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.

Journal of Virological Methods 304 (2022) 114522

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Concordance in RT-PCR detection of SARS-CoV-2 between samples preserved in viral and bacterial transport medium

Bia Peña [a,*], Mayra Ochoa [a], Omar Flores [a], Ana I. Gil [a], Lucie Ecker [a], Rubelio Cornejo [a], Claudio F. Lanata [a,b,c], Leigh M. Howard [d], Carlos G. Grijalva [d]

[a] Instituto de Investigación Nutricional, Lima 15024, Peru
[b] Vanderbilt University, Nashville, TN 37235, USA
[c] London School of Hygiene and Tropical Medicine, London WC1E, UK
[d] Vanderbilt University and Vanderbilt University Medical Center, Nashville, TN 37232, USA

ARTICLE INFO

Keywords:
SARS-CoV-2
Nasal swab
Nasopharyngeal swab

ABSTRACT

Background: While the detection of SARS-CoV-2 in samples preserved in viral transport medium (VTM) by RT-PCR is a standard diagnostic method, this may preclude the study of bacterial respiratory pathogens from the same specimen. It is unclear if the use of skim milk, tryptone, glucose, and glycerin (STGG) transport media, used for study of respiratory bacteria, allows an efficient and concurrent study of SARS-CoV-2 infections.
Objectives: To determine the concordance in SARS-CoV-2 detection by real time RT-PCR between paired nasopharyngeal (NP) swabs preserved in STGG and nasal (NS) swabs preserved in VTM.
Study design: Paired samples of NP and NS swabs were collected between December 2020 and March 2021 from a prospective longitudinal cohort study of 44 households and 132 participants from a peri-urban community (Lima, Peru). NP and NS swabs were taken from all participants once and twice per week, respectively, independent of respiratory symptoms. STGG medium was used for NP samples and VTM for NS samples. Samples were analyzed for SARS-CoV-2 by RT-PCR for N, S and ORF1ab targets. We calculated the concordance in detections between sample types and compared the RT-PCR cycle thresholds (Ct).
Results: Among the 148 paired samples, we observed a high concordance in detections between NP and NS samples (agreement = 94.59%; Kappa = 0.79). Median Ct values were statistically similar between sample types for each RT-PCR target: N, S and ORF1ab ($p = 0.11$, $p = 0.71$ and $p = 0.11$, respectively).
Conclusions: NP swabs collected in STGG medium are reliable alternatives to nasal swabs collected in VTM for the study of SARS-CoV-2.

1. Background

Previous influenza pandemics have been associated with a high burden of bacterial co-infections (Morens et al., 2008; Petersdor et al., 1959; Schwarzmann et al., 1971; Palacios et al., 2009), which have been associated with an increased mortality (Gupta et al., 2008). Understanding patterns of bacterial colonization and the frequency of bacterial co-infections during viral pandemics is of great public health interest.

SARS-CoV-2 infections spread rapidly throughout the world and disproportionately affected those with limited access to diagnostic services and medical care. As of November 2021, nearly 240 million cases have been reported worldwide and, in Peru at least 200,000 people have died with the associated coronavirus disease 2019 (COVID-19) (Johns Hopkins Coronavirus Resource Center, 2020). While bacterial co-infections have been proposed as possible contributors to the severity and mortality associated with COVID-19 (Mirzaei et al., 2020), existing studies suggest that secondary bacterial infections were uncommon during the COVID-19 pandemic. Nevertheless, while several studies have focused on symptomatic bacterial infections, few studies have evaluated the influence of SARS-CoV-2 viral infections on bacterial colonization.

The World Health Organization (WHO) considers nasopharyngeal (NP) swabs in viral transport medium (VTM) as one of the recommended types of sample for the detection of SARS-CoV-2 (WHO, 2020). Other health authorities, such as the U.S. Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC), supports the

* Correspondence to: Instituto de Investigación Nutricional, Av. La Molina 1885, Lima 15024, Peru.
E-mail address: biapena@iin.sld.pe (B. Peña).

https://doi.org/10.1016/j.jviromet.2022.114522
Received 11 December 2021; Received in revised form 28 February 2022; Accepted 7 March 2022
Available online 9 March 2022

Table 1
SARS-CoV-2 results by real-time RT-PCR in NP and NS samples after resolution of inconclusive results.

		Nasopharyngeal			Percent agreement	CI 95%	Kappa[a]	CI 95%
	Result	Negative	Positive	Total				
Nasal	Negative	121 (81.8%)	5 (3.4%)	126 (85.1%)	94.59%	0.90–0.98	0.79	0.66–0.93
	Positive	3 (2%)	19 (12.8%)	22 (14.9%)				
	Total	124 (83.8%)	24 (16.2%)	148 (100%)				

[a] Cohen's Kappa, Standard error= 0.082.

collection of anterior nasal (NS) swabs in VTM (Péré et al., 2020) for viral detections. However, these options generally preclude the study of bacterial co-detections, which would require collection of additional NP samples in specialized media, which is burdensome for patients and logistically challenging.

NP samples preserved in skim milk–tryptone–glucose–glycerol (STGG) medium are commonly used for the detection of *Streptococcus pneumoniae* and other respiratory bacteria (Satzke et al., 2013). Prior studies have evaluated STGG medium as an alternative for the study of respiratory viruses (Grijalva et al., 2014; Turner et al., 2011). We postulate that use of NP samples preserved in STGG medium is also adequate for the detection of SARS-CoV-2.

2. Objectives

To determine the concordance of SARS-CoV-2 detection by real time RT-PCR between paired nasopharyngeal (NP) swabs preserved in STGG and nasal (NS) swabs preserved in VTM.

3. Study design

3.1. Study population

Paired NP and NS samples were collected between December 2020 and March 2021 from a prospective cohort study of individuals in 44 households enrolled in the San Juan de Lurigancho district (Lima, Peru) as described elsewhere (Lanata et al., 2021). The cohort included at least a child (< 18 years of age), a young adult (18–50 years of age) and an older adult (> 50 years of age) from each household and encompassed 132 participants (44 participants < 18 years of age, 44 participants 18–50 years of age, and 44 participants > 50 years of age). Paired NP and NS swabs were collected from the same participant and on the same date, regardless of respiratory symptoms.

The study was approved by the independent ethics committee of *Instituto de Investigación Nutricional* (IIN) and Vanderbilt University Medical Center, and written informed consent was obtained from each participant at enrollment.

3.2. Sample collection

Trained field workers collected NP samples from study participants using rayon swabs (Puritan®) and preserved the swabs in 1 ml of STGG medium. STGG, the standard medium for pneumococcal colonization studies, was prepared at the laboratory of the Instituto de Medicina Tropical – Universidad Peruana Cayetano Heredia according to a previously established protocol (O'Brien et al., 2001). NS samples were collected using Polyester swabs (Puritan®) and preserved in 3 ml of VTM (Remel®). NS samples were transported in cold envelopes for their separation in aliquots and maintained refrigerated at 2–8 °C until their final storage at − 80 °C (within 24 h of collection). NP samples were stored in their original STGG medium at − 20 °C in a local processing site until their final storage at − 80 °C.

3.3. Identification of SARS-CoV-2 by real time RT-PCR

RNA extraction from both types of samples was performed using the KingFisher DUO Prime equipment with the MagMAX™ Viral/Pathogen Nucleic Acid Isolation kit following the manufacturer's instructions (Thermo-Fisher Scientific, 2021) The extraction process was performed using 200 µl of sample to obtain a volume of 50 µl of eluted RNA. Real-time RT-PCR was performed on the QuantStudio 5 equipment using the TaqPath™ COVID-19 CE-IVD RT-PCR kit (Thermo-Fisher Scientific, 2021) and following manufacturer's instructions (Thermo-Fisher Scientific, 2021). TaqPath™ COVID-19 CE-IVD RT-PCR kit (Thermo-Fisher Scientific, 2021) includes the MS2 phage, an internal RNA extraction control. The reported limit of detection for this kit is 10 genomic copy equivalents per reaction. If samples provided inconclusive results, a second RT-PCR was performed on those samples using the IDT 2019-NCOV RUO KIT (IDT, 2021) and following the CDC instructions of use (CDC, 2021).

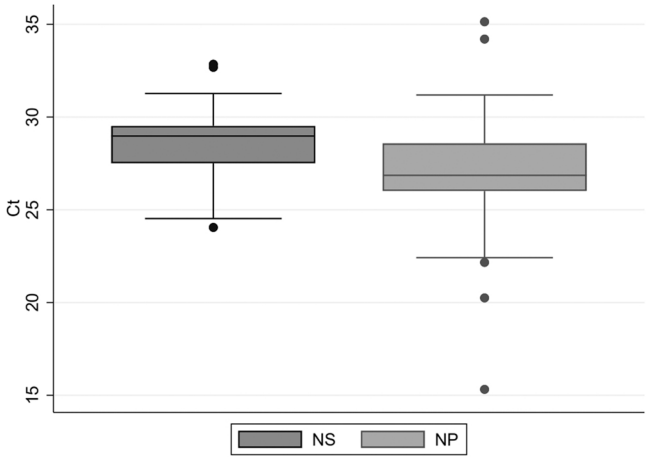

Fig. 1. Ct comparisons of the phage MS2 from NS and NP swabs (n = 148) using a Wilcoxon signed-rank test. The analysis showed; NS samples, median = 28.98 and IQR = 2.01, for NP swabs; median = 26.86 and IQR = 2.56.

3.4. Statistical analysis

We compared the results between NP and NS samples using overall percent agreement and calculated the Kappa statistic to determine their concordance. Additionally, we compared the cycle threshold (Ct) differences by the Ct mean between paired NP and NS samples using Bland-Altman plots (Bland and Altman, 1986). These examinations were done for each one of the SARS-CoV-2 target genes in the TaqPath™ COVID-19 CE-IVD RT-PCR kit (Thermo-Fisher Scientific, 2021) N, S and ORF1ab. To verify the quality of the RNA extracted from paired samples, we compared the Cts of the MS2 phage applying a Wilcoxon signed-rank test. Furthermore, we compared the mean Cts between paired tests for each target gene using the Wilcoxon signed-rank test. Statistical analyses were performed using the STATA 16.0 program.

4. Results

148 paired samples of NP and NS were included in this analysis.

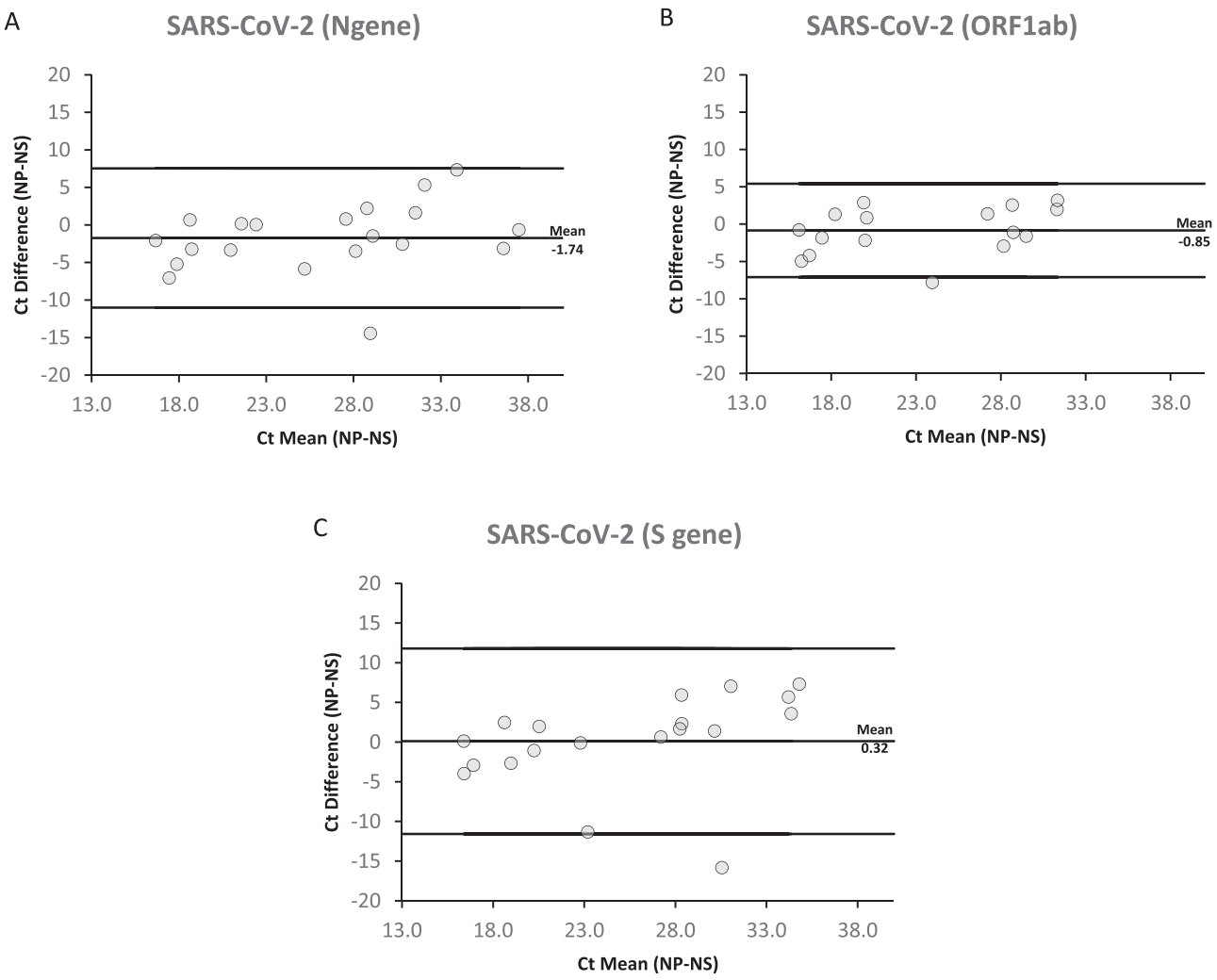

Fig. 2. Differences in Ct of NP and NS versus their mean using the Bland-Altman graphs for each evaluated gene with the TaqPath™ COVID-19 CE-IVD RT-PCR kit. (A) N gene target, 20 paired samples analyzed (B) ORF1ab gene target, 16 paired samples analyzed (C) S gene target, 19 paired samples analyzed. When the Ct difference in the y-axis is above 0, NS samples were more sensitive, Ct differences under 0 indicates NP swabs were more sensitive.

Table 2
Comparison of the distribution of Cts between NS and NP for each gene evaluated using the Wilcoxon signed-rank test.

Target	NS Median	IQR	NP Median	IQR	*p*
N	27.92	(30.51 – 21.26)	24.41	(31.12 – 19.12)	0.11
S	26.90	(29.47 – 19.55)	22.74	(31.31 – 17.65)	0.44
ORF1ab	23.81	(29.47 – 18.61)	20.93	(28.44 – 17.71)	0.43

There were 7 and 6 samples with initial inconclusive results for NS and NP swabs respectively (4.1% versus 4.7%). After resolution of the inconclusive results, a total of 22 NS and 24 NP samples were positive. The overall percent agreement was 94.59%, with a Cohen's Kappa of 0.79 (Table 1), indicating excellent agreement. (Fleiss et al., 2003).

The Ct comparisons of MS2 phage demonstrated a significant difference in the total RNA concentration between the two types of paired samples with lower median Ct in NP than NS ($p = 0.0001$; Wilcoxon signed-rank test) (Fig. 1). However, the Ct differences for the viral genes were small and close to 0 (< 10 cycles for N and ORF1ab, and < 12 cycles for S) (Fig. 2). The Cts between the three targets did not show statistically significant differences between the two types of samples (Table 2). The visual inspection of the Bland-Altman plots suggested no systematic trends in the agreement between NP and NS values, although the differences in Ct values for the S gene detections seemed to increase with increasing mean Ct values (Fig. 2).

5. Discussion

Our report demonstrates excellent concordance between real-time RT-PCR detection of SARS-CoV-2 RNA from NP samples preserved in STGG and NS samples preserved in VTM, supporting the use of NP specimens preserved in STGG to enable the simultaneous study of SARS-CoV-2 and respiratory bacterial co-detections.

Previous studies have reported high concordance between NP samples preserved in STGG medium and nasal aspirate or nasal swab specimens preserved in VTM for the detection of common respiratory viruses (Grijalva et al., 2014; Turner et al., 2011). A previous study described the use of STGG for identification of SARS-CoV-2 infections, but the reliability of this approach has remained unclear (Desmet et al., 2021).

Our study has important strengths. This is one of few studies that evaluated the performance of different paired specimens on detection of SARS-CoV-2 infections. We used RT-PCR and approved methodologies for viral detections. We complemented our agreement assessments with comparisons of semiquantitative Ct values obtained from the same RT-PCR testing platform. We also acknowledge some limitations. Although collecting separate NP swabs in VTM and STGG may have provided a more direct assessment of the performance of transport media, we considered that collecting additional NP swabs would have

resulted in unnecessary discomfort and burden for the participants, who were enrolled in a parent longitudinal cohort study that required twice per week collection of respiratory samples. Furthermore, the less invasive nasal swabs are well-accepted methods for detection of SARS-CoV-2 and viral infections (Péré et al., 2020). The overall number of specimens was small, which may have limited the precision of our agreement estimates. Furthermore, the limited number of specimens precluded comparisons by subgroups, for example, specimens collected when viral loads are low, or at the beginning or end of the infection, due to the replicative kinetics of SARS-CoV-2. Additional studies would be helpful to complement our findings. Finally, the study was conducted in a peri-urban population using specific research settings, and our findings may not be directly applicable to other populations or settings.

In conclusion, our results support the use of NP samples with STGG as an efficient sampling technique that enables the study of both SARS-CoV-2 and nasopharyngeal bacteria.

Funding

This work was supported by Consejo Nacional de Ciencia y Tecnología – CONCYTEC-PROCIENCIA, Lima, Peru (contract 082–2020-FONDECYT, Principal Investigator CFL); Vanderbilt University (Trans-Institutional Program (TIPs) grant, Principal Investigators CGG and LMH) and Instituto de Investigación Nutricional. CGG was supported in part by NIH-NIAID K24 AI148459.

Data sharing

Data without any participant's identifying information, could be provided after all final analysis are completed, if requested to the correspondence author. After approval, and the signature of a data transfer agreement form, data could only be used for the purpose requested, unless a new request is made.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors wish to thank the study participants which allowed us to conduct this study. In addition to the field and laboratory team involved in the conduct of the present study. To Mrs. Edda Franco for her editorial help in reviewing this manuscript. And finally, to the sponsors of this study.

Competing interests

CFL is a member of WHO COVID-19 vaccine effectiveness working group and WHO Product Development Advisory Group. CGG reports consultancy fees from Pfizer, Merck, and Sanofi-Pasteur; grants from Campbell Alliance/Syneos Health, CDC, NIH, the Food and Drug Administration, AHQR, and Sanofi, outside the submitted work. LMH reports grant funding from the Infectious Disease Society of America Education and Research Fund supported by Pfizer and from NIH, outside the submitted work.

References

Bland, J.M., Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1, 307–310.

CDC, 2021. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Retrieved April 29,2021, from: https://www.fda.gov/media/134922/download.

Desmet, S., Ekinci, E., Wouters, I., Decru, B., Beuselinck, K., Malhotra-Kumar, S., Theeten, H., 2021. No SARS-CoV-2 carriage observed in children attending daycare centers during the intial weeks of the epidemic in Belgium. J. Med. Virol. 93 (3), 1828–1831. https://doi.org/10.1002/jmv.26689.

Fleiss JL, Levin B., Paik MC. Statistical Methods for Rates and Proportions. 3rd. ed. Hoboken, NJ: J. Wiley; 2003.

Grijalva, C.G., Griffin, M.R., Edwards, K.M., Johnson, M., Gil, A.I., Verastegui, H., Lanata, C.F., Williams, J.V., 2014. Concordance between RT-PCR-based detection of respiratory viruses from nasal swabs collected for viral testing and nasopharyngeal swabs collected for bacterial testing. J. Clin. Virol. 60 (3), 309–312. https://doi.org/10.1016/j.jcv.2014.04.011.

Gupta, R.K., George, R., Nguyen-Van-Tam, J.S., 2008. Bacterial pneumonia and pandemic influenza planning. Emerg. Infect. Dis. 14 (8), 1187–1192. https://doi.org/10.3201/eid1407.070751.

IDT, 2021. SARS-CoV-2 Research Use Only Primer and Probe Sets. Retrieved April 5, 2021, from: https://www.idtdna.com/pages/landing/coronavirus-research-reagents/cdc-assays.

Johns Hopkins Coronavirus Resource Center, 2020. *ArcGIS Dashboards Classic*. COVID-19 Dashboard. Retrieved December 10, 2021, from https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6.

Lanata, C.F., Gil, A.I., Ecker, L., Cornejo, R., Rios, S., Ochoa, M., Peña, B., Flores, O., Howard, L.M., Grijalva, C.G., 2021. SARS-CoV-2 infections in households in a peri-urban community of Lima, Peru: A prospective cohort study. Influenza Other Respir Viruses. https://doi.org/10.1111/irv.12952.

Mirzaei, R., Goodarzi, P., Asadi, M., Soltani, A., Aljanabi, H.A.A., Jeda, A.S., Dashtbin, S., Jalalifar, S., Mohammadzadeh, R., Teimoori, A., Tari, K., Salari, M., Ghiasvand, S., Kazemi, S., Yousefimashouf, R., Keyvani, H., Karampoor, S., 2020. Bacterial co-infections with SARS-CoV-2. IUBMB Life 72 (10), 2097–2111. https://doi.org/10.1002/iub.2356.

Morens, D.M., Taubenberger, J.K., Fauci, A.S., 2008. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J. Infect. Dis. 198 (7), 962–970. https://doi.org/10.1086/591708.

O'Brien, K.L., Bronsdon, M.A., Dagan, R., Yagupsky, P., Janco, J., Elliott, J., Whitney, C. G., Yang, Y.H., Robinson, L.G., Schwartz, B., Carlone, G.M., 2001. Evaluation of a medium (STGG) for transport and optimal recovery of Streptococcus pneumoniae from nasopharyngeal secretions collected during field studies. J. Clin. Microbiol 39 (3), 1021–1024. https://doi.org/10.1128/JCM.39.3.1021-1024.2001.

Palacios, G., Hornig, M., Cisterna, D., Savji, N., Bussetti, A.V., Kapoor, V., Hui, J., Tokarz, R., Briese, T., Baumeister, E., Lipkin, W.I., 2009. Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. PLoS One 4 (12), e8540. https://doi.org/10.1371/journal.pone.0008540.

Péré, H., Podglajen, I., Wack, M., Flamarion, E., Mirault, T., Goudot, G., Hauw-Berlemont, C., Le, L., Caudron, E., Carrabin, S., Rodary, J., Ribeyre, T., Bélec, L., Veyer, D., 2020. Nasal swab sampling for SARS-CoV-2: a convenient alternative in times of nasopharyngeal swab shortage. J. Clin. Microbiol. 58 (6) https://doi.org/10.1128/JCM.00721-20 e00721-20.

Petersdor, R.G., Fusco, J.J., Harter, D.H., Albrink, W.S., 1959. Pulmonary infections complicating Asian influenza. AMA Arch. Intern. Med. 103 (2), 262–272. https://doi.org/10.1001/archinte.1959.00270020090010.

Satzke, C., Turner, P., Virolainen-Julkunen, A., Adrian, P.V., Antonio, M., Hare, K.M., Henao-Restrepo, A.M., Leach, A.J., Klugman, K.P., Porter, B.D., Sá-Leão, R., Scott, J. A., Nohynek, H., O'Brien, K.L., WHO Pneumococcal Carriage Working Group, 2013. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: Updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. Vaccine 32 (1), 165–179. https://doi.org/10.1016/j.vaccine.2013.08.062.

Schwarzmann, S.W., Adler, J.L., Sullivan, R.J., Marine, W.M., 1971. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968-1969: Experience in a city-County hospital. Arch. Intern. Med. 127 (6), 1037–1041. https://doi.org/10.1001/archinte.1971.00310180053006.

Thermo-Fisher Scientific, 2021. TaqPath™ COVID-19 CE-IVD RT-PCR Kit2021. Retrieved April 29, 2021, from: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0019215_TaqPathCOVID-19_CE-IVD_RT-PCR%20Kit_IFU.pdf.

Thermo-Fisher Scientific, 2021. TaqPath™ COVID-19 Combo Kit and TaqPath™ COVID-19 Combo Kit Advanced. Retrieved April 29, 2021, from: https://www.fda.gov/media/136112/download.

Turner, P., Po, L., Turner, C., Goldblatt, D., Nosten, F., 2011. Detection of respiratory viruses by PCR assay of nasopharyngeal swabs stored in skim milk-tryptone-glucose-glycerol transport medium. J. Clin. Microbiol. 49 (6), 2311–2313. https://doi.org/10.1128/JCM.00224-11.

WHO, 2020. Laboratory testing of 2019 novel coronavirus (2019-nCoV) in suspected human cases: Interim guidance, 17 January 2020. Retrieved October 07, 2021, from: https://www.who.int/publications/i/item/laboratory-testing-of-2019-novel-coronavirus-(-2019-ncov)-in-suspected-human-cases-interim-guidance-17-january-2020.