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WHITE PAPER

COVID-19, FLU A, FLU B, & RSV
COLLECTED SALIVA 10-SECOND VIRAL INACTIVATION

The creation, stabilization, and preservation of a robust and safe biomaterial used in clinical diagnostic and screen-based testing.

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EXECUTIVE SUMMARY

Pioneering a new era of at-home specimen self-collection for viral infections, an independent study has demonstrated the greater than or equal to 99.99% neutralization of the SARS-CoV-2 (COVID-19), Flu A (H1N1), Flu B, and HRSV (Human Respiratory Syncytial Virus) live viruses in 10 seconds using Spectrum Solutions' patented preservation agent at room temperature.

Until recently, industry "gold-standard" upper respiratory viral testing methods depended on the nasopharyngeal (NP) swab using the extended nasal cavity and the oropharyngeal (OP) swab that uses the throat for specimen sample collection. These specimen collection methods both require the assistance of a healthcare professional to be performed correctly and minimize patient discomfort. According to the Center of Disease Control (CDC) NP and OP specimens are not appropriate for self-collection[1]. In contrast, saliva sample collection using Spectrum's SDNA device has proven to be easier and more comfortable for patients through incorporating a more simple, noninvasive sample self-collection protocol. This whole saliva collection method benefits from needing no additional collection supplies and doesn't require any direct interaction form healthcare workers.

Saliva collection reduces the need for masks, gowns, gloves, and other personal protective equipment (PPE) that would be necessary for health care professionals to collect other sample types. In mid-April (2020), the FDA granted emergency authorization for the first saliva-based test exclusively using the Spectrum SDNA Saliva Collection

Devices and patented CV3 preservation chemistry. The SDNA is non-invasive and easy to use intended for the self-collection of saliva samples with weeks of extended in-device post-collection biosample stability, storage, and sample transport all at room temperature.

2022 testing performed by an independent third-party laboratory, evaluated the virucidal properties of six replicates of each from 3 lots when challenged with severe acute respiratory syndrome related Coronavirus 2 (SARS-CoV-2), Flu A H1n1, Flu B, and Human Respiratory Syncytial Virus (RSV). Under the conditions of this viral inactivation evaluation samples were plated in quadruplicate. Key take aways from this evaluation demonstrated successful viral inactivation within 10 seconds of chemistry exposure and maintained its inactivation status when tested again 60 minutes later. Key study take-aways are illustrated in the highlighted Log Reduction per sample below in Table 1.

Table 1	Virus Control	Log_{10} Reduction	Average Log_{10} Reduction	Average Percent Reduction	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control
SARS-CoV-2	0000 6.50	≥ 4.00	≥ 4.00	≥ 99.99%	5.75	6.00	≤ 2.50
FLU A H1N1	8.00	≥ 5.50	≥ 5.50	≥ 99.99%	7.75	7.75	≤ 2.50
FLU B	6.50	≥ 4.00	≥ 4.00	≥ 99.99%	6.00	6.00	≤ 2.50
HRSV	6.50	≥ 4.00	≥ 4.00	≥ 99.99%	5.75	6.25	≤ 2.50

[1] https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

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THE PROBLEM

The risk of undo exposure to active viral loads when collecting, transporting, and processing biospecimens represents a serious containment vulnerability, which carries across healthcare teams[2] and into the community. During the pandemic, it became abundantly clear that current protocols did little to mitigate unnecessary exposure to the active virus during collection and were heavily dependent on already critically low supplies, which created a major strain on supply chains. As the restrictions began to ease and we entered the endemic, the new normal of self-collection presented further risk with spike in respiratory viruses, such as influenza and RSV. It is critical that we promote a method to inactivate infectious material immediately upon collection, which in turn will provide piece of mind to all individuals handling of the sample that they are not being exposed, while also lowering costly logistics for hazardous shipments and time-consuming decontamination processes upon arrival at the laboratory. The method to achieving this must not sacrifice the quality of the molecular material and allow for both quantitative and qualitative readouts.

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THE BACKGROUND

COVID-19, Flu A, Flu B, or RSV viruses inactive and preserves the viral RNA for transport to a reference laboratory for molecular analysis.

Upon arrival at the laboratory, the viral RNA is extracted from the saliva sample. Independent studies[3,4] have shown when using saliva for molecular analysis the essential step of extraction and purification delivers the needed sensitivity boost required for optimal accuracy. The viral RNA was subjected to a semi-quantitative multiplex RT-PCR test to qualitatively identify three independent viral transcripts used to determine whether a patient is actively infected and in danger of potentially posing a risk of infection to others.

Given the scientific, safety, and experiential advantages to saliva collection for COVID-19, Flu A, Flu B, or RSV, it is also important to ensure that the potentially infectious material provided by any given patient is rendered safe for downstream sample transport and handling once it arrives at the laboratory. Currently, all swab collections are placed in viral or universal transfer media that supports an environment where any infectious virus retains its potential to infect those handling the sample; this is also a concern for dry swabs and unpreserved saliva as SARS-Cov-2 is a very robust virus. In contrast, saliva collection using the SDNA and patented CV3 preservation solution renders any infectious COVID-19, Flu A, Flu B, or RSV viruses completely inactive allowing for a safer laboratory experience and a more robust direct-to-bench sampling and extracting process.

[2] Hazard Recognition published by US Dept of Labor, Occupational Safety and Health Administration. Available at https://www.osha.gov/coronavirus/hazards

[3] Sensitivity of SalivaDirect compared to saliva tested using a standard qRT-PCR assay. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836249/

[4] Challenges in use of saliva for detection of SARS CoV-2 RNA in symptomatic outpatients. Objective: To compare SARS CoV-2 positivity on paired NPS and saliva samples. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7392849/

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THE SDNA-CV3 STUDY

2020 Study Parameters

This white paper describes a series of studies that support the above viral inactivation claims. Viral inactivation in 2020 was determined by measuring cytopathic effect (CPE) focused solely on SARS-CoV-2. New 2022 study data performed by an independent testing laboratory includes not only SARS-CoV-2 but Flu A, Flu B, and RSV viral inactivation findings.

Virus activity and infection are measured by evaluating a primary clinical sample in the context of a feeder layer of cells which simulates an environment that would support viral infection in humans. In order to perform these types of studies an intact and replication competent virus is cultured and used for experimentation in a BSL3 laboratory environment. The virus is exposed to the SDNA-CV3 preservation chemistry to simulate a clinical saliva sample collection. The approached used for removing any cellular toxic components in the preservation agent was published (Burton et al., 2017) and was validated herein as an effective approach to measure virus activity.

The virus was then cultured and added to either media with no preservation agent (experimental control) or the SDNA-CV3 preservation agent. In addition, media and preservation agent were tested without the addition of live virus as additional controls. Virus were added to both media and preservation agent to simulate an active infection to test the ability of the preservation chemistry to inactivate virus in the most highly infectious conditions. Once the samples were prepared each condition was either subject to filtration (to remove any cell growth inhibition components) or applied neat to the Vero cell cultures in a series of limiting dilutions. Once the cultures were treated with the dialyzed and neat sample conditions (virus alone, virus+media/saliva, virus+SDNA-CV3 preservation agent) the cells were cultured for 72-hours and subjected to both CPE and RT-PCR analysis. Following the first analysis, cells were passaged and retested 72-hours later simulating a time course similar to a persistent infection environment. All cultures were tested with both analyses at the conclusion of that second time point.

	Primary Culture	Passaged Culture Results
CPE		
SARS-2/SDNA-1000/Amicon filtration	No CPE (no effect of lysis buffer or virus on cell sheet)	No CPE (no effect of lysis buffer or virus on cell sheet)
SARS-2/PBS/Amicon filtration	CPE +++ through 10^-3 (infectious sample)	CPE +++ through 10^-3.5 (infectious sample)
SARS-2/no Amicon filtration (control)	CPE+++ through 10^-3 (infectious sample)	CPE+++ through 10^-3.5 (infectious sample)
BA (-) Saliva/SDNA-1000/Amicon filtration	No CPE	No CPE
SARS-2/SDNA-1000/no Amicon filtration	cell sheet dead at <10^-2-3 (lysis buffer kills cells)	cell sheet dead at <10^-1 (lysis buffer kills cells)
RT-PCR		
SARS-2/SDNA-1000/Amicon filtration (10^0 dilution day 0)	Ct= 25	Ct = ND
SARS-2/PBS/Amicon filtration (10^0 dilution day 0)	Ct = 14	Ct = 17
SARS-2/SDNA-1000/Amicon filtration (10^0 dilution day 3)	Ct = 32	Ct = ND
SARS-2/SDNA-1000/Amicon filtration (10^0 dilution passage 1 d3)	Ct = 33	Ct = ND

CPE analysis is a measurement of structural changes to host cells that are caused by viral infection. The infection can cause lysis of host cells due to the cells inability to reproduce as a function of viral infection. Both of these outcomes are considered CPE and were scored manually by a pathological review of each culture. RT-PCR analysis is a measurement of viral RNA transcripts in a given sample. The process for this analysis requires the lysis of virus in the sample followed by RNA extraction. The RNA can then be measured qualitatively and in some instance quantitatively (via qPCR) to assess whether the sample in question has been exposed to and is infected by COVID-19, Flu A, Flu B, or RSV. When combined these

measurements provide a complete and sensitive assessment of viral activity and infectivity as a function of sample collection scenarios.

2022 Study Parameters

This study, performed by an independent third-party laboratory, evaluated virucidal properties of six replicates from 3 lots when challenged with Severe Acute Respiratory Syndrome related Coronavirus 2 (SARS-CoV-2), Flu A H1n1, Flu B, and Human Respiratory Syncytial Virus (RSV). The testing was based upon ASTM El052-20, Standard Practice to Assess the Activity of Microbicides against Viruses in Suspension. All testing was performed in accordance with Food and Drug Administration Good Laboratory Practices, as specified in 21 CFR Part 58. Characterization of the identity, strength, purity, composition, stability, and solubility of the test articles remained the responsibility of Spectrum Solutions and was not performed by the testing facility (GLP 58.105).

In addition, third-party testing laboratory quality assurance conducted in-phase audits of the critical test procedures over the course of testing and on completion of testing, QA performed an audit of the raw data and of the final report, in its entirety.

LEGEND	
+	CPE Present
0	CPE Not Detected
NT	Not Tested
n/a	Not Applicable
Rep.	Replicate

Table 1: Test SARS-CoV-2 Viral Inactivation Data

(Cell Control: 0000)	10 sec.	60 min.	Dilution (- Log_{10})	Virus Control	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control
Rep 1	0000	0000	-3	++++	NT	NT	0000
Rep 2	0000	0000	-4	++++	++++	++++	0000
Rep 3	0000	0000	-5	++++	++++	++++	0000
Rep 4	0000	0000	-6	0+++	0+00	00+0	NT
Rep 5	0000	0000	-7	+000	0000	0+00	NT
Rep 6	0000	0000	-8	0000	0000	0000	NT
$TCID_{50}$ (Log_{10})	≤ 2.50			6.50	5.75	6.00	≤ 2.50
Log10 Reduction	≥ 4.00						
Average Log_{10} Reduction	≥ 4.00						
Percent Reduction	≥ 99.99%						
Average Percent Reduction	≥ 99.99%						

Under the conditions of this evaluation the patented preservation chemistry reduced the infectivity of the SARS-CoV-2, Omicron variant, by an average of ≥4.00 log10 (≥99.99%) following a 10-second exposure and by an average of ≥4.00 log10 (≥99.99%) following a 60-minute exposure.

Table 2: Test FLU A H1N1 Viral Inactivation Data

(Cell Control: 0000)	10 sec.	60 min.	Dilution (- Log_{10})	Virus Control	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control
Rep 1	0000	0000	-3	++++	NT	NT	0000
Rep 2	0000	0000	-4	++++	++++	++++	0000
Rep 3	0000	0000	-5	++++	++++	++++	0000
Rep 4	0000	0000	-6	0+++	0+00	00+0	NT
Rep 5	0000	0000	-7	+00	0000	0+00	NT
Rep 6	0000	0000	-8	0	0000	0000	NT
$TCID_{50}$ (Log_{10})	≤ 2.50			8.00	7.75	7.75	≤ 2.50
Log_{10} Reduction	≥ 5.50						
Average Log_{10} Reduction	≥ 5.50						
Percent Reduction	≥ 99.99%						
Average Percent Reduction	≥ 99.99%						

Under the conditions of this evaluation, the patented CV3 preservation chemistry reduced the infectivity Influenza A H1N1 strain by ≥ 5.50 log10 (≥ 99.99%) following a 10-second exposure and by ≥ 5.50 log10 (≥ 99.99%) following a 60-minute exposure.

Table 3: Test FLU B Viral Inactivation Data

(Cell Control: 0000)	10 sec.	60 min.	Dilution (- Log_{10})	Virus Control	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control
Rep 1	0000	0000	-3	++++	NT	NT	0000
Rep 2	0000	0000	-4	++++	++++	++++	0000
Rep 3	0000	0000	-5	++++	++++	++++	0000
Rep 4	0000	0000	-6	0+++	0+00	00+0	NT
Rep 5	0000	0000	-7	+00	0000	0+00	NT
Rep 6	0000	0000	-8	0	0000	0000	NT
$TCID_{50}$ (Log_{10})	≤ 2.50			6.50	6.00	6.00	≤ 2.50
Log_{10} Reduction	≥ 4.00						
Average Log_{10} Reduction	≥ 4.00						
Percent Reduction	≥ 99.99%						
Average Percent Reduction	≥ 99.99%						

Under the conditions of this evaluation the patented CV3 preservation chemistry reduced the infectivity of Influenza B strain by ≥ 4.00 log10 (≥ 99.99%) following a 10-second exposure and by ≥ 4.00 log10 (≥ 99.99%) following a 60-minute exposure.

Table 4: Test HRSV Viral Inactivation Data

(Cell Control: 0000)	10 sec.	60 min.	Dilution (- Log_{10})	Virus Control	Neutralization Control	Neutralizer Toxicity Control	Cytotoxicity Control
Rep 1	0000	0000	-3	++++	NT	NT	0000
Rep 2	0000	0000	-4	++++	++++	++++	0000
Rep 3	0000	0000	-5	++++	++++	++++	0000
Rep 4	0000	0000	-6	0+++	0+00	00+0	NT
Rep 5	0000	0000	-7	+00	0000	0+00	NT
Rep 6	0000	0000	-8	0	0000	0000	NT
$TCID_{50}$ (Log_{10})	≤ 2.50			6.50	5.75	6.25	≤ 2.50
Log_{10} Reduction	≥ 4.00						
Average Log_{10} Reduction	≥ 4.00						
Percent Reduction	≥ 99.99%						
Average Percent Reduction	≥ 99.99%						

Under the conditions of this evaluation the patented CV3 preservation chemistry reduced the infectivity of Human Respiratory Syncytial Virus strain by ≥ 4.00 log10 (≥ 99.99%) following a 10-second exposure and by ≥ 4.00 log10 (≥ 99.99%) following a 60-minute exposure.

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THE CONCLUSION

Under the conditions of this evaluation with samples plated in quadruplicate, the patented preservation chemistry reduced the infectivity of the SARS-CoV-2, Omicron variant, by an average of ≥4.00 log10 (≥99.99%) following a 10-second exposure and by an average of ≥ 4.00 log10 (≥99.99%) following a 60-minute exposure. Test results for the Influenza A H1N1 strain reduced the infectivity by ≥ 5.50 log10 (≥ 99.99%) following a 10-second exposure and by ≥ 5.50 log10 (≥ 99.99%) following a 60-minute exposure. For Influenza B, test results reduced the infectivity of the strain by ≥ 4.00 log10 (≥ 99.99%) following a 10-second exposure and by ≥ 4.00 log10 (≥ 99.99%) following a 60-minute exposure. Results from testing the Human Respiratory Syncytial Virus strain demonstrated a reduced infectivity by ≥ 4.00 log10 (≥ 99.99%) following a 10-second exposure and by ≥ 4.00 log10 (≥ 99.99%) following a 60-minute exposure (see Table 1 in Executive Summary).

Results of this study successfully concluded no evidence of viral growth in presence of SDNA-CV3 lysis buffer by either CPE read out or RT-PCR. The complete lack of CPE in any sample mixed with SDNA-CV3 lysis buffer demonstrates a greater than 6-log order reduction in viral activity in Vero cultured cells. Additionally, the lack of viral load increase (as measured by RT-PCR) across several days of cell culture indicates that there is no viral growth or infection following exposure to the SDNA preservation agent. It was confirmed that the SDNA-CV3 preservation agent itself is toxic to feeder cells so dialysis of buffer components was required to perform viral inactivation studies. PBS/media/saliva controls that were spiked with live virus retained both infectivity as measured by CPE and RT-PCR following the same dialysis procedure that was used to remove any cellular toxic components in the preservation agent. This data supports the complete inactivation of the COVID-19, Flu A, Flu B, or RSV viruses in the presence of the SDNA-CV3 patented preservation chemistry in 10 seconds at room temperature.

The inactivation of the virus in the SDNA Saliva Collection Devices creates the most robust and safest biomaterial collection approach for the detection of COVID-19, Flu A, Flu B, or RSV infections and leads the way to a new era of at-home biosample self-collection for the diagnosis of viral infections.

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THE BENEFITS

There are several advantages to using saliva collected with an SDNA Saliva Collection Device as the primary source of COVID-19, Flu A, Flu B, or RSV detection for molecular analysis. The following summation highlights the key benefits.

First, the pain-free SDNA saliva collection system mitigates all risk of infection to those individuals administering the test since it does not require the close contact with healthcare professional like nasopharyngeal swab-based collection does as well as additionally safeguarding those professionals downstream with testing sample handling and processing applications. At the start of the pandemic, the nation experienced a global shortage of both testing supplies and the personal protective equipment (PPE) required for those collections. COVID-19 testing using Spectrum's saliva collection device with the inactivation of the live virus delivered a greater than 90% reduction in the use/need for PPE. A successful testing benefit back then delivering direct global relief that's expanded today to proactively include Flu A, Flu B, and RSV.

Second, it safely expands access to testing for more populations. We witnessed how supply shortages created limited and priority-based testing scenarios that threatened many vulnerable population segments. According to a 2021 National Institute of Health (NIH) report by Emily Savela, SARS-CoV-2 RNA first appears in saliva and high-sensitivity saliva testing can detect an infection up to 4.5 days before viral loads in nasal swabs

are able to reach the limit of detection. The published report also states how the early detection of SARS-CoV-2 infection is critical to reduce the asymptomatic and pre-symptomatic spread of COVID-19. Detection requires access to repeat testing. The non-invasive self-collection of salvia using a Spectrum SDNA device delivers the at-home testing opportunity direct to a patient's door and then safely back to the laboratory for processing. In addition, the SDNA system removes the need for any temperature-controlled storage or transport of saliva samples and offers weeks of safe post-collection sample stability for COVID-19, Flu A, Flu B, or RSV viral testing.

Third, saliva is a more robust biomaterial to facilitate molecular testing. There is less sample variability using the SDNA for collecting saliva while rendering maximum sensitivity and optimal testing accuracy.

Lastly, using an SDNA device renders any infectious COVID-19, Flu A, Flu B, or RSV virus completely inactive. This offers a better, pain-free patient experience when compared to invasive swab sample collections, the SDNA device additionally provides for a safer laboratory experience as well. Viral inactivation at ambient temperatures significantly reduces the time spent in a laminar flow cabinet and ultimately increases lab process efficiencies facilitating the use of automation at the very beginning of the sample handling process.

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ABOUT SPECTRUM SOLUTION®

Headquartered in Salt Lake City, Utah, Spectrum Solutions believes when you bridge the gap between science and real medical solutions you deliver innovation with the practical power to impact and change outcomes.

As a Laboratory Medicine clinical science and sampling kit development partner, our expert medical device ideation, superior point-of-collection innovation, and clinical kitting services are modernizing direct-to-consumer healthcare access and pioneering a new era of multi-omic understanding. For more information please visit spectrumsolution.com.

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