RT-qPCR Testing for Covid-19 RNA

SUMMARY
SGS offers lab-based testing for the presence of SARS-CoV-2, the virus responsible for COVID-19 on environmental surfaces and air samples. Testing for the presence of SARS-CoV-2 viral RNA is the most direct and definitive test for ensuring completeness of disinfection procedures. The COVID-19 virus analysis is based on the Centers for Disease Control and Prevention(1), adapted and validated for environmental samples in consideration of test equipment and consumables. Especially in critical high-risk scenarios, and for targeted testing of areas of known contamination, appropriately validated RT-qPCR methods can provide the highest level of confidence.

FREQUENTLY ASKED QUESTIONS

WHAT IS RT-QPCR?
RT-qPCR, or Reverse Transcription, quantitative-Polymerase Chain Reaction measurement is a technique used to measure RNA. It works by converting the RNA into its complementary DNA using a transcriptase enzyme, and then amplifying the DNA using the polymerase chain reaction. A fluorophore (or fluorescent chemical compound) is added to the mixture to be able to read the fluorescence of the amplified cDNA. In the Covid-19 RT-qPCR test, the specific primers and probes used ensure that only RNA from the Covid-19 virus is detected.

WHAT DOES THE RT-QPCR TEST REPORT?
The test reports the presence/absence of RNA characteristic of the SARS-COV2 virus that causes COVID-19. The test is based on CDC RT-qPCR panel(1) and measures the same gene targets as the clinical tests used for Covid-19 testing in humans. As this RT-qPCR assay tests for the presence of RNA fragments that are unique to the Covid-19 virus, and no other virus, it is the most direct, specific and sensitive test for the presence of Covid-19 RNA on a surface.

WHY USE RT-QPCR?
While coronaviruses are easily destroyed by the application of soap and multiple other cleaning and disinfection agents, their presence on a surface is the most definitive measure of incomplete cleaning. For critical areas, RT-qPCR testing provides the greatest confidence on the efficacy of a cleaning and disinfection protocol.

WHAT MATRICES ARE THE TEST OFFERED IN?
The RT-qPCR test for the SARS-CoV-2 virus is currently offered in:
- Surface swabs, typically 5 cm x 5 cm surface wiped per swab
- Air samples from endotoxin-free polycarbonate cassettes and PTFE cassettes. The polycarbonate cassettes can be sampled at up to 15L/min, at least 2 hrs is recommended. The PTFE cassettes, SKC PTFE Filter, 0.3 µm, 37 mm. For example require a lower flow rate and longer sampling time (~ 2-4L/min, and 10 hr sampling time)
RT-qPCR Testing for Covid-19 RNA

FREQUENTLY ASKED QUESTIONS

WHAT IS THE REPORTING THRESHOLD OF THE RT-qPCR TEST?

The reporting threshold is 10 genome units per test, which after dilution translates to 72 genome units/swab.

WHAT DOES A POSITIVE RESULT MEAN?

Like other coronaviruses, the SARS-COV-2 virus is viable and infectious when its lipid cell membrane and nucleocapsid (protein layer) are intact. A positive swab test indicates the presence of this viral RNA in a stable, hence potentially infectious form. Unprotected, viral RNA is unstable in the environment as RNA-degrading enzymes can destroy it rapidly. A positive test on a surface is indicative of the presence of infectious Covid-19 virus, or very recent presence (if the RNA has not degraded yet). However, the correlation between detectable viral RNA and infection potential is a function of many factors including total load, exposure route and number of viral units needed to infect an individual. Positive tests are best viewed as metrics for cleaning success rather than as a direct estimator of infection potential.

HOW LONG CAN RT-qPCR SAMPLES BE STORED PRIOR TO ANALYSIS?

Storage stability of RT-qPCR samples depends on the viral transport medium used. Samples shipped currently need to be stored and shipped on ice, and analyzed within 72 hours of sample receipt. Work is underway to increase the stability.

CAN RT-qPCR SAMPLES BE POOLED?

RT-qPCR samples can be pooled in order to increase the effective surface area cleared as disinfected per lab test. Up to 4 swabs can be pooled into one sample run. There are trade-offs involved with reporting limits per test increasing with increasing pooling. This pooling technique involves the collection of one swab per sample area, that are pooled in the laboratory for analysis. The other big disadvantage of pooling is the possibility that an inhibitor present on one of the swabs invalidates the results of all the swabs due to pooling.

Other techniques that have been suggested to increase effective surface area per swab include re-using the same swab on multiple surfaces. This approach needs a very careful examination of the validation data supporting the process, as swab capture efficiency is typically validated for a single swab, and extraction efficiency of the swab will most likely change with each swabbing as the amount of liquid on the swab changes. This approach also increases the likelihood of spreading potentially infectious material across multiple spaces, and hence need to be considered very carefully before adoption.

ARE THERE INTERFERENCES IN RT-qPCR ANALYSIS? HOW CAN THEY BE MITIGATED?

RT-qPCR analysis, while being the definitive test for the presence of SARS-Cov-2, can be affected by interferences and inhibitors. A PCR inhibitor prevents the amplification of nucleic acids and affects PCR through interaction with DNA or interference with the DNA polymerase. RT-qPCR can also be affected by inhibition of the fluorescence reaction. So, in the presence of an inhibitor, the viral RNA present in a sample does not magnify, resulting in a false negative result. There have been multiple inhibitors identified in PCR analysis of environmental samples including metal ions such as calcium and iron, metallic iron, debris, fulvic acids, humic materials and polyphenol.

The best approach to mitigation involves using the RT-qPCR test after cleaning to ensure that a clean
surface is being tested. Most common disinfection agents such as bleach, hydrogen peroxide and quaternary ammonium agents are not expected to interfere with the test. Communication on the location/surface type of the sample tested, and information on the type of cleaning and disinfection agents used will help in understanding and troubleshooting issues that arise. Specific sample preparation techniques such as the use of magnetic beads for nucleic acid isolation can also greatly reduce inhibition potential.

Sample specific inhibition can be detected using internal positive controls. A synthetic oligo/plasmid target is added to each sample and is processed along with the target RNA. Inhibitors present in the sample also inhibit the amplification of the positive control, so a failure of the positive control in a negative sample will indicate the presence of an inhibitor, or some other issue with the process. A failure in the internal control for a positive test does not invalidate the result as a positive test is still considered definitive evidence of the presence of viral RNA.
ATP Testing

SUMMARY

ATP, or Adenosine Triphosphate is the energy molecule found in the cells of all living organisms. Therefore, the presence of ATP on a surface can be used as a metric of cleaning efficiency. If cleaning has been properly executed and is adequate to remove ATP, it is consequently considered a reasonable proxy for removal of human-borne microbial matter. ATP testing can only be an indirect measure of cleaning efficiency and not as a marker for viral presence, as viruses do not use ATP for metabolism. Also, the presence of ATP does not mean the presence of virus. It can only mean the presence of cellular matter from living organisms.

FREQUENTLY ASKED QUESTIONS

HOW IS ATP MEASURED?

ATP is measured using the light produced through its reaction with the firefly enzyme Luciferase.

The amount of light produced is read using a device called a Luminometer and is directly proportional to the amount of ATP present in the sample.

WHAT IS THE ADVANTAGE OF USING ATP TESTING FOR CLEANING?

ATP testing is suited for validation of cleaning due to its speed. Depending on the equipment used, a surface can be swabbed and results available in a few minutes, with the extraction and data generation steps taking a minute. As ATP tests for living matter, it is a quick indicator for the effectiveness of cleaning.

WHAT ARE THE DISADVANTAGES OF USING ATP TESTING?

ATP testing is an indirect measure of cleaning as it is not testing for viral presence or load. ATP testing is also subject to interferences from disinfection agents, and other sources.

So, ATP testing is to only be carried out prior to disinfection, by rinsing off disinfection agents prior to testing, or by validating the use with disinfectants (only recommended with 2-step ATP). The Indoor Air Quality Association of Australia (IAQAA) in its interim guidance on cleaning validations post Covid-19 (6) cleaning recommends a minimum of 5%, or 1 per surface confirmation by microscopic lift testing and visual debris analysis.
ATP Testing

WHAT ARE THE OPTIONS FOR MEASURING ATP?
There are two field-based ATP testing options.

1-STEP SEMI-QUANTITATIVE TESTING
This is the most commonly used ATP test and is available from multiple vendors. SGS uses the Hygiena Ensure platform and the Supersnap swabs for maximum sensitivity and best reproducibility. In this test, pre-moistened swab is swabbed on a pre-determined surface area (5 x 5, or 10 x 10 cm²). The swab is then activated in a solution that both extracts and reacts the ATP with luciferase. The activated swab is inserted into the luminometer, which provides a reading in Relative Light Units (RLU). The higher the RLU, the higher the ATP measured. This test is considered semi-quantitative as the reading is relative to the particular unit used. This video from Hygiena demonstrates the use of the test on surfaces.

2-STEP QUANTITATIVE TESTING
This test, available through Luminultra, uses the same principles for ATP measurement, but separates the extraction portion of the ATP test from the luciferase reaction portion. This separation enables optimization of both steps of the process, and quantitative measurement as a standard of known ATP concentration is used to calibrate the instrument. So, the luminometer readout in RLU can be converted into a concentration (pg/cm²) using the calibration standard, enabling a direct comparison against a concentration-based threshold. This video from Luminultra demonstrates the use of the test on surfaces. The two-step test has several advantages over the one-step tests especially in more complex matrices such as wastewater, ballast water, etc. due to the use of a more comprehensive extraction step and a calibrated quantitative result. For cleaning validation protocols applicable across multiple sites and locations, and based on a standardized ATP concentration threshold, quantitative

HOW DO THESE TWO ATP TESTS COMPARE?

<table>
<thead>
<tr>
<th></th>
<th>1-STEP SEMI-QUANTITATIVE</th>
<th>2-STEP QUANTITATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of test</td>
<td>1-2 minutes per sample</td>
<td>10 minutes per sample</td>
</tr>
<tr>
<td>Ease of test</td>
<td>Very easy</td>
<td>Easy: Requires pipetting and transfer steps</td>
</tr>
<tr>
<td>Measurement</td>
<td>Relative light units, semi-quantitative</td>
<td>Concentration, calibrated and quantitative</td>
</tr>
<tr>
<td>Completeness of extraction</td>
<td>Potentially lower, as both extraction and enzyme activation are combined. In cleaner environments and surface testing, it is considered adequate.</td>
<td>High, as extraction buffer is separate from enzymatic step and optimized</td>
</tr>
<tr>
<td>Interference from disinfectants (See discussion)</td>
<td>High potential for interference, so testing must always be conducted before disinfection. See (5) for data on the effects of disinfectants on common ATP tests used in the field (does not include the 2-step quantitative test from Luminultra)</td>
<td>Low potential for interference Test is more resistant to bleach and many other common disinfectants, hence is less prone to interference. However, there is not definitive data proving lack of interference against all disinfection agents when used in surface testing. But, interference potential can be validated in the field</td>
</tr>
<tr>
<td>Confirmation using microscopic lift testing</td>
<td>At a 5% sampling rate to confirm cleanliness</td>
<td>Not determined. So, same recommendation to confirm at a 5% rate will apply</td>
</tr>
<tr>
<td>Cost</td>
<td>Consumables are $3-5 per sample</td>
<td>Consumables are $10-12 per sample</td>
</tr>
</tbody>
</table>
ATP Testing

HOW DO DISINFECTION PRODUCTS INTERACT WITH ATP TESTING?

The underlying technology of 1-step and 2-step ATP testing are the same, that is, the reaction of luciferase with ATP to produce light that is then read by a luminometer. So, the underlying interference by any disinfectant on the process is the same, it either interferes with the luciferase or hydrolyzes ATP so fast to ADP (this is the process that makes energy in cells) that it cannot react with the luciferase.

The biggest difference between 1st and 2nd Gen ATP is the 10-fold dilution step prior to reaction, which means all things being equal, which is a reasonable assumption, the 2-step Luminultra is 10 times less vulnerable to disinfection agents than 1st generation. Initial assessment indicates that second generation should be free of interference from bleach, peroxide and isopropanol, but there are significant uncertainties around other disinfectants such as quaternary products, phenols and citric acid.

A simple test of compatibility with the disinfectant and an ATP standard using the protocol from a disinfectant study reference (1) can be carried out to confirm compatibility. If this is done to confirm the disinfectant as fit for use, there is much higher certainty on the use of the ATP test after disinfection. The test should not take more than half an hour per disinfectant used and can be performed prior to validating a disinfectant for use in a facility or set of facilities, and in conjunction with calibration and instrument suitability checks.

AVAILABLE DATA

Omidbakhsh and Kenny (1) performed a comprehensive review of the abilities and limitations of the first generation 1-step ATP, including testing of quenching by disinfectants. This is how the test was done:

“Any chemical interference through quenching or enhancement of bioluminescence was tested by placing 10 µL of the appropriate dilution of ATP standard solution onto the tip of a swab followed by placement of 10 µL of the test disinfectant. The baseline ATP solution concentrations used above were individually determined for each of the luminometers, selecting the aliquot with ATP concentration that fell between the ATP meters’ true maximum and minimum detection limits based on their obtained linearity standard curves. Also, the volume of dispensed disinfectant on the swabs, 10 µL, was determined by testing the average volume of water required to keep 50% of a 10 cm x 10 cm hard non-porous surface (a typical surface area dimension recommended by ATP meter manufacturers to be swabbed) wet for 3 minutes. The calculated average volume required was 80 µL in ambient room temperatures. This volume was reduced to 10 µL to compensate for the evaporation of the volatile ingredients. To account for the repeatability of the results, all the tests have been performed in triplicates.”
## ATP Testing

Results of 1-step ATP disinfectant interference testing. The higher the number, worse the performance. Those with worse than 30% suppression marked in grey.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>ACTIVE INGREDIENT</th>
<th>KIKKOMAN</th>
<th>3M</th>
<th>HYGIENA</th>
<th>CHARM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaviCide</td>
<td>Isopropyl alcohol, 17.2%; 2-butoxyethanol, 1–5%; Diisobutyl – phenoxo-ethoxy-ethyl-dimethyl-benzyl ammonium chloride, 0.28%</td>
<td>23.2%</td>
<td>83.7%</td>
<td>62.8%</td>
<td>31.6%</td>
</tr>
<tr>
<td>PCS 1000</td>
<td>Sodium hypochlorite, 0.1%</td>
<td>15.3%</td>
<td>53.5%</td>
<td>12.9%</td>
<td>13.7%</td>
</tr>
<tr>
<td>Sani-Cloth Plus</td>
<td>Isopropanol, 10–20%; 2-butoxyethanol, 1–4%; Benzyl-C12–18-alkyldimethylammonium chlorides &lt;0.125%, C12–18-alkyl[(ethylphenyl)methyl] dimethyl chlorides, &lt;0.125%</td>
<td>13.2%</td>
<td>52.2%</td>
<td>12.1%</td>
<td>14.8%</td>
</tr>
<tr>
<td>Accel TB</td>
<td>Hydrogen peroxide, 0.5%</td>
<td>37.1%</td>
<td>65.7%</td>
<td>51.4%</td>
<td>44.5%</td>
</tr>
<tr>
<td>CleanCide</td>
<td>Citric acid, 0.6%</td>
<td>99.9%</td>
<td>89.6%</td>
<td>99.9%</td>
<td>-0.4%</td>
</tr>
<tr>
<td>Clorox Hydrogen Peroxide Wipes</td>
<td>Hydrogen peroxide, 1.4%;</td>
<td>51.4%</td>
<td>78.0%</td>
<td>92.8%</td>
<td>55.3%</td>
</tr>
<tr>
<td>Clorox Clean-up disinfectant</td>
<td>Sodium hypochlorite, 1.84%</td>
<td>26.3%</td>
<td>93.5%</td>
<td>60.4%</td>
<td>77.0%</td>
</tr>
<tr>
<td>IPA, 70%</td>
<td>Isopropyl alcohol, 70% v/v</td>
<td>32.3%</td>
<td>32.2%</td>
<td>22.1%</td>
<td>24.2%</td>
</tr>
<tr>
<td>0.5% H2O2</td>
<td>Hydrogen peroxide, 0.5% w/w</td>
<td>-0.1%</td>
<td>-6.8%</td>
<td>5.4%</td>
<td>-2.8%</td>
</tr>
<tr>
<td>Ultra Clorox Bleach (1:10)</td>
<td>Sodium hypochlorite, 5–8%</td>
<td>-0.2%</td>
<td>91.2%</td>
<td>27.4%</td>
<td>45.3%</td>
</tr>
<tr>
<td>Accel PREVention RTU</td>
<td>Hydrogen peroxide, 0.5%</td>
<td>-5.7%</td>
<td>48.3%</td>
<td>36.1%</td>
<td>-38.8%</td>
</tr>
<tr>
<td>Virox 5 RTU</td>
<td>Hydrogen peroxide, 0.5%</td>
<td>2.7%</td>
<td>23.3%</td>
<td>30.7%</td>
<td>-17.9%</td>
</tr>
<tr>
<td>BTC 50 (1:125)</td>
<td>Alkyl dimethyl benzyl ammonium chloride(C12–18) 50–51.5%, Ethanol 5–5.5%</td>
<td>14.8%</td>
<td>9.5%</td>
<td>9.5%</td>
<td>19.3%</td>
</tr>
<tr>
<td>Sporicidin</td>
<td>Phenol, 1.58%, sodium phenate, 0.06%</td>
<td>95.2%</td>
<td>40.1%</td>
<td>99.1%</td>
<td>80.2%</td>
</tr>
</tbody>
</table>
Results from testing of 2-step ATP disinfectant interference testing. 
Note, this testing was carried out in water containing the active ingredient at the concentration listed below.

<table>
<thead>
<tr>
<th>BIOCIDE (PPM ACTIVE INGREDIENT)</th>
<th>SUPPRESSION (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isothiazolone (CMIT/MIT, 1000ppm Active Ingredient)</td>
<td>0%</td>
</tr>
<tr>
<td>Methylene bis(thiocyanate) (MBT, 1000ppm Active Ingredient)</td>
<td>3%</td>
</tr>
<tr>
<td>DBNPA (1000ppm Active Ingredient)</td>
<td>4%</td>
</tr>
<tr>
<td>Bronopol (1000ppm Active Ingredient)</td>
<td>2%</td>
</tr>
<tr>
<td>Phenol (100mg/L Active Ingredient)</td>
<td>11%</td>
</tr>
<tr>
<td>Sodium Hypochlorite (15mg/L FAC)</td>
<td>12%</td>
</tr>
<tr>
<td>Hydrogen Peroxide (200 mg/L)</td>
<td>0</td>
</tr>
</tbody>
</table>
Microscopic Lift Testing

SUMMARY

SGS will provide surface lift kits and perform indirect surface microscopic analysis for surface debris and other evidence of incomplete cleaning in accordance to ASTM D7910:14 - Standard Practice for Collection of Fungal Material from Surfaces by Tape Lift(7). Developed by SGS Forensic Laboratories, the JF2000 Sampling Kits are a unique and innovative approach to particulate sampling and analysis. The JF2000 Sampling Kits contain five ready-for-use microscope slides prepared with a specially formulated transparent tape used to assist industrial hygienists in effectively identifying dust components during an IAQ evaluation survey.

The IAQA cleaning guidance recommends the use of confirmatory microscopic lift testing, especially when ATP screening using a traditional test such as the Hygiena ultrasnap is performed. The guidance recommends a 1 in 20 confirmation sample rate of microscopic testing for every traditional ATP test.

Lift testing will look for epithelial/skin cells as a marker for cleaning, and limited fibre analysis.

REFERENCES

Whether you are looking for COVID-19 surface cleanliness testing, passive or active sampling of disinfectants, portable or continuous area instruments, or indoor air quality analysis, we have the services and equipment Industrial Hygienists need to make the COVID-19 recovery process smooth.

**COVID-19 MICROBIOLOGY: SAMPLING & ANALYSIS (SURFACE CLEANLINESS)**

**RT-qPCR Testing**
In partnership with Dr. James Scott and Sporometrics, SGS provides environmental sampling kits with RNA-preservation and RT-qPCR lab-based testing for the presence of SARS-CoV-2. This validated method provides the highest level of confidence for validation of surface decontamination.

**ATP Screening – Semi-quantitative**
Our Hygiena Ensure Luminometer with the Supersnap Test Kit provides a quick and sensitive screening level estimate of ATP on a surface using ATP equipment that is familiar to most industry professionals.

**ATP Testing – Quantitative 2nd Generation**
Our premium, 2nd-generation ATP test from Luminultra ensures high-quality, quantitative ATP field results (pg/cm2) while maximizing ATP extraction efficiency and minimizing interference from cleaning and disinfection agents.

**Microscopic Lift Testing**
Our JF2000 Sampling Kits provide a unique approach to particulate sampling and analysis. Each kit contains 5 ready-for-use microscope slides prepared with a specially formulated transparent tape to effectively identify dust components during an IAQ evaluation survey.

**DISINFECTANTS: SAMPLING & ANALYSIS (AIR: PERSONAL & AREA)**

**AMMONIA**
- Passive Sampling (Area/Personal) Mod. OSHA ID-188/ID-164; ISE
- Active Sampling (Area/Personal) Mod. OSHA ID-188/ID-164; ISE
- Portable Instruments (Area/Personal) ToxiRae Pro Single Gas
- Continuous Area Monitors (Area) RKI GD-70D-NH3
- Web-Based Continuous Area Monitors (Area) Smart Sense RKI NH3

**CHLORINE**
- Active Sampling (Area/Personal) Mod. NIOSH 6011; IC
- Portable Instruments (Area) ToxiRae Pro Single Gas
- Continuous Area Monitors (Area) RKI GD-70D-CL2
- Web-Based Continuous Area Monitors (Area) Smart Sense RKI CL2

**CHLORINE DIOXIDE**
- Active Sampling (Area/Personal) Mod. OSHA ID-202; IC
- Portable Instruments (Area) ToxiRae Pro Single Gas
- Continuous Area Monitors (Area) RKI GD-70D-CLO2
- Web-Based Continuous Area Monitors (Area) Smart Sense RKI CLO2
### DISINFECTANTS: SAMPLING & ANALYSIS (AIR: PERSONAL & AREA) continued

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Passive Sampling (Area/Personal)</th>
<th>Active Sampling (Area/Personal)</th>
<th>Active Sampling (Area/Personal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETHYL ALCOHOL</td>
<td>Mod. NIOSH 1400; GC/FID BADGE</td>
<td>Mod. NIOSH 1400; GC/FID</td>
<td>Mod. OSHA 5001; GC/FI</td>
</tr>
<tr>
<td>FORMALDEHYDE</td>
<td>Mod. OSHA 1007; HPLC/UV</td>
<td>Mod. NIOSH 2016; HPLC/UV</td>
<td></td>
</tr>
<tr>
<td>HYDROCHLORIC ACID</td>
<td>Mod. NIOSH 7907</td>
<td>Mod. OSHA ID-165SG; IC</td>
<td></td>
</tr>
<tr>
<td>HYDROGEN PEROXIDE</td>
<td>Mod. OSHA VI-6; Colorimetric</td>
<td>Mod. OSHA 1019; Colorimetric</td>
<td></td>
</tr>
<tr>
<td>ISOPROPYL ALCOHOL</td>
<td>Mod. NIOSH 1400; GC/FID BADGE</td>
<td>Mod. NIOSH 1400; GC/FID</td>
<td></td>
</tr>
<tr>
<td>HYDROGEN PEROXIDE</td>
<td>Mod. OSHA 1019; Colorimetric</td>
<td>Mod. OSHA ID-165SG; IC</td>
<td></td>
</tr>
</tbody>
</table>

### WATER QUALITY TESTING

Buildings that have been closed for extended periods of time, can have water quality issues around stagnation and contaminant build-up. SGS offers a comprehensive set of services including lead, legionella, and other critical water quality parameters.

- **Lead, Legionella**

SGS is the world’s leading inspection, verification, testing and certification company. We are recognized as the global benchmark for quality and integrity. With more than 94,000 employees, we operate a network of more than 2,600 offices and laboratories around the world.

**TO FIND OUT MORE ABOUT ENVIRONMENT, HEALTH & SAFETY SERVICES CONTACT**

EHS.CLIENTCARE@SGS.COM OR VISIT
WWW.SGS.COM/EHSNAM

© SGS North America Inc - 2020 – All rights reserved - SGS is a registered trademark of SGS Group Management SA.