Rapid Surface Testing with the COV-Hygien Express Kit to Control the Spread of COVID-19

SUMMARY

The current COVID-19 pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which can spread directly through contact with an infected individual or indirectly by touching a surface contaminated by an infected individual. The COV-Hygien Express kit was designed to help mitigate the risk of spreading the virus by detecting and monitoring SARS-CoV-2 contamination of environmental surfaces. Because our kit uses test strips and a premixed buffer solution to test for surface contamination, it can be performed on-site by any user, with no need for specialized training or equipment, facilitating the frequent testing and rapid results that are key to effective COVID-19 surveillance (Larremore et al. 2020).

The kit is based on detection of the SARS-CoV-2 nucleocapsid (N) protein, which is thought to be one of the most abundant and immunogenic proteins expressed by the virus based on similarity with SARS-CoV-1 (He et al. 2004, Leung et al. 2004). The limit of detection (LOD) for the COV-Hygien Express kit is 0.25 ng/mL for the N protein and 5×10^3 pfu/mL for the virus itself. Detection of the SARS-CoV-2 N protein does not necessarily indicate the presence of live virus, as the protein can exist in damaged or noninfectious viral particles. Studies have shown that viral concentrations in samples taken from the lower and upper respiratory tract at different points during infection can range from 1×10^4 copies of the viral genome per mL (Pan et al. 2020) to as high as 7×10^8 copies per throat swab (Wölfel et al. 2020), suggesting that the COV-Hygien Express kit may be sensitive enough to detect viral titers lower than those deposited on surfaces contaminated by infected individuals.

Importantly, droplets and aerosols generated by sneezing, coughing, and even speaking can transmit high titers of SARS-CoV-2 to environmental surfaces. A recent modeling study calculated that approximately one-third of all respiratory droplets produced by an infected individual contain at least one viral particle (Stadnytskyi et al. 2020), and simulation has shown that droplets and aerosols can be propelled up to 8 m (Bourouiba 2020), thereby potentially contaminating surfaces within a wide radius of a patient with COVID-19. Once introduced to the environment, SARS-CoV-2 can survive for hours to days, depending on the surface. Hard, smooth surfaces are particularly prone to retaining live virus over a long period of time, with one study reporting recovery of SARS-CoV-2 from plastic and stainless steel 72 hours after exposure to aerosol (van Doremalen, et al. 2020), and another study finding that SARS-CoV-2 persisted on stainless steel and plastic for up to 7 days (Chin et al. 2020). The swabs provided with the COV-Hygien Express kit reliably recover approximately 50% of SARS-CoV-2 particles on surfaces such as hard plastic, metal, or glass when they are used correctly. Thus, the COV-Hygien Express kit can be used to quickly and easily check for the presence of SARS-CoV-2 on environmental surfaces hours to days after they were contaminated by an infected individual.

In summary, the COV-Hygien Express kit detects low concentrations of the SARS-CoV-2 N protein that may correspond to clinically relevant viral titers. The user-friendly test format can be easily implemented to monitor surfaces for SARS-CoV-2 contamination and help reduce the risk of spreading the disease. When used appropriately, the COV-Hygien Express kit identifies realistic levels of potentially threatening viral contamination on surfaces, and is a helpful tool for risk prevention.

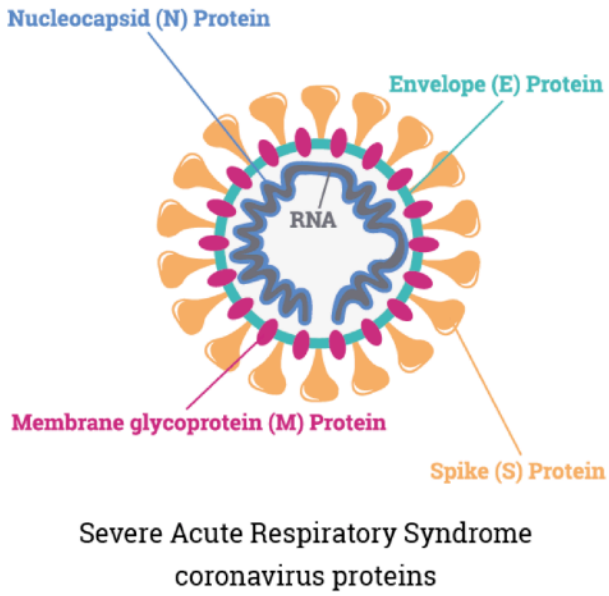
Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible for the current COVID-19 pandemic. The virus spreads between individuals either directly through contact with an infected individual's saliva or respiratory excretions, or indirectly by touching a surface contaminated by an infected individual. It is therefore important to monitor surfaces in public areas to mitigate the risk of spreading the virus through contaminated surfaces.

The COV-Hygien Express kit was designed to help detect and monitor SARS-CoV-2 contamination of environmental surfaces using a simple antibody-based technology that can be performed by anyone, with no need for special training or equipment, to enable you to quickly and easily check surfaces for contamination.

The SARS-CoV-2 virus nucleocapsid (N) protein

SARS-CoV-2 is closely related to SARS-CoV-1, which caused an outbreak of respiratory disease originating in China in 2002. SARS-CoV-2 is an enveloped, single-stranded, positive-sense RNA virus, which means that its genome is composed of RNA (instead of DNA, like in humans), and that each viral particle is surrounded by a membrane (or 'envelope') composed of lipids. SARS-CoV-2 expresses four main proteins: the spike (S) protein, membrane (M) protein, and envelope (E) protein, which are exposed on the surface of the viral envelope, and the nucleocapsid (N) protein, which binds closely to the viral genome contained within the viral envelope (Schoeman et al. 2019).



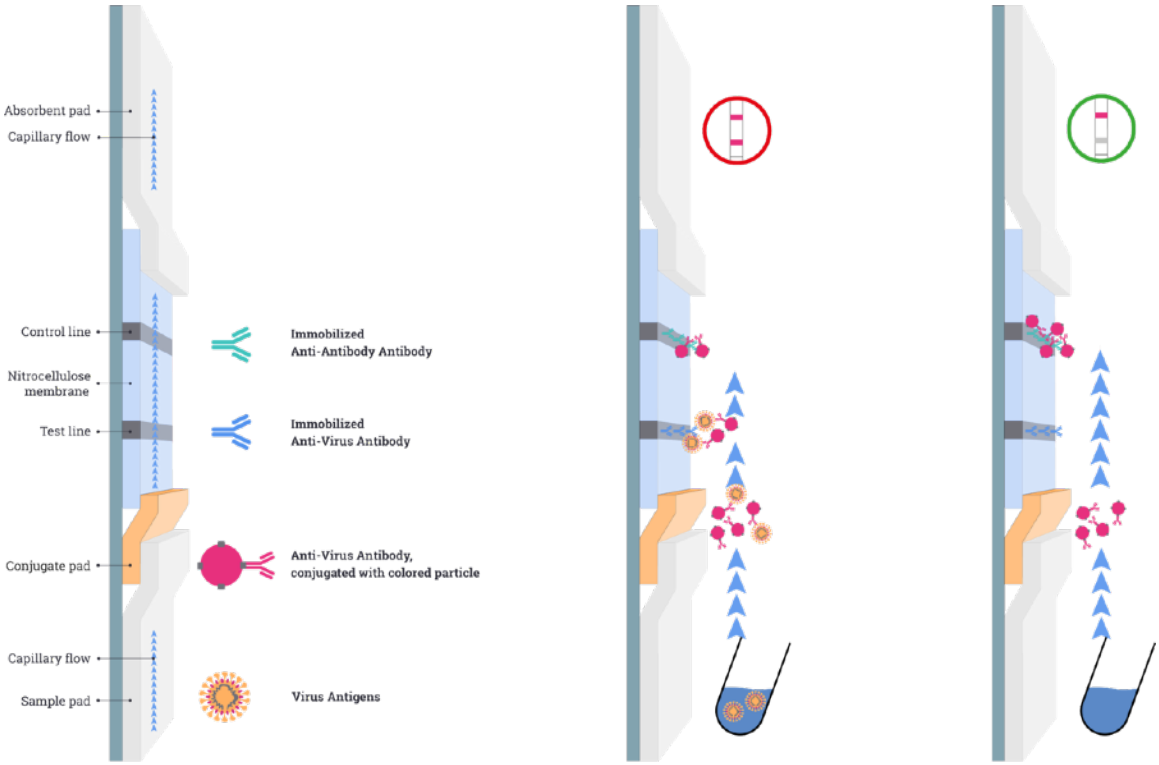
[Figure 1: virus, N protein]

The COV-Hygien Express kit was designed to detect the presence of the SARS-CoV-2 N protein in environmental samples. The N protein is the most abundant protein in SARS-CoV-1 (He et al. 2004), and a structural analysis estimated that each SARS-CoV-1 particle contains approximately 730 to 2200 copies of N (Neuman 2011). Given the similarities between the two viruses, it is likely that N is also expressed at high levels in SARS-CoV-2. In addition, a study of patients infected with SARS-CoV-1 showed that the N protein was the most immunogenic protein, meaning that the body recognized and mounted an immune response to this protein more strongly than to other viral proteins, suggesting that N is a strong indicator of the presence of

virus (Leung et al. 2004). Due to the high abundance and immunogenicity of N during infection, researchers have suggested designing vaccines (Dutta et al. 2008) and diagnostic tools (Lee et al. 2008) based on this protein. Furthermore, the SARS-CoV-1 N protein has been proposed as an early diagnostic marker for infection based on its high abundance and strong immunogenicity (Che et al. 2004). While much of our knowledge of the N protein is based on SARS-CoV-1, a recent study showed that screening for an immune response to the SARS-CoV-2 N protein effectively identified patients with COVID-19 infection (Guo et al. 2020), suggesting that N is also a reliable and appropriate marker for SARS-CoV-2.

How the COV-Hygien Express kit works

The kit is based on lateral flow technology, which involves dipping a paper test strip into a liquid test sample and reading the results based on a color change on the strip (much like a pregnancy test). To use the kit, the surface to be tested is rubbed gently with a premoistened swab, which is then wrung out in the test tube provided with the kit, thereby transferring any virus or viral proteins from the surface into the liquid buffer in the test tube. The test strip is then placed in the test tube, at which point the liquid travels up the strip due to capillary action, crossing two lines of antibodies embedded in the strip. One of these lines serves as a control to confirm that the test was performed correctly, and the other line contains antibodies specific to the SARS-CoV-2 N protein. If this second line turns color, this indicates that the sample contains the viral protein.



[Figure 2: depiction of how the strip works]

Unlike other SARS-CoV-2 tests based on quantitative real-time polymerase chain reaction that detect the viral genome and require specialized training and equipment to perform, the COV-Hygien Express kit, which detects a viral protein, can be performed quickly and easily by an untrained user. This means that the test can be carried out on-site, and the results are available within 30 to 60 minutes. This is particularly important given that a recent, non–peer-reviewed

modeling study demonstrated that frequent testing with rapid results is key to effective COVID-19 surveillance (Larremore et al. 2020).

COV-Hygien Express kit detection capabilities

The COV-Hygien Express kit has a limit of detection (LOD) of 0.25 ng/mL for recombinant SARS-CoV-2 N protein and 5×10^3 pfu/mL for the virus itself, as determined by the Public Health Agency of Sweden (Folkhälsomyndigheten, Stockholm) ('pfu' stands for 'plaque-forming units', which is a way of estimating the number of infectious viral particles in a sample). Because these values were determined under laboratory conditions, they may differ somewhat from performance in the real world, as the protein LOD results can vary based on how the recombinant protein was produced and stored, while the virus LOD results can vary depending on how the virus was grown and processed for use in the test assays. In addition, detection of the SARS-CoV-2 N protein does not necessarily indicate the presence of live virus, as the protein can exist in dead, damaged and/or noninfectious viral particles.

Regarding the relevance of the LOD to actual viral concentrations on contaminated surfaces, several recent studies have investigated the number of viral particles present in patients infected with SARS-CoV-2. A study of nine patients from a hospital in Munich reported viral concentrations of 7.11×10^8 RNA copies per throat swab on the fourth day of infection (Wölfel et al. 2020). Furthermore, two patients evaluated in Beijing were found to have 10^4 to 10^7 copies of the viral genome per mL in throat swab and sputum samples at the peak of infection (Pan et al. 2020), and an additional 80 patients investigated in this study exhibited viral loads $>1 \times 10^6$ copies per mL shortly after the onset of symptoms (Pan et al. 2020). The first two patients in Korea, who had mild to moderate infections, had viral loads of approximately 5×10^4 and 4.6×10^7 copies/mL in the upper respiratory tract and 4×10^5 and 9×10^6 copies/mL in the lower respiratory tract early in the course of infection (Kim et al. 2020). SARS-CoV-2 is also present in the saliva (To et al. 2020), although it is unclear how levels in the saliva correspond to levels in the sinuses and lungs, with one study reporting equivalent levels (Iwaskai et al. 2020) and other, non–peer-reviewed studies reporting higher (Wyllie et al. 2020) or lower (Becker et al. 2020) levels in the saliva compared to nasopharyngeal swabs. Thus, the COV-Hygien Express kit LOD may be sensitive enough to detect the high titers of SARS-CoV-2 carried by infected individuals.

SARS-CoV-2 survival on environmental surfaces

SARS-CoV-2 can be spread by sneezing, coughing, speaking, singing, and any other activity that introduces viral particles from the throat and upper respiratory tract into the air by generating droplets and aerosols. Droplets, being larger, are also heavier, and tend to fall out of the air, potentially contaminating surfaces close to the source of the cough or sneeze; while aerosols stay in the air longer and can thus transmit live virus over larger distances. Indeed, a recent simulation study showed that strong exhalations can create a cloud of droplets and aerosols extending up to 8 m from the source (Bourouiba 2020). Furthermore, two recent modeling studies calculated that each large (50-µm-diameter) droplet has an approximately 37% chance of containing at least one viral particle (Stadnytskyi et al. 2020), and that breathing and coughing can contaminate the air with 0.0000049 and 0.277 copies/cm^3, respectively (Riediker et al. 2020), suggesting that high titers of SARS-CoV-2 can be transmitted to environmental surfaces from infected individuals.

SARS-CoV-2 on surfaces, whether deposited by coughing, sneezing, or other aerosol- and droplet-generating activities, can persist for hours to days, depending on the surface. A recent study generated aerosols mimicking the concentrations of SARS-CoV-2 found in patients' respiratory tracts and found that the aerosols persisted in the air for over 3 hours (van Doremalen et al. 2020). Furthermore, while no virus could be detected on copper surfaces after 4 hours or on cardboard after 24 hours, SARS-CoV-2 was still detectable on plastic and stainless

steel 72 hours after these surfaces were exposed to the aerosol (van Doremalen, et al. 2020). Another study found that, under normal environmental conditions, SARS-CoV-2 applied directly to glass could still be detected 4 days later, and that in the case of stainless steel and plastic the virus was detectable for up to 7 days (Chin et al. 2020). Thus, virus shed by infected individuals can survive on various surfaces for hours to days after the initial exposure, presenting a risk of infection to anyone who subsequently comes into contact with those surfaces.

[Figure 3: sneeze cloud]

The COV-Hygien Express kit is most suitable for sampling smooth surfaces such as hard plastic, metal, or glass, on which SARS-CoV-2 survives for an extended period of time. It is more difficult to recover virus from surfaces that are soft, rough, or porous, such as unfinished wood or soft smartphone covers, so the test is not recommended for these applications. In addition, the ability of the test to detect SARS-CoV-2 is affected by the amount of virus recovered by the swab and transferred to the test tube. In our tests, we have found that approximately 50% of the virus deposited on surfaces is picked up by the swab and effectively transferred to the tube. It is therefore important to follow the sampling instructions closely, swabbing the recommended surface size in the manner described, to ensure obtaining the most accurate results.

Conclusions

In conclusion, monitoring surfaces for SARS-CoV-2 contamination is important for reducing the risk of spread of the disease. Virus shed through sneezing, coughing, or speaking, or through contact with surfaces contaminated by an infected person, has the potential to infect others through contact with the same surfaces. The COV-Hygien Express kit is capable of detecting small numbers of the SARS-CoV-2 N protein that may correspond to clinically relevant viral titers based on current estimates of viral content in the respiratory tract and saliva, as well as the amount of virus transferred in aerosols or respiratory droplets, suggesting that the test is sensitive enough to be useful in risk prevention. Given the variation in test sensitivity based on surface type and texture, it is recommended to verify that the kit is suitable for a specific application. When used correctly, the COV-Hygien Express kit may be capable of detecting realistic levels of potentially threatening viral contamination on surfaces, and is a useful tool for identifying contaminated surfaces that can be disinfected to help prevent future infections.

References

Schoeman, D., Fielding, B.C. Coronavirus envelope protein: current knowledge. *Virol J* **16,** 69 (2019). https://doi.org/10.1186/s12985-019-1182-0

Wölfel, R., Corman, V.M., Guggemos, W. *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* **581,** 465–469 (2020).

Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis*. 2020;20(4):411-412. doi:10.1016/S1473-3099(20)30113-4

Chin, A. W. H., Chu, J. T. S., Perera, M. R. A., Hui, K. P. Y., Yen, H.-L., Chan, M. C. W., … Poon, L. L. M. (2020). Stability of SARS-CoV-2 in different environmental conditions. The Lancet Microbe, 1(1), e10. doi:10.1016/s2666-5247(20)30003-3

He, Y., Zhou, Y., Wu, H., Kou, Z., Liu, S., & Jiang, S. (2004). Mapping of Antigenic Sites on the Nucleocapsid Protein of the Severe Acute Respiratory Syndrome Coronavirus. Journal of Clinical Microbiology, 42(11), 5309–5314. doi:10.1128/jcm.42.11.5309-5314.2004

Kim, J. Y., Ko, J.-H., Kim, Y., Kim, Y.-J., Kim, J.-M., Chung, Y.-S., … Chin, B. S. (2020). Viral Load Kinetics of SARS-CoV-2 Infection in First Two Patients in Korea. Journal of Korean Medical Science, 35(7). doi:10.3346/jkms.2020.35.e86

Bourouiba, L. (2020). Turbulent Gas Clouds and Respiratory Pathogen Emissions. JAMA. doi:10.1001/jama.2020.4756

Leung, D. T. M., Tam, F. C. H., Ma, C. H., Chan, P. K. S., Cheung, J. L. K., Niu, H., … Lim, P. L. (2004). Antibody Response of Patients with Severe Acute Respiratory Syndrome (SARS) Targets the Viral Nucleocapsid. The Journal of Infectious Diseases, 190(2), 379–386. doi:10.1086/422040

Dutta, N. K., Mazumdar, K., Lee, B.-H., Baek, M.-W., Kim, D.-J., Na, Y.-R., … Park, J.-H. (2008). Search for potential target site of nucleocapsid gene for the design of an epitope-based SARS DNA vaccine. Immunology Letters, 118(1), 65–71. doi:10.1016/j.imlet.2008.03.003

Lee HK, Lee BH, Dutta NK, et al. Detection of antibodies against SARS-Coronavirus using recombinant truncated nucleocapsid proteins by ELISA. *J Microbiol Biotechnol*. 2008;18(10):1717-1721.

Che, X.-Y., Hao, W., Wang, Y., Di, B., Yin, K., Xu, Y.-C., … Yuen, K.-Y. (2004). Nucleocapsid Protein as Early Diagnostic Marker for SARS. Emerging Infectious Diseases, 10(11), 1947–1949. doi:10.3201/eid1011.040516

Guo, L., Ren, L., Yang, S., Xiao, M., Chang, D., Yang, F., … Wang, J. (2020). Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clinical Infectious Diseases, 71(15), 778–785. doi:10.1093/cid/ciaa310

Neuman, B. W., Kiss, G., Kunding, A. H., Bhella, D., Baksh, M. F., Connelly, S., … Buchmeier, M. J. (2011). A structural analysis of M protein in coronavirus assembly and morphology. Journal of Structural Biology, 174(1), 11–22. doi:10.1016/j.jsb.2010.11.021

To, K. K.-W., Tsang, O. T.-Y., Yip, C. C.-Y., Chan, K.-H., Wu, T.-C., Chan, J. M.-C., … Yuen, K.-Y. (2020). Consistent Detection of 2019 Novel Coronavirus in Saliva. Clinical Infectious Diseases, 71(15), 841–843. doi:10.1093/cid/ciaa149

Iwasaki, S., Fujisawa, S., Nakakubo, S., Kamada, K., Yamashita, Y., Fukumoto, T., … Teshima, T. (2020). Comparison of SARS-CoV-2 detection in nasopharyngeal swab and saliva. Journal of Infection, 81(2), e145–e147. doi:10.1016/j.jinf.2020.05.071

Wyllie, A. L., Fournier, J., Casanovas-Massana, A., Campbell, M., Tokuyama, M., Vijayakumar, P., … Ko, A. I. (2020). Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. doi:10.1101/2020.04.16.20067835

Becker, D., Sandoval, E., Amin, A., De Hoff, P., Diets, A., Leonetti, N., … Lu, J. (2020). Saliva is less sensitive than nasopharyngeal swabs for COVID-19 detection in the community setting. doi:10.1101/2020.05.11.20092338

Stadnytskyi, V., Bax, C. E., Bax, A., & Anfinrud, P. (2020). The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission. Proceedings of the National Academy of Sciences, 117(22), 11875–11877. doi:10.1073/pnas.2006874117

van Doremalen, N. et al. (2020) Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med 2020; 382:1564-1567.DOI: 10.1056/NEJMc2004973

Larremore, D. B., Wilder, B., Lester, E., Shehata, S., Burke, J. M., Hay, J. A., … Parker, R. (2020). Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv 2020.06.22.20136309; doi:10.1101/2020.06.22.20136309

Riediker, M., & Tsai, D.-H. (2020). Estimation of Viral Aerosol Emissions From Simulated Individuals With Asymptomatic to Moderate Coronavirus Disease 2019. JAMA Network Open, 3(7), e2013807. doi:10.1001/jamanetworkopen.2020.13807

Scientific writing support was provided by Emily Crow of ETC Science Communication.