal swab and saliva testing using VTM also were shown to be comparable to nasopharyngeal swabs for detection of the virus.[3,4] As the COVID-19 pandemic has swept across the world, availability of VTM has become severely limited, impairing local and regional capacity for diagnosis. Because SARS-CoV-2 has capped RNA with a 5′ GTP resembling host RNA, and the virus single-guide RNA manifests remarkable stability,[5] real-time quantitative PCR (qPCR) detection of SARS-CoV-2 in specimens preserved in phosphate-buffered saline (PBS), which is readily available, was posited as being comparable to those in VTM. The current article reports that sample preservation in PBS or VTM is comparably effective for the preservation of SARS-CoV-2 in endotracheal secretions.

Materials and Methods

Source Materials

For transport media, procedures outlined in standard references were followed.[5–8] PBS, a water-based salt solution containing disodium hydrogen phosphate, sodium chloride, potassium chloride, and potassium dihydrogen phosphate, pH 7.2, was used (Sigma Aldrich, St. Louis, MO). VTM was derived according to the Centers for Disease Control and Prevention coronavirus outbreak response (*https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf*, last accessed April 29, 2020). In brief, a solution with Hanks's balanced salt solution, heat-inactivated fetal bovine serum (final concentration, 2%), gentamicin, 100 μg/mL, and amphotericin B, 0.5 μg/mL, was prepared and aliquoted into 2-mL screw-top vials.[6] Tubes then were stored at 4°C until use.

Experimental Protocols

Respiratory secretions from 16 confirmed COVID-19–positive subjects were collected over a 4-day period from an intensive care unit at Robert Wood Johnson University Hospital (New Brunswick, NJ), according to a protocol approved by the Rutgers Institutional Review Board (protocol number Pro2020000800). All subjects were mechanically ventilated for acute hypoxemic respiratory failure due to confirmed COVID-19 pneumonia. Specimens were collected into a sterile container via closed-circuit, in-line catheter suction of respiratory secretions from the endotracheal tube, as part of routine clinical care. Swabs were then dropped into vials containing PBS or VTM and transported to the Rutgers University Cell and DNA Repository laboratory for analysis. To test intrapatient and interpatient variation in efficacy of detecting SARS-CoV-2 from endotracheal tube–derived samples, three experimental procedures were performed. Eight samples from two subjects (four from each subject) were harvested at the same time and transported in either VTM or PBS. Samples were processed immediately (at 0 hours) or after 2 hours at room temperature. qPCR was performed on one sample from each transport medium at each incubation time (0 or 2 hours), and Ct values for the SARS-CoV-2 *nucleocapsid* (*N*), *open reading frame 1ab* (*ORF1ab*), and *spike protein* (*S*) genes were compared; *bacteriophage MS2* (*MS2*) spiked into the samples was used as a positive control. In a parallel experiment, the stability of detection of SARS-CoV-2 single-guide RNA by qPCR in samples transported in PBS and VTM also was examined after remaining at room temperature for time points ranging from 0 to 18 hours. These experiments mimicked field conditions, in which specimens remain in transport at room temperature for periods up to 18 hours. Twenty samples from each of the two subjects were kept for 0, 2, 4, or 6 hours or overnight at room temperature in VTM or PBS to mimic these real-world transport condition. Ct values were again compared for the qPCRs for the three viral genes described above across the indicated time points. To examine intersubject variance, samples were examined from an additional 12 patients whose endotracheal tube secretions were transported in either PBS or VTM; qPCR was performed immediately on arrival in the laboratory for these 24 samples, and Ct values were again compared between those transported in VTM or PBS.

Viral RNA Extraction

After removal of the collection swab, 300 μL of VTM or PBS sample was transferred to a 96-well Deep Well Processing plate (PerkinElmer, Waltham, MA), and extractions were performed on a Chemagic 360 instrument using the Chemagic Viral DNA/RNA 300 Kit H96 (PerkinElmer), as described by the manufacturer. This system eliminates manual sample handling, reduces risk of cross-contamination, and ensures rapid and consistent processing. In brief, extraction included 4 μL of poly(A) RNA, 10 μL of proteinase K, 300 μL of lysis buffer, and 8 μL of MS2 phage internal control added to each sample. Then, 300 μL of TaqPathCOVID-19C Positive Control and 300 μL of nCoV Negative Control (Thermo Fisher Scientific, Waltham, MA) were added to designated wells to be extracted alongside the subject samples. Magnetic beads (150 μL) were added, followed by 900 μL RNA binding buffer. The beads/RNA mixture was washed with 500 μL wash buffer 3, then with 500 μL wash buffer 4. Elution buffer was added, and residual beads were washed in 500 μL water, followed by a second elution step to a final volume of 50 μL.

SARS-CoV-2 assays were conducted under Food and Drug Administration–approved EUA number 200090 at Rutgers University Cell and DNA Repository Infinite Biologics (Piscataway, NJ). A total of 5 μL of each extracted RNA sample was aliquoted into a 384-well plate with 5 μL of positive and no template controls aliquoted into designated wells. Amplification was performed on the ABI QuantStudio

5 Real-Time PCR System using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific) following the manufacturer's directions. This assay detects the SARS-CoV-2 *ORF1ab* (FAM labeled), *S* (VIC labeled), and *N* (ABY labeled) genes with an *MS2 phage* control (JUN labeled). Data were interpreted and Ct values were generated using ABI QuantStudio5 PCR Real Time PCR software version 1.3 (Thermo Fisher Scientific). Reactions were in 20-μL volumes and run using the cycling protocol: 25°C for 2 minutes, 53°C for 10 minutes, 95°C for 2 minutes, then 95°C for 3 seconds, and 60°C for 30 seconds, collecting the fluorescence signal during the final 60°C step, and repeated for a total of 40 cycles. Assays were performed in triplicate, and there are positive and negative assay controls with the MS2 phage as a positive control of nucleic acid extraction and PCR. The lower limit of SARS-CoV-2 detection is 200 copies/mL, and the assay exhibits no cross-reactivity with 43 organisms and viruses that were tested.

Interpretation

The TaqPath COVID-19 Combo Kit provided negative, positive, and internal controls to monitor the reliability of the results for the entire batch of specimens from sample extraction to PCR amplification, according to the manufacturer's instructions. *MS2* Ct <37 was considered as positive, and *N*, *ORF1ab*, and *S* Ct >37 was considered negative.

Statistical Analysis

Pearson correlation was used to quantify association between repeated samples within subject, between transport media, and between the three genes. Linear mixed-effect models were used to address several of the research questions. These models were fitted separately for each of the three genes. The outcomes were the Ct values, whereas the predictors included the transport media (VTM versus PBS) and hours in storage (0, 2, 4, 6, or 18 hours). The models included a random intercept for each subject, to account for repeated observations (within-subject correlation). lme4 and ggplot2 packages in R were used for the linear mixed-effects models and plots, respectively (*https://www.R-project.org*, last accessed April 29, 2020).[9]

Results

Analysis of Repeated Samples from the Same Subject

Paired identical swab specimens were obtained from two unique subjects and transported in PBS and VTM. A total of 39 samples was analyzed, representing 10 for each subject in PBS and 9 in VTM, and the variation in Ct was plotted (Figure 1). As expected, SARS-CoV-2 was detected in all samples with Ct values significantly below the negativity cutoff of 37 in each assay. For each transport medium, there was strong correlation of values between the 9 or 10 replicates in each gene assay. These analyses indicate the consistency of the transport and measurements for samples that were obtained and stored in identical manners.

Comparison of VTM and PBS

First, scatterplots of the values from all 16 subjects tested, including all five times studied, were generated for a comparison of 26 values for both PBS and VTM for each of the three genes studied (Figure 2). The correlations ranged from 0.83 for the *ORF1ab* gene to 0.93 for the *N*, and all were significant ($P < 0.05$). Because some individuals contributed samples at multiple time points, there were repeated measures per individual. To examine a single measurement per individual, the analysis was restricted to the data obtained at time 0 only. The correlation for the three genes did not change substantially and remained significant. Across the time 0 samples from the 16 individual subjects, the SDs varied with respect to the gene studied, but did not vary significantly according to the transport medium. Thus, using PBS did not significantly skew the distribution of values in relation to the VTM (data not shown).

Next, a linear mixed-effects model (see *Materials and Methods*) was used to compare the sensitivity of the two transport media in the timed samples, from 0 hours to overnight (18 hours) storage. For all three genes, there were no significant differences, and in each case, the VTM values

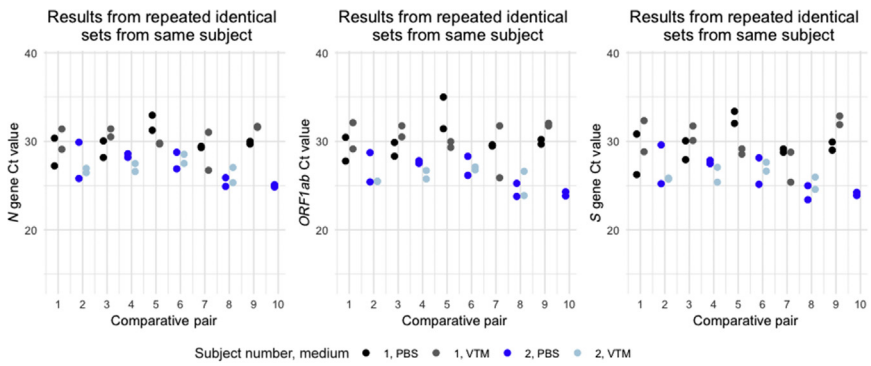

Figure 1 Ct counts from replicate samples from two individuals. **Black/gray points** are from Subject 1, and **dark blue/light blue points** are from Subject 2. **Dark points** and **light points** indicate the two types of transport media, phosphate-buffered saline (PBS) and viral transport medium (VTM), respectively. The horizontal axis, comparative pair, refers to replicate samples (ie, same individual, same length of storage time).

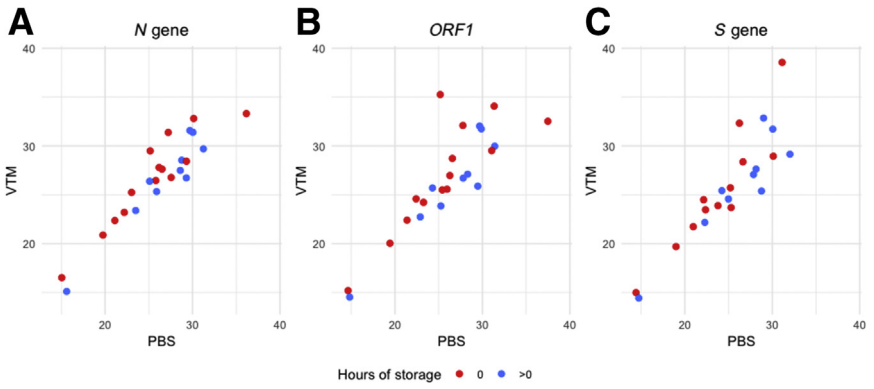

Figure 2 Comparison of Ct values in real-time quantitative PCR assays for three severe acute respiratory syndrome coronavirus 2 genes for samples transported in viral transport medium (VTM) or phosphate-buffered saline (PBS). A total of 26 samples that were transported in the two media are shown: *N* (**A**), *ORF1ab* (**B**), and *S* (**C**). The correlations are 0.93 for the *N*, 0.83 for *ORF1ab*, and 0.91 for the *S*, and all are significant ($P < 0.05$).

trended higher than for PBS. Next, it was evaluated whether across individual samples, the values obtained in testing one gene correlated with the results for the other genes. In addition, whether the choice of transport medium made a difference was tested. In total, for the 13 samples transported in VTM, the pairwise correlations between the three genes ranged from 0.947 to 0.956. For the 14 samples transported in PBS, the correlations ranged from 0.963 to 0.991. Thus, the results for the three genes were highly correlated independent of transport medium type.

Decay of the Viral Signal Over Time for Specimens Transported in the Two Media

Next, it was determined whether there was decay in viral signal over time, and whether it differed according to the transport medium used. From the prior analyses, the coefficient of hours of storage was estimated to be negative in the models of all three genes tested (approximately −0.03 for each gene) and was not significantly different from zero (all *P* values >0.5). These data indicate that storage at room temperature for up to 18 hours had little effect on the values detected in the qPCRs for the three SARS-CoV-2 genes tested. However, these models did not include an interaction term for the transport medium used for storage and time, and there could be differences between the media. To assess this possibility, the same analyses as above were performed, but interactions between hours of storage and transport medium used were included. For each of the three genes tested, both the main effect of time and the interaction between time and the transport medium were not statistically significant (all *P* values >0.5) and estimates were close to zero. Therefore, over the time interval studied, no decay in the viral signal or differential decay by transport medium was detected.

Discussion

During pandemics, molecular diagnostics are crucial to obtaining accurate and timely data to influence public health policy decisions in real time.[10] However, mounting demand for testing has caused a depletion of the viral transport medium needed to perform SARS-CoV-2 PCR testing.[11] Thus, in the midst of the SARS-CoV-2 pandemic, the Food and Drug Administration has allowed laboratories to consider testing alternative transport media (*https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2*, last accessed April 29, 2020). In the current study, experiments using clinical samples demonstrate the efficacy of PBS as a transport medium and its applicability to clinically relevant conditions, such as overnight storage at room temperature. First, SARS-CoV-2 qPCR detection with PBS as a transport medium was determined to have high intrapatient reliability. Next, using PBS for transport, strong interpatient reliability of SARS-CoV-2 qPCR was demonstrated. Strong correlation of Ct values from specimens transported in either PBS or VTM was also found across multiple subjects with unknown viral loads. These results establish PBS as a dependable transport medium for use with clinical samples. The data are consistent with the recent demonstration that PBS is equivalent to VTM when each medium is spiked with known quantities of SARS-CoV-2.[12]

With little decay in signal over storage times up to 18 hours, PBS also has utility for laboratories that test for several SARS-Cov-2 genes that have different specimen processing times. Testing can focus on any or all of the four SARS-Cov-2 structural proteins, including the spike, envelope, membrane, and nucleocapsid proteins, or on any of their protein domains.[13] The fact that results for all three viral genes tested were strongly correlated across samples from multiple subjects supports the robustness of the entire testing pathway, including transport. Furthermore, delays in getting samples to the testing laboratory often occur in busy clinical settings.[11] As such, the findings that PBS acts as a stable storage medium with lack of significant viral decay for up to 18 hours at room temperature before qPCR is advantageous.

This study is limited in that tracheal secretions from mechanically ventilated patients were used, and the extent to which these results can be extended to nasopharyngeal swabs, oropharyngeal swabs, and/or saliva testing is unknown. Given the severity of illness in the subjects, they may

have had higher viral loads than patients with milder disease in whom increased sensitivity of detection may be needed. However, the stability of the signal, with minimal changes in intensity for 18 hours, indicates the robustness of the method. The stability of SARS-CoV-2 in the environment,[5] which contributes to its widespread dissemination, may diminish the need for rapid transport of clinical specimens.

The extent to which clinical laboratories can respond to the COVID-19 pandemic is tied to the ability to develop and deploy proper diagnostic procedures.[10] Early SARS-CoV-2 detection allows prompt treatment of infected patients and rapid implementation of control measures to limit viral transmission.[14] Expanded testing capacity would also facilitate more widespread surveillance and containment of infectious transmission in communities, which could support policies to relax restrictions in work, travel, and social distancing. The current study establishes PBS as a clinically useful transport medium with the potential to increase viral detection capacity, thus improving clinical care and surveillance efforts.

Acknowledgments

We acknowledge the tireless work of the entire Rutgers Corona Cohort study team as well as the Rutgers Biomedical and Health Sciences leadership for facilitating this work. We thank (in alphabetical order): Tracy Andrews, Judith Argon, Joseph Barone, Kathleen Black, Brian Buckley, Kryst Cedeno, Debra Chew, Karen Dragert, Mark Einstein, Shereef Elnahal, Cecile Feldman, John Gantner, Sununda Gaur, Patricia Greenberg, Matthew Leonardelli, Deborah McCloskey, Helaine Novek, Veenat Parmar, Ami Patel, Nancy Reilly, Brian Strom, and Helmut Zarbl; Marten/UPS and dfYoung for in-kind logistics support; and our courageous colleagues serving on the frontlines in the clinic.

References

1. Phelan AL, Katz R, Gostin LO: The novel coronavirus originating in Wuhan, China: challenges for global health governance. JAMA 2020. doi:10.1001/jama.2020.1097, [Epub ahead of print]
2. Gralinski LE, Menachery VD: Return of the coronavirus: 2019-nCoV. Viruses 2020, 12:E135
3. To KK-W, Tsang OT-Y, Chik-Yan Yip C, Chan K-H, Wu T-C, Chan JMC, Leung W-S, Chik TS-H, Choi CY-C, Kandamby DH, Lung DC, Tam AR, Poon RW-S, Fung AY-F, Hung IF-N, Cheng VC-C, Chan JF-W, Yuen K-Y: Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis 2020. doi:10.1093/cid/ciaa149, [Epub ahead of print]
4. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C: Virological assessment of hospitalized patients with COVID-2019. Nature 2020. doi: 10.1038/s41586-020-2196-x, [Epub ahead of print]
5. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ: Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020, 382:1564−1567
6. Leland D. Concepts of Clinical Diagnostic Virology. Edited by Lennette EH. ed 2. New York, NY: Marcel Dekker Inc, 1992
7. Johnson FB: Transport of viral specimens. Clin Microbiol Rev 1990, 3:120−131
8. US Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health: Biosafety in Microbiological and Biomedical Laboratories (5th ed.). Edited by Chosewood LC, Wilson DE. ed 5. Washington, DC: US Government Printing Office, 2007
9. R Core Team: R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing, 2019
10. Nowak JA, Kaul KL: The role of community molecular diagnostics laboratories in the H1N1 pandemic. J Mol Diagn 2009, 11:369−370
11. Babiker A, Myers CW, Hill CE, Guarner J: SARS-CoV-2 testing. Am J Clin Pathol 2020. doi:10.1093/ajcp/aqaa052, [Epub ahead of print]
12. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, Boerger A, Schuetz AN, Yao JD, Binnicker MJ: Evaluation of saline, phosphate buffered saline and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. J Clin Microbiol 2020. doi:10.1128/JCM.00590-20
13. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, Zhou Y, Du L: Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol 2020. doi: 10.1038/s41423-020-0400-4. Epub ahead of print]
14. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, Chen H, Mubareka S, Gubbay JB, Chan WCW: Diagnosing COVID-19: the disease and tools for detection. ACS Nano 2020, 14:3822−3835