



Journal of Health and Medical Sciences

Rajendra, R., Tuba, S., & Sulaiman, S. A. S. (2023), Comparison of the Effectiveness in Nasopharyngeal, Throat, Saliva, and Nasal Swab Sample Media of Detection SARS-Cov-2 using RT-PCR. *Journal of Health and Medical Sciences*, 6(2), 72-78.

ISSN 2622-7258

DOI: 10.31014/aior.1994.06.02.270

The online version of this article can be found at:

<https://www.asianinstituteofresearch.org/>

Published by:
The Asian Institute of Research

The *Journal of Health and Medical Sciences* is an Open Access publication. It may be read, copied, and distributed free of charge according to the conditions of the Creative Commons Attribution 4.0 International license.

The Asian Institute of Research *Journal of Health and Medical Sciences* is a peer-reviewed International Journal. The journal covers scholarly articles in the fields of Medicine and Public Health, including medicine, surgery, ophthalmology, gynecology and obstetrics, psychiatry, anesthesia, pediatrics, orthopedics, microbiology, pathology and laboratory medicine, medical education, research methodology, forensic medicine, medical ethics, community medicine, public health, community health, behavioral health, health policy, health service, health education, health economics, medical ethics, health protection, environmental health, and equity in health. As the journal is Open Access, it ensures high visibility and the increase of citations for all research articles published. The *Journal of Health and Medical Sciences* aims to facilitate scholarly work on recent theoretical and practical aspects of Health and Medical Sciences.



ASIAN INSTITUTE OF RESEARCH
Connecting Scholars Worldwide

Comparison of the Effectiveness in Nasopharyngeal, Throat, Saliva, and Nasal Swab Sample Media of Detection SARS-Cov-2 using RT-PCR

Raka Rajendra¹, Syahrul Tuba¹, Syed Azhar Syed Sulaiman²

¹ Faculty of Military Pharmacy, Indonesia Defense University, Sentul, Bogor, Indonesia

² School of Pharmaceutical Science, University Sains Malaysia, Pulau Penang, Malaysia

Correspondence: Syahrul Tuba, Faculty of Military Pharmacy, The Republic of Indonesia Defense University, Sentul, 16810, Indonesia. Tel: +6281296882703. E-mail: syahrulpharm@gmail.com

Abstract

To evaluate effectivity results among Nasopharyngeal, Throat, Saliva, and Nasal Swab Sample Media for Detection of SARS-Cov-2 virus using RT-PCR. SARS-CoV-2 is a coronavirus microorganism found in humans. A known viral infection causes the covid-19 disease to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Covid-19 has confused the public because of the different places where the samples were taken. Sampling was taken from the Nasopharynx, Throat, Saliva, and nasal Swab. This study used mini-review journals from several leading search engine journals such as PubMed, Elsevier, Jama Network, BMJ, Cochrane, Wiley, medRxiv, Lancet, and others, as well as from government websites such as WHO selected between 2020 and 2021 in the English language. Each sampling place has its advantages and disadvantages. Any place that is used as the gold standard is the nasal swab and nasopharyngeal. This paper attempts to compare the efficacy of four sample media to find the best method for detecting the SARS-CoV-2 virus. It is hoped that repeating this paper can make us aware of every method that we can use to detect the SARS-CoV-2 virus and reduce the spread of this virus, which is increasingly widespread.

Keywords: SARS-CoV-2, RT-PCR, Covid-19

1. Introduction

SARS-CoV-2 is a microorganism of coronavirus found in humans. A known viral infection causes the covid-19 disease to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Viruses enter cells and begin infecting them by connecting to receptors on the cell surface that they recognize. The expression and distribution of viral invagination receptors influence their orientation and determine which tissue it infects and thus the pathogenesis of the disease. SARS-CoV-2 is the third human coronavirus to invade cells using the embryo peptidase angiotensin-converting enzyme 2 (ACE2). From early SARS-CoV-2 infection to severe coronavirus disease in 2019, the interaction between SARS-CoV-2 and ACE2 is critical for controlling tissue orientation and development (Matheson & Lehner, 2020). SARS-CoV-2 drew extreme attention to the world that originated in Wuhan, Hubei Province, China. SARS-CoV-2 is rapidly spreading, which causes all countries in the world to be

exposed to the SARS-CoV-2 (Astuti & Ysrafil, 2020; Chan et al., 2020; Yamayoshi et al., 2020). As of March 31, 2021, there were 128 million confirmed cases by WHO and roughly 2 million deaths [<https://covid19.who.int/>]. The standard gold method of detecting RNA viruses with reverse transcriptase-polymerase chain reaction test (RT-PCR) is used in detecting SARS-CoV-2 infection. The RT-PCR test is used to verify connections to minimize the chances of Covid-19 cases re-emerging (Dogan et al., 2020; Torres et al., 2020; Wikramaratna et al., 2020). RT-PCR is a method of detecting and measuring the expression of associated genes. Real-time polymerase chain reaction (RT-PCR) is conducted on nasopharyngeal, throat, saliva, and nasal swab sample media (Deepak et al., 2007).

In detecting saliva samples on SARS-CoV-2, most studies are equally Stable and open with nasopharyngeal samples. Saliva detected that there are viral nucleic acids found in the salivary gland ducts indicating infection of the glands. In saliva, we can find out that there are live viruses by the method of viral culture. From the data obtained on live viral infections, it is predicted that in the throat sample, harmful levels are incorrect from the SARS-CoV RT-PCR study (Medeiros da Silva et al., 2020). In the nasal swab sample, there is viral infection data. It is predicted that in the throat sample, harmful levels are incorrect from the SARS-CoV RT-PCR study (Xiao et al., 2020).

Therefore, each approach has benefits and drawbacks of its own. This paper seeks to compare the efficacy of the four-sample media to find the best method for detecting the SARS-COV-2 virus. It is hoped that with the best detection method, the dissemination of covid-19 will be reduced.

2. Research Method

We conducted a mini-review of scientific literature in the PubMed, Elsevier, Jama Network, BMJ, Cochrane, Wiley, medRxiv, Lancet, and others, as well as from government websites such as WHO selected between 2020 and 2021 in the English language databases about the experiences who survived COVID-19, with the following descriptors being used: COVID-19 AND Nasopharyngeal AND Throat AND Saliva AND Nasal Swab AND Detection SARS-Cov-2 AND RT-PCR.

The criteria for inclusion in this study comprised of primary research articles and reviews that were published within the past decade. Initially, the articles were selected based on their titles, followed by a thorough reading of the abstracts. Any duplicated articles were subsequently excluded from consideration. Following this, a comprehensive examination of the articles was conducted, with a focus on identifying those that satisfied the established criteria for inclusion. The authors conducted a qualitative thematic analysis of the texts and subsequently utilized data triangulation to identify three distinct categories.

3. Result and Discussion

RT-PCR is a technique that uses viral RNA to diagnose SARS-CoV-2 infection (la Marca et al., 2020). To imitate and persist in the SARS-CoV-2 genomic sequence, researchers employed RT-PCR. RT-PCR is a quantitative test that can determine an imitation of any sequence of genome forms, and the total copies of RNA obtained on PCR will increase and equal to the first material, the viral load. In this RT-PCR method, the viral RNA model will be replaced to form cDNA, which is complementary DNA through the DNA polymerase enzyme based on RNA or reverse transcriptase. Then the cDNA will be assisted by the PCR polymerase chain through stages, namely the condition of the double strand of template DNA, which is split into single strands at denaturation at 95°C. The next step is that the DNA double bonds are broken and become a single strand on each DNA template which will cool down to a temperature of 60°C, this is what makes a gap for the primary pair of the primary annealing, and the probe will attach to every single strand of DNA. Furthermore, the DNA polymerase primer, which operates at 72°C, will cause RNA extension. There will be an addition of 3' In the primer attached to the single strand of the template DNA (Afzal, 2020).

3.1. Mechanism of action of RT-PCR on nasopharyngeal, throat, saliva, and nasal swab sample.

3.1.1. Mechanism of action on nasopharyngeal samples

In the nasopharyngeal sample, it is precisely the lining of the nose that is the basis of infection and the basis of the spread of SARS-CoV-2. The receptor used by SARS-CoV-2 is ACE2 which can support the SARS-CoV-2 virus to enter host cells. These receptors make the SARS-Cov-2 virus have the power to move from one human to another. When infected, the SARS-CoV-2 virus enters, relying on the attachment of protein spikes due to cellular proteases (Pondaven-Letourmy et al., 2020).

3.1.2. Mechanism of action on saliva samples

SARS-CoV-2 in salivary gland duct epithelial cells can be infected in rhesus macaques. The appearance of Covid-19 in saliva causes infection of the salivary glands. Saliva comprises saliva generated by the central and minor salivary glands and secretions that descend from the nasopharynx or lungs via cilia activity on the airway. (To et al., 2020). The saliva samples were carried out using several methods, namely the standard heating inactivation of SARS-CoV-2 at a temperature of 60°C. In killing the virus, it was carried out by direct RT-qPCR, which did not involve the extraction of RNA when it was impaled at room temperature. For which no SARS-CoC-2 gene was observed (Ranoa et al., 2020).

3.1.3. Mechanism of action on throat samples and nasal swabs

Nasal and throat swab samples in immunosuppressed people on viral culture were carried out in the first seven days of the onset of indications with an increase in viral load. The virus could increase in the respiratory tract of people shown in the first week after the onset of symptoms (Perera et al., 2020; Rabaan et al., 2021; van Kampen et al., 2020). Then the chances of SARS-CoV-2 will decrease after seven days (Rabaan et al., 2021; Young et al., 2020). The nasal and throat swab samples were carried out by looking at the viral load that occurred. The increase in viral load will differ from the computed tomography (Ct) values observed after the onset of the disease. Using the increased viral load, it is more legible on the nose and throat. What occurs is that when viral nucleic acids are released, they become infected with SARS-CoV-2. The viral load can be found in asymptomatic people (Zou et al., 2020).

3.2. How to take sampling from all three sample media

3.2.1. How to take the nasopharyngeal sample

In the nasopharyngeal, sampling is carried out in a sitting position, and the head is upright by following the bridge of the nose perpendicular to the face. The head is placed on a holder on the head of the chair to ensure that the head performs the reflex movements. Look at the bridge of the nose to find out the right place. Then from the nasal space, it will be obtained right on the contact in the nasopharynx, precisely on the posterior wall, which will be rotated to take samples at about 8-10 cm for adults and 6-7 cm for children. The grip of the nasopharyngeal swab is mandatory for holding the pen (**Fig. 1**). After the swab has been taken, at the end of the swab, there will be a long mark that can be detected, but there is also no sign (Pondaven-Letourmy et al., 2020).

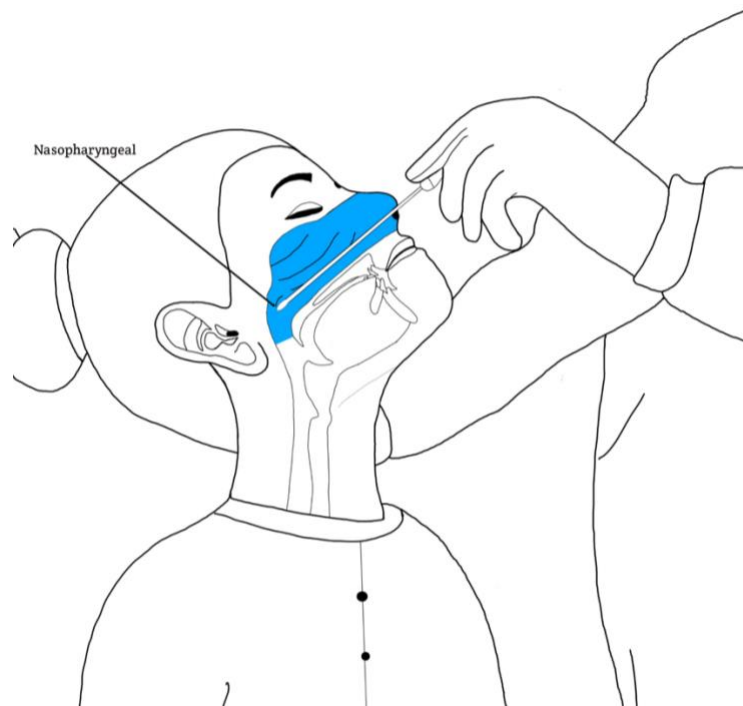


Figure 1. How to take the nasopharyngeal sample

3.2.2. How to take the throat sample

In the throat or oropharynx, the sample is taken by taking it on the edge of the back wall of the posterior pharynx just below the uvula and oropharynx (**Fig. 2**), then before removing it, turn the swab a maximum of 3 times (Berenger et al., 2020).

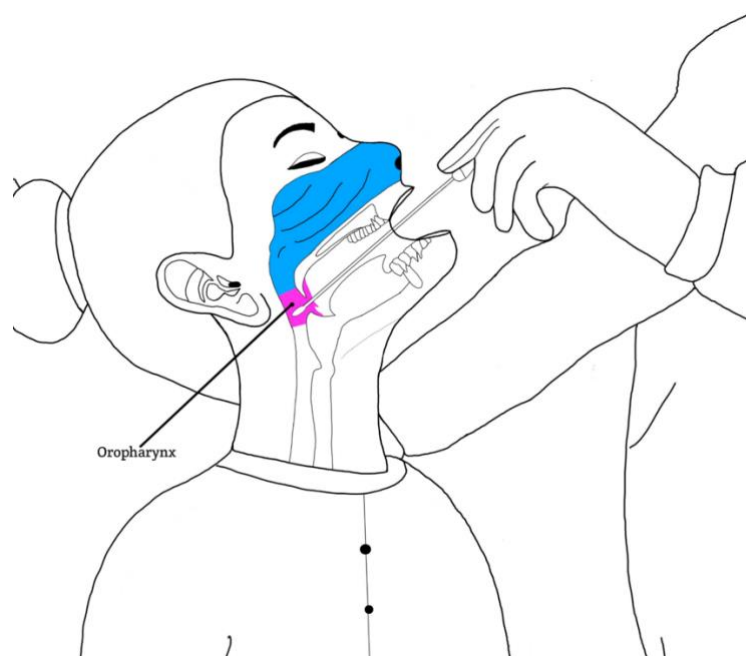


Figure 2. How to take the throat sample

3.2.3. How to collect saliva samples

In **fig. 3**, taking samples of saliva to take samples using a cotton swab that has been prepared then put under the tongue to keep away from contamination from breathing when put under the tongue directed not to cough (Tsujimoto et al., 2021).



Figure 3. How to collect saliva samples

3.2.4. How to collect nasal swab sample

In taking the sample, the nose swab (**Fig. 4**) is carried out with the head tilted 70° towards the back of the cotton or the swab that has been prepared is inserted and taken into the nostrils between 3 cm or until resistance occurs on the turbinate which is rotated at least three times (Berenger et al., 2020).

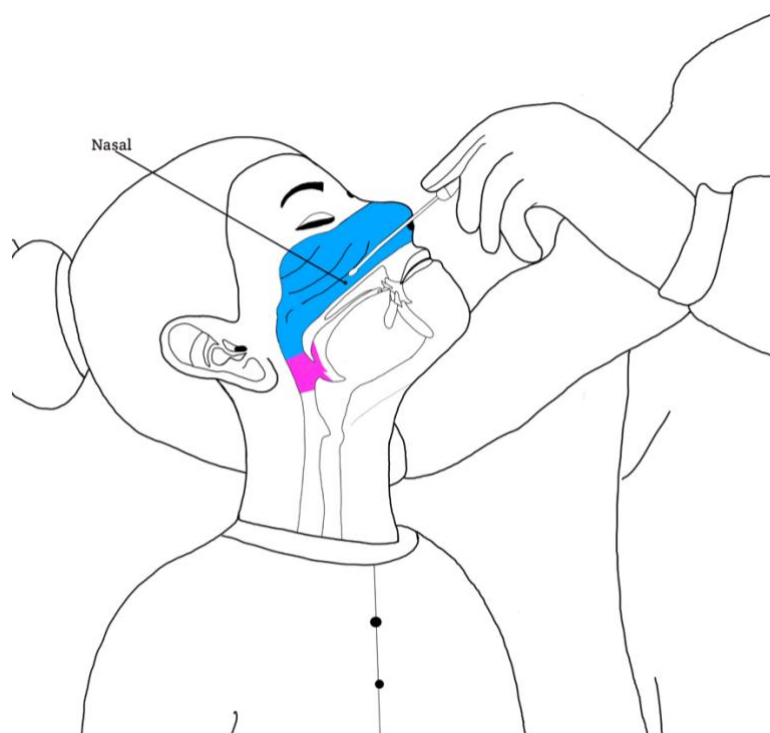


Figure 4: How to collect nasal swab sample

3.3. Comparison of samples

Based on the results data obtained from journals, there are no significant results from nasopharyngeal samples and saliva samples, which in practice saliva samples are more widely used, in the advantage that they can be easily

obtained and do not require more energy. However, it takes many saliva and nasopharyngeal samples to make comparisons and assess the presence of the SARS-CoV-2 virus, which is done using many techniques (Nasiri & Dimitrova, 2021).

In the combination of throat swab and nose swab, the sensitivity results are classified as the same in detecting SARS-CoV-2 as a nasopharyngeal swab, although on computed tomography (Ct), the nasopharyngeal smear is down. For nasal swab samples and nasopharyngeal samples have the same position, which is used as the gold standard in detecting SARS-CoV-2. The nasal swab samples showed great sensitivity and qualification (Péré et al., 2020).

4. Conclusion

Each approach has its benefits and drawbacks. This article compares the efficiency of four sample media to determine the most effective approach for identifying the SARS-CoV-2 virus. It is hoped that repeating this paper can make us aware of every method that we can use to detect the SARS-CoV-2 virus and reduce the spread of this virus, which is increasingly widespread.

Acknowledgment

The authors state that they do not have any competing interests.

References

- Afzal, A. (2020). Molecular diagnostic technologies for COVID-19: Limitations and challenges. In *Journal of Advanced Research* (Vol. 26, p. 149-159). Elsevier B.V. <https://doi.org/10.1016/j.jare.2020.08.002>
- Astuti, I., & Ysrafil. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 14(4), 407-412. <https://doi.org/10.1016/j.dsx.2020.04.020>
- Berenger, B. M., Fonseca, K., Schneider, A. R., Hu, J., & Zelyas, N. (2020). Sensitivity of nasopharyngeal, nasal and throat swab for the detection of SARS-CoV-2. In *medRxiv*. medRxiv. <https://doi.org/10.1101/2020.05.05.20084889>
- Chan, J. F. W., Yuan, S., Kok, K. H., To, K. K. W., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C. C. Y., Poon, R. W. S., Tsoi, H. W., Lo, S. K. F., Chan, K. H., Poon, V. K. M., Chan, W. M., Ip, J. D., Cai, J. P., Cheng, V. C. C., Chen, H., ... Yuen, K. Y. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *The Lancet*, 395(10223), 514-523. [https://doi.org/10.1016/S0140-6736\(20\)30154-9](https://doi.org/10.1016/S0140-6736(20)30154-9)
- Deepak, S. A., Kottapalli, K. R., Rakwal, R., Oros, G., Rangappa, K. S., Iwahashi, H., Masuo, Y., & Agrawal, G. K. (2007). Real-Time PCR: Revolutionizing Detection and Expression Analysis of Genes. In *Current Genomics* (Vol. 8).
- Dogan, O. A., Kose, B., Agaoglu, N. B., Yildiz, J., Alkurt, G., Demirkol, Y. K., Irvem, A., Doganay, G. D., & Doğanay, L. (2020). Does sampling saliva increase detection of SARS-CoV-2 by RT-PCR? Comparing saliva with oro-nasopharyngeal swabs. In *medRxiv*. medRxiv. <https://doi.org/10.1101/2020.07.26.20158618>
- la Marca, A., Capuzzo, M., Paglia, T., Roli, L., Trenti, T., & Nelson, S. M. (2020). Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. In *Reproductive BioMedicine Online* (Vol. 41, Numéro 3, p. 483-499). Elsevier Ltd. <https://doi.org/10.1016/j.rbmo.2020.06.001>
- Matheson, N. J., & Lehner, P. J. (2020). How does SARS-CoV-2 cause COVID-19? *Science*, 369(6503), 510. <https://doi.org/10.1126/science.abc6156>
- Medeiros da Silva, R. C., Nogueira Marinho, L. C., de Araújo Silva, D. N., Costa de Lima, K., Pirihi, F. Q., & Luz de Aquino Martins, A. R. (2020). Saliva as a possible tool for the SARS-CoV-2 detection: A review. In *Travel Medicine and Infectious Disease* (Vol. 38). Elsevier Inc. <https://doi.org/10.1016/j.tmaid.2020.101920>
- Nasiri, K., & Dimitrova, A. (2021). Comparing saliva and nasopharyngeal swab specimens in the detection of COVID-19: A systematic review and meta-analysis. In *Journal of Dental Sciences*. Association for Dental Sciences of the Republic of China. <https://doi.org/10.1016/j.jds.2021.01.010>
- Péré, H., Péré, H., Péré, H., Podglajen, I., Podglajen, I., Wack, M., Wack, M., Flamarion, E., Mirault, T., Mirault, T., Goudot, G., Goudot, G., Hauw-Berlemont, C., Le, L., Le, L., Caudron, E., Caudron, E., Carrabin, S.,

- Rodary, J., ... Veyer, D. (2020). Nasal swab sampling for SARS-CoV-2: A convenient alternative in times of nasopharyngeal swab shortage. In *Journal of Clinical Microbiology* (Vol. 58, Numéro 6). American Society for Microbiology. <https://doi.org/10.1128/JCM.00721-20>
- Perera, R. A. P. M., Tso, E., Tsang, O. T. Y., Tsang, D. N. C., Fung, K., Leung, Y. W. Y., Chin, A. W. H., Chu, D. K. W., Cheng, S. M. S., Poon, L. L. M., Chuang, V. W. M., & Peiris, M. (2020). SARS-CoV-2 virus culture and subgenomic RNA for respiratory specimens from patients with mild Coronavirus disease. *Emerging Infectious Diseases*, 26(11), 2701-2704. <https://doi.org/10.3201/eid2611.203219>
- Pondaven-Letourmy, S., Alvin, F., Boumghit, Y., & Simon, F. (2020). How to perform a nasopharyngeal swab in adults and children in the COVID-19 era. *European Annals of Otorhinolaryngology, Head and Neck Diseases*, 137(4), 325-327. <https://doi.org/10.1016/j.anorl.2020.06.001>
- Rabaan, A. A., Tirupathi, R., Sule, A. A., Aldali, J., Mutair, A. al, Alhumaid, S., Muzaaheed, Gupta, N., Koritala, T., Adhikari, R., Bilal, M., Dhawan, M., Tiwari, R., Mitra, S., Emran, T. bin, & Dhama, K. (2021). Viral Dynamics and Real-Time RT-PCR Ct Values Correlation with Disease Severity in COVID-19. *Diagnostics*, 11(6), 1091. <https://doi.org/10.3390/diagnostics11061091>
- Ranao, D. R. E., Holland, R. L., Alnaji, F. G., Green, K. J., Wang, L., Brooke, C. B., Burke, M. D., Fan, T. M., & Hergenrother, P. J. (2020). Saliva-based molecular testing for SARS-CoV-2 that bypasses RNA extraction. In *bioRxiv*. bioRxiv. <https://doi.org/10.1101/2020.06.18.159434>
- To, K. K.-W., Tsang, O. T.-Y., Yip, C. C.-Y., Chan, K.-H., Wu, T.-C., Chan, J. M.-C., Leung, W.-S., Chik, T. S.-H., Choi, C. Y.-C., Kandamby, D. H., Lung, D. C., Tam, A. R., Poon, R. W.-S., Fung, A. Y.-F., Hung, I. F.-N., Cheng, V. C.-C., Chan, J. F.-W., & Yuen, K.-Y. (2020). Consistent Detection of 2019 Novel Coronavirus in Saliva. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 71(15), 841-843. <https://doi.org/10.1093/cid/ciaa149>
- Torres, I., Albert, E., & Navarro, D. (2020). Pooling of nasopharyngeal swab specimens for SARS-CoV-2 detection by RT-PCR. In *medRxiv*. medRxiv. <https://doi.org/10.1101/2020.04.22.20075598>
- Tsujimoto, Y., Terada, J., Kimura, M., Moriya, A., Motohashi, A., Izumi, S., Kawajiri, K., Hakkaku, K., Morishita, M., Saito, S., Takumida, H., Watanabe, H., Tsukada, A., Morita, C., Yamaguchi, Y., Katsuno, T., Kusaba, Y., Sakamoto, K., Hashimoto, M., ... Sugiyama, H. (2021). Diagnostic accuracy of nasopharyngeal swab, nasal swab and saliva swab samples for the detection of SARS-CoV-2 using RT-PCR. *Infectious Diseases*. <https://doi.org/10.1080/23744235.2021.1903550>
- van Kampen, J. J. A., van de Vijver, D. A. M. C., Fraaij, P. L. A., Haagmans, B. L., Lamers, M. M., Okba, N., van den Akker, J. P. C., Endeman, H., Gommers, D. A. M. P. J., Cornelissen, J. J., Hoek, R. A. S., van der Eerden, M. M., Hesselink, D. A., Metselaar, H. J., Verbon, A., de Steenwinkel, J. E. M., Aron, G. I., van Gorp, E. C. M., van Boheemen, S., ... van der Eijk, A. A. (2020). Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants. *medRxiv*, 2020.06.08.20125310. <https://doi.org/10.1101/2020.06.08.20125310>
- Wikramaratna, P. S., Paton, R. S., Ghafari, M., & Lourenço, J. (2020). Estimating the false-negative test probability of SARS-CoV-2 by RT-PCR. In *medRxiv*. medRxiv. <https://doi.org/10.1101/2020.04.05.20053355>
- Xiao, A. T., Tong, Y. X., Gao, C., Zhu, L., Zhang, Y. J., & Zhang, S. (2020). Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: A descriptive study. *Journal of Clinical Virology*, 127. <https://doi.org/10.1016/j.jcv.2020.104346>
- Yamayoshi, S., Sakai-Tagawa, Y., Koga, M., Akasaka, O., Nakachi, I., Koh, H., Maeda, K., Adachi, E., Saito, M., Nagai, H., Ikeuchi, K., Ogura, T., Baba, R., Fujita, K., Fukui, T., Ito, F., Hattori, S. I., Yamamoto, K., Nakamoto, T., ... Kawaoka, Y. (2020). Comparison of Rapid Antigen Tests for COVID-19. *Viruses*, 12(12). <https://doi.org/10.3390/v12121420>
- Young, B. E., Ong, S. W. X., Ng, L. F. P., Anderson, D. E., Chia, W. N., Chia, P. Y., Ang, L. W., Mak, T.-M., Kalimuddin, S., Chai, L. Y. A., Pada, S., Tan, S. Y., Sun, L., Parthasarathy, P., Fong, S.-W., Chan, Y.-H., Tan, C. W., Lee, B., Röttschke, O., ... Team, S. 2019 N. C. O. R. (2020). Viral Dynamics and Immune Correlates of Coronavirus Disease 2019 (COVID-19) Severity. *Clinical Infectious Diseases*. <https://doi.org/10.1093/cid/ciaa1280>
- Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., Yu, J., Kang, M., Song, Y., Xia, J., Guo, Q., Song, T., He, J., Yen, H.-L., Peiris, M., & Wu, J. (2020). SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *The New England Journal of Medicine*, 382(12), 1177-1179. <https://doi.org/10.1056/NEJMc2001737>