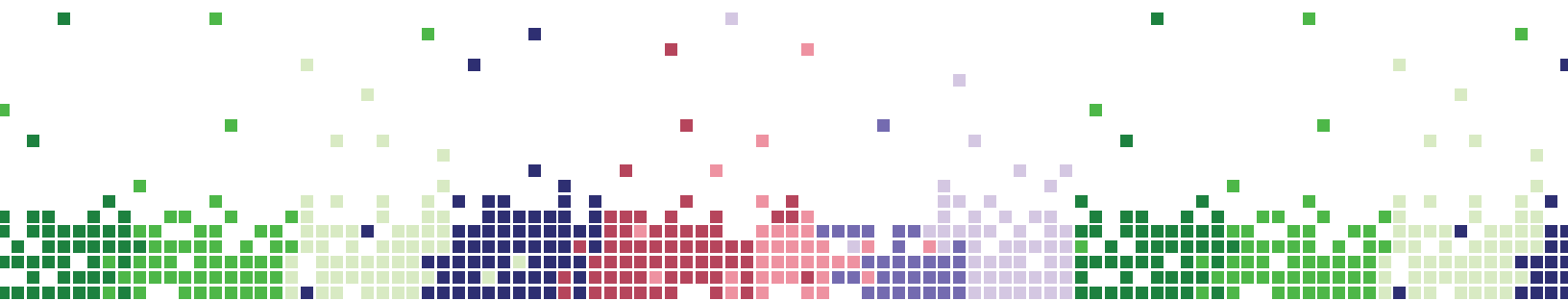
phylagen®

# Phylagen Surface™

## A Sensitive PCR Test for SARS-CoV-2 in the Environment

June 2020

Bradford J. Taft, Nick J. Fantin, Katie Spring,
Juan A. Ugalde, Vivian Yuen, Jessica L. Green



Executive Summary 3

Introduction 4

**Phylagen Surface**™ Product Description 4

**Phylagen Surface**™ Development and Validation 5

- Development: Internal Process Control 5
- Validation 6
  - Assay Design 6
  - Wet Lab RT-qPCR Limit of Detection 6
  - **Phylagen Surface**™ Limit of Detection 7
  - Blind Testing of Proxy Surfaces 8
  - Built Environment Validation following occupation by COVID-19 positive individuals 9
  - Field Testing 9
  - Customer Case Study 9
  - Results 9

Conclusions 10

References 11

## Executive Summary

Phylagen Surface™ allows anyone to test surfaces for the presence of SARS-CoV-2, the coronavirus that causes COVID-19. The test detects if SARS-CoV-2 is present on an environmental surface swab utilizing real time RT-PCR (polymerase chain reaction), the same molecular diagnostic technique used to test if a patient is infected with COVID-19. The limit of detection (LOD) of Phylagen Surface™ has been validated with 95% confidence to be 1,000 viral particles per surface. This is to our knowledge the most sensitive SARS-CoV-2 surface test LOD that takes into account inherent sampling and extraction inefficiencies.

In real-world case studies, Phylagen Surface™ results have shown that surfaces in the vicinity of infected people are often positive for the virus, with a range of 5% to 57% positive samples per tested building. In rooms with known COVID-19 carriers, high surfaces that are rarely touched (e.g. the top of a vending machine or window sill) consistently tested positive for the virus, supporting evidence that it may spread via aerosols. Surface testing also detected the virus in non-obvious places that can be touched by COVID-19 carriers, like the underside of a bench in a locker room. It is also shown that cleaning reduces the frequency of virus-positive samples.

# Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the ongoing coronavirus disease (COVID-19) pandemic. The virus primarily spreads from person to person through respiratory droplets. These droplets land on surfaces, and infected persons can also transmit droplets to surfaces by touching them. Other people can then become infected if they touch these surfaces and then touch their mouth, eyes, or nose. Research suggests that viral particles (virions) from respiratory droplets can remain infectious on environmental surfaces for up to three days[1].

The presence of the virus on environmental surfaces is most easily, quickly and reliably detected using a genomic technology known as RT-qPCR (reverse transcription quantitative polymerase chain reaction), which identifies the novel coronavirus and distinguishes it from other viruses through its unique RNA sequence. This is the same technology used to diagnose patients with COVID-19 based on samples from nasal swabs or saliva.

# Phylagen Surface™ Product Description

Phylagen Surface™ for SARS-CoV-2 is a test kit that allows anyone to test surfaces in the built environment for the presence of SARS-CoV-2. The test relies on RT-qPCR to sensitively detect the novel coronavirus. The kit includes all materials the user needs to swab 25 surface test points, protect the samples in plastic tubes, and safely return them to Phylagen for processing. Phylagen prepares the samples, adds positive and negative controls, performs the RT-qPCR and returns results to customers within 24 hours, via our secure online portal. For each sample, Phylagen returns a result of Present, Not Detected, or Undetermined.

Phylagen Surface™ test kits are easy-to-use: anyone can perform the sampling by following the simple instructions. Phylagen also provides a mobile app that conveniently collects additional information about each surface tested (name of location, timestamp, optional photo of surface) to assign reported results to each specific location swabbed.

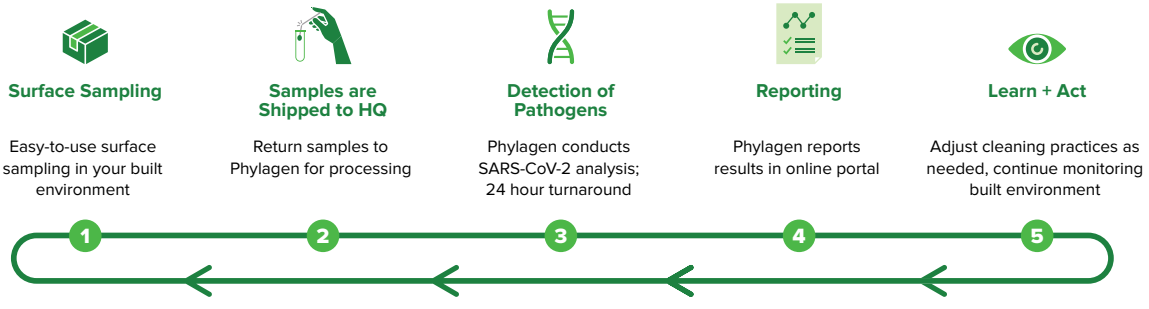



**Figure 1.** Phylagen Surface™ workflow.

# Phylagen Surface™ Development and Validation

Phylagen developed **Phylagen Surface**™ for SARS-CoV-2 by incorporating CDC designed assays[2] into Phylagen's pre-existing and proven technology platform for characterizing DNA and RNA on surfaces. (The CDC primers and probes are DNA sequences complementary to the SARS-CoV-2 RNA sequences that have been shown to uniquely identify SARS-CoV-2 with high sensitivity and specificity.) After developing the test and an internal process control, Phylagen conducted a 5-part validation of the product [Figure 2]: (1) determining PCR assay specificity in silico, (2) quantifying the wet-lab RT-qPCR Limit of Detection, (3) quantifying the Surface Test Limit of Detection, (4) blind testing of proxy surfaces, (5) field testing in facilities known to have COVID-19 positive occupants.

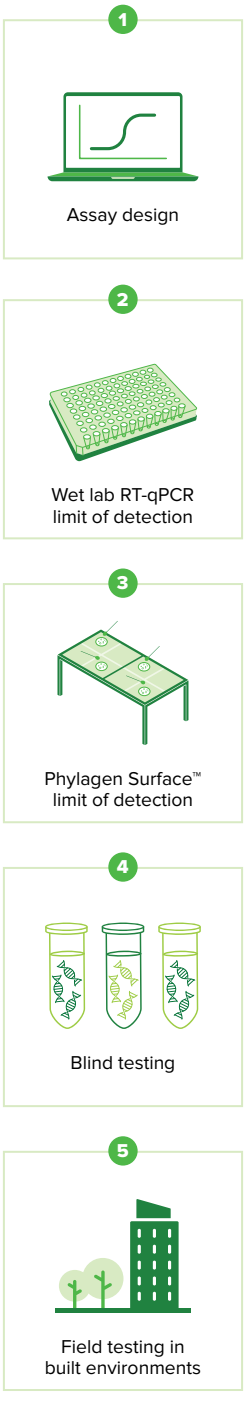



**Figure 2.**
Validation Strategy

## Development: Internal Process Control

An internal process control (IPC) is commonly used in RT-qPCR test design to control for sample preparation failures or PCR failures. Surface samples can originate from a wide range of environments, so the target RNA is typically mixed together with various other chemical, physical, and biological substances. These substances can potentially inhibit or prevent target detection in the sample. The IPC is designed to be impacted by these interfering substances in the same way as the SARS-CoV-2 target. The IPC allows us to distinguish between (i) the case where SARS-CoV-2 is not detected because it is truly not present (reported as "not detected") and (ii) the case where the virus (which may or may not be present) cannot be detected due to interfering substances (reported as "undetermined").

To implement an IPC for **Phylagen Surface**™, a calibrated quantity of a second virus, whose RNA sequence is different from that of SARS-CoV-2, is added to each sample upon arrival at Phylagen's labs. For each sample, the IPC RNA sequence is assayed in a separate PCR reaction in order to determine if the sample preparation protocol was successful for that sample. If RT-qPCR indicates that SARS-CoV-2 is not present but confirms presence of the IPC RNA, then we report with high confidence that SARS-CoV-2 was "not detected" in the sample at the limit of detection. If RT-qPCR indicates that neither SARS-CoV-2 nor the IPC RNA are present, then it is not possible to determine whether the SARS-CoV-2 is present, and the sample is classified as "undetermined". "This process controls for and minimizes the frequency of false negative results. Positive SARS-CoV-2 controls are also included in each batch of samples processed.

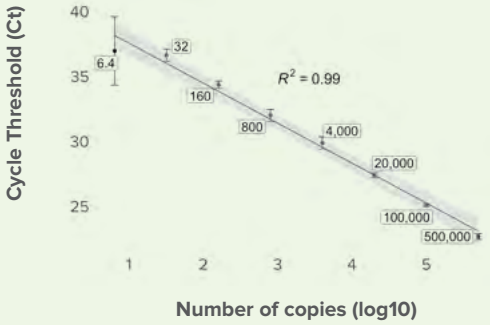



**Figure 3.** Wet lab RT-qPCR performance for a range of SARS-CoV-2 RNA copy inputs (number of copies specified in the rectangles). Error bars represent standard deviation across 6 technical replicates of the indicated copy number, and the purple shade is the 95% confidence interval of the linear regression.

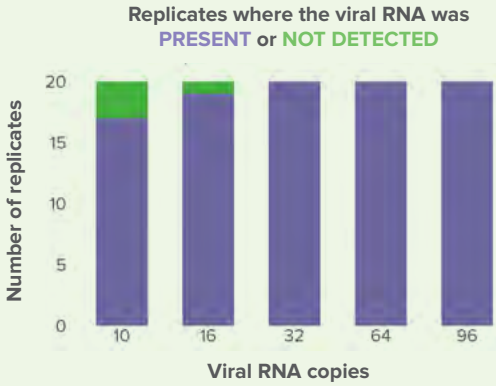



**Figure 4.** Wet labRT-qPCR Limit of Detection (LOD). To accurately determine the PCR LOD, 20 replicate PCR reactions at known inputs above and below our anticipated LOD were run. At a 10 copy input, the RNA was detected in 17 of 20 replicates, at a 16 copy input, the RNA was detected in 19 of 20 replicates. At all inputs above 16 copies, the virus was detected in all 20 replicates. A sample is positive for SARS-CoV-2 if it's amplification curve crosses the threshold below cycle 40.

# Validation

## Assay Design

Phylagen evaluated the CDC assay design for its ability to distinguish SARS-CoV-2 from other viruses. We tested for potential off-target amplification, which would lead to false positives, using an *in silico* PCR approach against a dataset of 2,904 viral genomes retrieved from the National Center for Biotechnology Information (NCBI) database[3] (June 1st, 2020). The list of assessed sequences included all available genomes from the Coronavirus family (excluding SARS-CoV-2), Human Metapneumovirus, Human Parainfluenza Virus Types (PIV) 1, 2, 3, and 4, Influenza A and B, Enterovirus D68, Respiratory syncytial virus (RSV), and Rhinovirus. In addition, we evaluated 11,074 bacterial genomes from the RefSeq[4] representative dataset (June 1st, 2020).

When allowing up to two mismatches on each primer sequence against the reference sequence, none of these viral or bacterial genomes resulted in an *in silico* amplification of the target region, indicating an **assay specificity of 100% for SARS-CoV-2.**

In the context of *in silico* analysis, assay sensitivity measures how well the assay detects all known SARS-CoV-2 variants. This was evaluated using all available SARS-CoV-2 genomes from the Global Initiative on Sharing All Influenza Data (GISAID) database[5] (June 1st, 2020). A total of 23,203 genomes were selected based on completeness (more than 29,000 nucleotides in length) and depth of coverage (as defined by GISAID). *In silico* PCR resulted in detection of 23,193 of the target SARS-CoV-2 genomes, indicating an **assay sensitivity of 99.96% for SARS-Cov-2**. Because the number of available genomes on GISAID increases daily, assay sensitivity is continually evaluated against newly deposited sequences.

## Wet Lab RT-qPCR Limit of Detection

After validating the performance of the SARS-CoV-2 assay *in silico*, development and testing moved to the wet lab to integrate these assay designs with Phylagen's sample preparation systems. The RT-qPCR assay was tested against synthetic SARS-CoV-2 RNA across a wide range of target inputs (six technical replicates) to assess performance against the anticipated viral load in real samples (Figure 3). The PCR Limit of Detection (LOD) is the number of viral RNA copies necessary in a PCR reaction to reliably detect the presence of the virus. The PCR limit of detection was determined by running RNA concentrations designed to bracket the anticipated LOD (20 technical replicates per concentration). The PCR LOD, the input concentration at which at least 95% (19 of 20) replicates are correctly identified as positive, was determined to be 16 copies per reaction (Figure 4).

## Phylagen Surface™ Limit of Detection

The wet lab RT-PCR reaction is just one piece of determining the **Phylagen Surface**™ Limit of Detection. Sampling efficiency (what percentage of available RNA on the surface is picked up by the swab), extraction efficiency (the percentage of RNA that is recovered following sample extraction), and the amount of the recovered sample that is tested in each reaction all play important roles in determining the **Phylagen Surface**™ LOD.


**Figure 5.** Steps impacting a PCR test for SARS-CoV-2 in the environment. The Phylagen Surface LOD takes into account all of the steps necessary to test for SARS-CoV-2; swabbing, RNA extraction, and RT-qPCR.

**Phylagen Surface™ LOD, the lowest number of viral copies detectable on a surface (with a 95% confidence level)**, differs from the RT-qPCR LOD because of the steps required to recover SARS-CoV-2 RNA from a surface. Phylagen has defined an overall LOD which encompasses the entire workflow necessary to detect the virus.

To test the performance of the complete end-to-end workflow of **Phylagen Surface**™ for different concentrations of SARS-CoV-2 we simulated as follows. Unmounted laminate countertops were divided into 10 cm x 10 cm squares. The counters were thoroughly cleaned with bleach, isopropyl alcohol, treated with UV-light, and an RNAse remover. Synthetic SARS-CoV-2 analogues, pseudo-viral particles assembled from viral coat proteins and RNA sequences, were diluted in 1X TE buffer, deposited and spread on the squares in the following quantities: 0, 1, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, and $10^7$ molecules per 100 $cm^2$ square. We sampled each square using the materials as provided in the **Phylagen Surface**™ for SARS-CoV-2 kit, following the provided sampling instructions. RNA from all six replicates of each seeded square down to 1,000 molecules ($10^3$) were detected. Representative results across the range of inputs are shown in Figure 6.

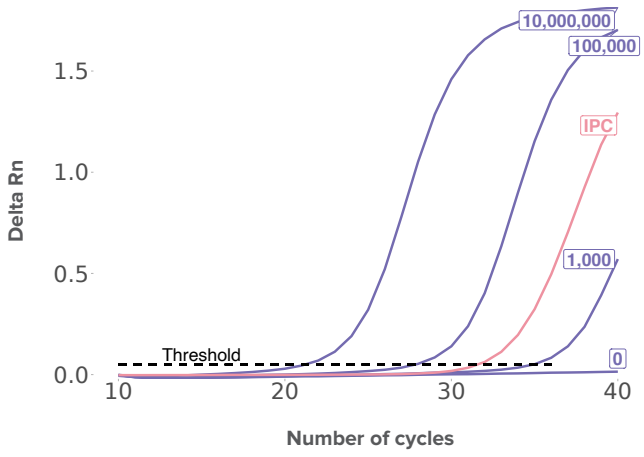



**Figure 6.** Phylagen Surface™ RT-qPCR curves. SARS-CoV-2 analogues were recovered from all areas swabbed with more densely seeded areas returning proportionately more RNA. Delta Rn is the change in fluorescent signal from the baseline signal. The purple curves each represent the average of 6 replicate swabs of synthetic SARS-CoV-2 analogues deposited on 100 cm2 areas, recovered using **Phylagen Surface**™. The red curve is the average of 60 replicates of 1,000 Plaque Forming Units of IPC. The dashed line is the threshold of the RT-qPCR run. The threshold is the set fluorescence value from the run that is statistically significant from the background noise. The threshold cycle (Ct) is the PCR cycle at which a sample's fluorescence intersects the threshold thereby confirming the presence of the target.

After evaluating **Phylagen Surface**™ against the broad range of virion input quantities, the LOD was determined by seeding 20 replicates at three low concentrations of the synthetic SARS-CoV-2 analogues (500, 1,000, and 2,000 molecules per 100 cm² square). The LOD was determined by finding the lowest concentration at which at least 19 of the 20 replicates were detected (95% confidence level). This was determined to be 1,000 molecules (Figure 7). The **Phylagen Surface**™ LOD encompasses swabbing efficiency, sample-preparation, PCR reaction sample input, and the wet lab PCR LOD described above in Section 2.

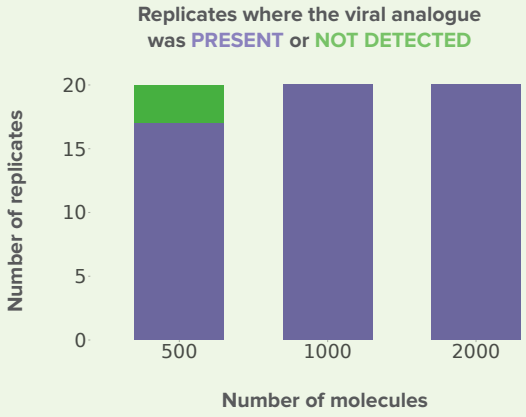



**Figure 7.** The **Phylagen Surface**™ Limit of Detection, defined as the smallest amount of synthetic SARS-CoV-2 analogue that can be detected in 95% of tests performed with the **Phylagen Surface**™ kit, was determined to be 1,000 molecules on a surface. The 500 molecule input was detected in 17 of 20 replicates. All replicates with 1,000 molecules or greater input were detected in 20 of 20 attempts.

## Blind Testing of Proxy Surfaces

A blinded experiment was conducted to evaluate the potential impact of operator bias in the validation experiments and ultimately, the performance of **Phylagen Surface**™. Positive samples were seeded on various surfaces by a single operator. A second operator, blind to the sample status, swabbed using the **Phylagen Surface**™ kit materials and allowed the samples to sit for 18 to 36 hours (corresponding to simulated transit time between collection and arrival at Phylagen for analysis) before processing them. In total, twenty-three 100 cm² countertop surfaces were seeded with synthetic SARS-CoV-2 analogues at amounts representing 25% of the LOD, 250 times the LOD and 25,000 times the LOD. The results of the independent swabbing and processing of these samples are represented in Figure 8.

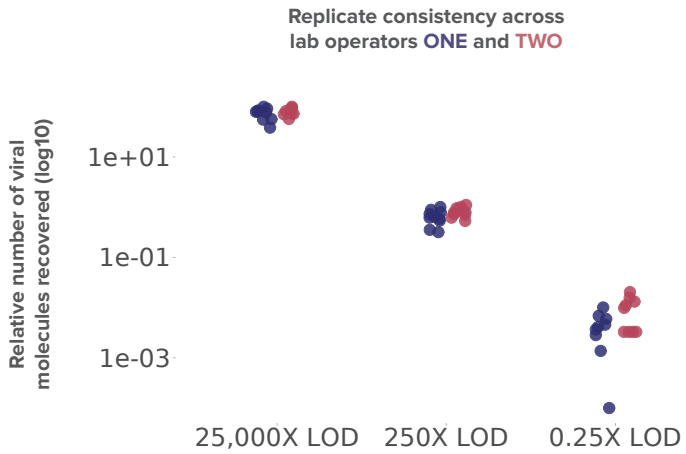



**Figure 8:** **Phylagen Surface**™ SARS-CoV-2 RNA recovery by two independent lab operators across a wide range of viral analogue inputs. Relative viral molecule recovery is defined as the ratio of viral molecules detected in a given sample divided by the highest number of molecules detected in any sample of the same surface seeding amount.

# Built Environment Validation following occupation by COVID-19 positive individuals

## Field Testing

To further validate Phylagen Surface™ for SARS-CoV-2, a field test was designed using two apartments within the same multi-tenant residential building: one occupied by a patient with COVID-19, and the other occupied by a person who had not tested positive for COVID-19 and had no symptoms. Forty-eight samples were collected per occupied environment including frequently and rarely touched surfaces. In the environment occupied by the COVID-19 patient, 57% of the samples tested were positive for SARS-CoV-2 (Figure 9). Conversely, in the other environment, SARS-CoV-2 was not detected in any of the samples. An "undetermined" result indicates that neither SARS-CoV-2, nor the IPC were detected.

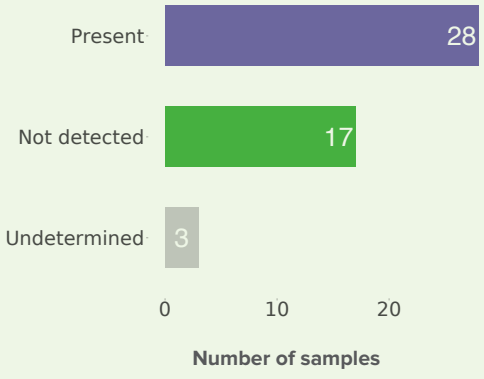



**Figure 9.** Detection of SARS-CoV-2 in a real-world built-environment. 48 samples were collected using Phylagen Surface™ in an environment known to be occupied by a COVID-19 positive individual. The virus was detected in 28 of the samples, not detected in 17, and 3 samples had undetermined results.

## Customer Case Study

**Auditing cleaning effectiveness and monitoring for the presence of SARS CoV-2 in a factory environment**

A Phylagen customer collected 50 samples from its factory after at least one COVID-19-positive employee had been reported. The customer followed Phylagen's test plan guidelines to determine where and when to conduct the sampling. Samples were taken from frequently touched surfaces (e.g. door handles), rarely touched surfaces (e.g. window sills), and low and high traffic rooms, as summarized in Table 1. Samples were also taken before and after the janitorial staff executed the shift-change cleaning protocols. Care was taken to ensure that no infected individuals were present in between the cleaning and the collection of the second set of samples.

**Table 1.** Examples of surfaces sampled

| FREQUENTLY TOUCHED |
|---|
| Vending machine, enter button |
| Refrigerator door handle |
| Microwave touchpad |
| Toaster oven timer knob |
| Coffee machine handle |
| Faucet handle |
| Bench seat, edge |
| Toilet seat |
| Toilet flush handle |
| Countertop |
| Door handle |

| RARELY TOUCHED |
|---|
| Top of vending machine |
| Top of locker |
| Bench leg near floor |
| Hands-free paper towel dispenser |
| Window sill |

## Results

Positive SARS-CoV-2 results for samples collected prior to cleaning aligned with the locations frequented by the COVID-19 positive worker. In many locations where SARS-CoV-2 was detected prior to cleaning, samples taken after cleaning showed that the cleaning was effective. However, in some locations (e.g. the restroom and locker room entrance doors, locker room bench bottom edge, tops of lockers, paper towel dispensers, and top of a vending machine in the kitchen), SARS-CoV-2 was detected both before and after cleaning, indicating the need for more thorough and targeted cleaning of various frequently touched surfaces throughout the facility.

After receiving this report, the customer made changes to their cleaning protocols, including increasing focus on the critical areas pointed out in the report that were insufficiently cleaned. Further, the client scheduled regular follow up testing to re-evaluate how employees and cleaning crew are performing the new protocols and procedures.

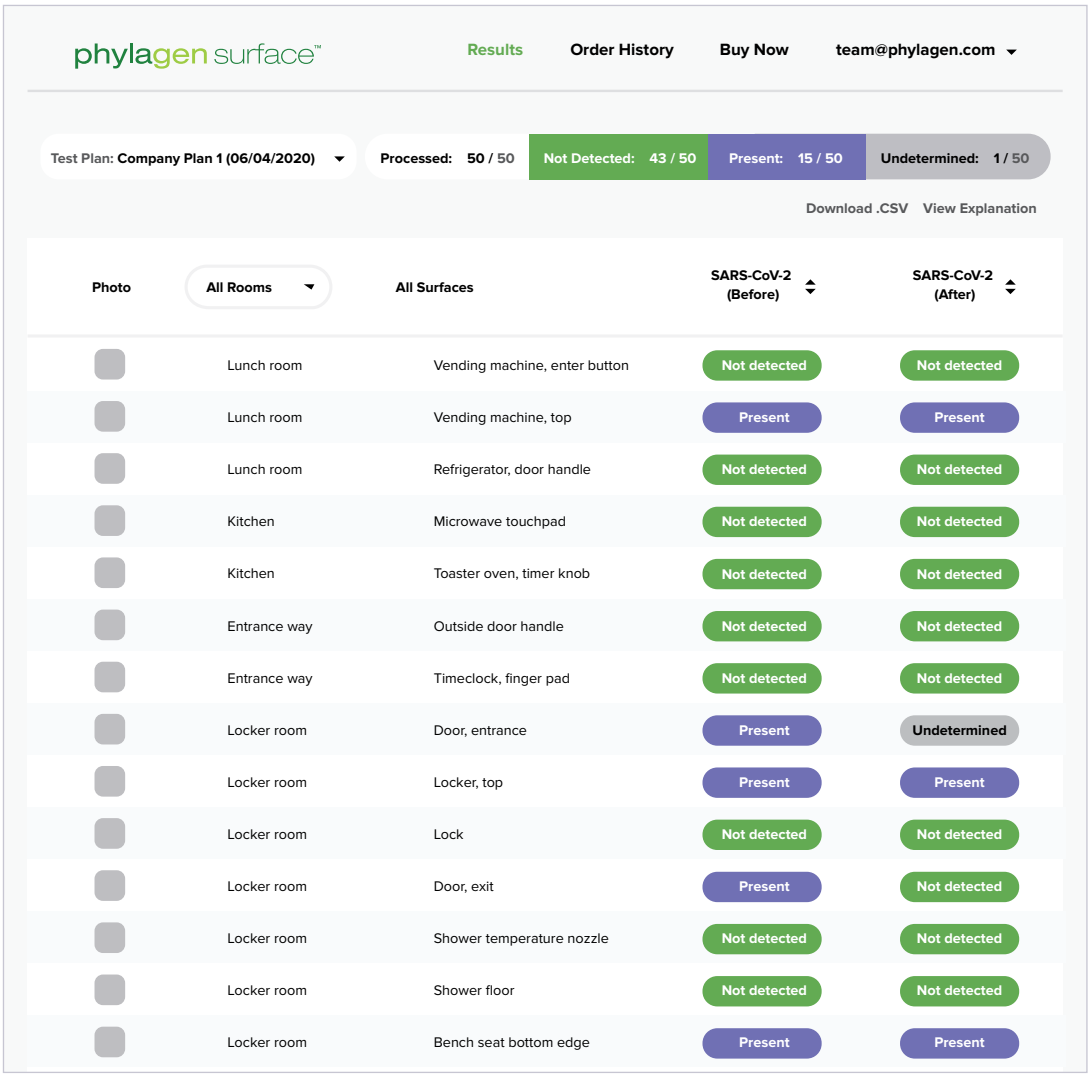



**Figure 10.** Sample Phylagen Surface™ for SARS-CoV-2 analysis report

# Conclusions

Monitoring for early detection of SARS-CoV-2 by regularly testing high risk areas may signal the presence of an infected person in the facility before they would be identified through other means. Research suggests that many individuals with COVID-19 are asymptomatic or presymptomatic[6]. These individuals may be shedding SARS-CoV-2 into the environment, potentially infecting other people without knowing it[7]. Even if infected individuals ultimately develop symptoms, they may not realize they are sick for many days. The virus they shed will nonetheless accumulate on surfaces. Surface monitoring can thus serve as an early sentinel of asymptomatic and presymptomatic transmission risk in the built environment: offices, schools, stores, restaurants, hotels, warehouses, factories, etc. As part of a robust surveillance plan, positive **Phylagen Surface**™ results should trigger a more thorough individual testing with the aim of reducing COVID-19 infections and the associated time that facilities will need to close for disinfecting.

### For more information:

product@phylagen.com
www.phylagen.com/covid-19

# References

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

2. Vogels, Chantal B.F. *et al.* "Analytical sensitivity and efficiency comparisons of SARS-COV-2 qRT-PCR assays." medRxiv (2020). https://doi.org/10.1101/2020.03.30.20048108

3. NCBI Resource Coordinators. "Database Resources of the National Center for Biotechnology Information". *Nucleic Acids Res.* (2018). 46: D8-D13. https://doi.org/10.1093/nar/gkx1095

4. O'Leary, N.A., *et al.* "Reference Sequence (RefSeq) Database at NCBI: Current Status, Taxonomic Expansion, and Functional Annotation (2016). 44: D733 - D745. https://doi.org/10.1093/nar/gkv1189

5. Shu, Y. and McCauley, J. "GISAID: Global initiative on sharing all influenza data - from vision to reality". *Euro Surveill.* (2017). 22: 30494. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494

6. Furukawa, N.W., Brooks, J.T., Sobel, J. "Evidence supporting transmission of severe acute respiratory syndrome coronavirus 2 while presymptomatic or asymptomatic". *Emerg Infect Dis.* (2020). https://doi.org/10.3201/eid2607.201595

7. Arav, Y., Klausner, Z., Fattal, E. "Understanding the indoor pre-symptomatic transmission mechanism of COVID-19". medRxiv (2020). https://www.medrxiv.org/content/10.1101/2020.05.12.20099085v1