White Paper

Inactivation of SARS-CoV-2 (COVID-19) in Saliva Collected using Spectrum's SDNA-1000

The creation of a robust and safe biomaterial for diagnostic and screen-based testing

August 01, 2020

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Executive Summary

Until recently, traditional viral testing methods depended on the nasopharyngeal swab approach to sample collection. This uncomfortable biosample collection method requires a healthcare professional to perform and includes the insertion of a long swab in the nose to the back of the throat where the sample is located. In mid-April (2020), the FDA granted emergency authorization for a **saliva-based test exclusively using the Spectrum SDNA-1000 saliva collection device.** The SDNA-1000 is a simple to use and self-administered device that is intended for non-invasive saliva collection.

In contrast to many different swab collections, saliva sample collection with the SDNA-1000 proved to be easier and more comfortable for patients through the simple self-collection of passive spit. The SDNA-1000 requires no additional collection supplies or any direct interaction form healthcare workers, saliva collection effectively reducing the need for masks, gowns, gloves, and other personal protective equipment (PPE) that would be required if a health care professional was necessary to administer a sample collection. Pioneering a new era of at-home biosample self-collection for viral infections and adding to the growing list of benefits using Spectrum's saliva collection device this study, initiated by Spectrum, is to evaluate and demonstrate the **100% neutralization of the SARS-CoV-2 (COVID-19) live virus when collected in the SDNA-1000 saliva device using its optimized and validated preservation agent.**

The Problem

Under the spotlight of a global pandemic, COVID-19 easily demonstrated as testing needs increased 1000-fold so too did the demand for critical biosample collection supplies and PPE. The risk of undo exposure for frontline support when collecting biosamples, the subsequent transportation, and processing of samples for viral testing is always a serious containment vulnerability. As testing supplies and PPE began to run out the threat of exposure to the virus during sample collection grew not only for healthcare teams, but additionally for the public at large. Not only did the lack of testing supplies directly impacted the ability to make testing widely available it also left those with possible exposure but asymptomatic, untested, undiagnosed, and unaware of the potentially risk of furthering infection to those in direct and close contact.

It became abundantly clear that in order to deliver a viable solution to the biosample collection problem at hand three things had to happen. First, the solution had to ***not*** incorporate any of the already critically low supply elements. Second, it needed to deliver a form of relief from the supply strain while extinguishing the threat of undo exposure. Third, the solution needed to provide an avenue of delivering tests to patients instead of patients to tests.

The Background

The SDNA-1000 by Spectrum Solutions is intended for non-invasive saliva specimen collection. In this study, the SDNA-1000 device is used to collect saliva samples, preserve the viral COVID-19 RNA, inactivate the live virus, and safely transport the biosample to the laboratory for molecular testing.

Saliva is an authorized and preferred method of sample collection for COVID-19 molecular detection. The Rutgers Clinical Genomics Laboratory (RCGL), now Infinity BiologiX, received FDA Emergency Use Authorization (FDA EUA #200090) on April 10th, 2020 authorizing the first use of saliva collected exclusively using the Spectrum

Solutions SDNA-1000 for the analysis and detection of COVID-19. This authorized process requires the collection of a minimal amount of saliva by spitting into the SDNA-1000 collection tube up to the demarcation line. Spectrum's SDNA-1000 contains a preservation chemistry that renders any COVID-19 virus 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 using a bead-based nucleic acid extraction chemistry that is optimized for viral RNA purification. Independent studies 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 is subjected to multiplex RT-PCR 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 those in direct and close contact.

Given the scientific, safety, and experiential advantages to saliva collection for COVID-19, it is also important to ensure that the potentially infectious material provided by any given patient is safe for both transportation from collection to the lab and the material is safe for 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-1000 device renders any infectious COVID-19 virus completely inactive allowing for a safer laboratory experience and more robust automation process for sampling and extracting from the collection device.**

The Study

This white paper describes a series of studies that support the above viral inactivation claims. Viral inactivation was determined by measuring both cytopathic effect (CPE) and viral transcript detection using RT-PCR as direct measurements of infectivity.

COVID-19 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 COVID-19 virus is cultured and used for experimentation in a BSL3 laboratory environment. The virus is exposed to the SDNA-1000 preservation agent to simulate a clinical saliva sample collection. The preservation agent contains a chaotropic agent that kills cultured eukaryotic cells on its own so a dialyzing procedure was used with Amicon filters to remove any buffer components that would lead to the destruction of feeder cells (Vero) and would ultimately prevent the measurement of potential infection following 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 in buffers that are toxic to cell culture on their own.

The COVID-19 virus was cultured and added to either media/saliva with no preservation agent (experimental control) or SDNA-1000 preservation agent. In addition, media/saliva and preservation agent were tested without the addition of live virus as additional controls. Virus at varying concentrations were added to both media/saliva and preservation agent to simulate an active infection at different viral loads with an

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emphasis on high viral titers to truly test the ability of the preservation 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+SDNA1000 preservation agent) the cells were cultured for 72-hours and subjected to both cytopathic effect (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.

Cytopathic effect analysis (CPE) is a measurement of structural changes to host cells that are caused by viral infection. The infection can cause lysis of host cells or death 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. When combined these measurements provide a complete and sensitive assessment of viral activity and infectivity as a function of sample collection scenarios.

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	Primary Culture Results	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

The Conclusion

Results of this study successfully concluded no evidence of viral growth in presence of SDNA-1000 lysis buffer by either CPE read out or RT-PCR. The complete lack of CPE in any sample mixed with SDNA-1000 lysis buffer demonstrates a greater than 6-log order reduction in viral activity in Vero cultured cells. Additionally, the lack of viral load

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increase (as measured by RT-PCR) across several days of cell culture indicates that there is no COVID-19 growth or infection following exposure to the SDNA preservation agent. It was confirmed that the SDNA-1000 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 virus in the presence of SDNA-1000 preservation agent.

The inactivation of the virus in the SDNA-1000 saliva collection device creates the most robust and safest biomaterial collection approach for the detection of COVID-19 infections and leads the way to a new era of at-home biosample self-collection for the diagnosis of viral infections.

The Benefits

There are several advantages to using saliva collected with the SDNA-1000 as the primary source of COVID-19 detection for molecular analysis. The following summation highlights the key benefits.

First, the pain-free SDNA-1000 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 swab-based collection does.

Second, there is a greater than 90% reduction in the use/need for personal protective equipment (PPE) compared to the current usage for swab collections providing direct relief to the global shortage of both testing supplies and PPE required for those collections.

Third, saliva is a more robust biomaterial to facilitate molecular testing. There is less sample variability using the SDNA-1000 for collecting saliva while rendering maximum sensitivity and optimal testing accuracy.

Lastly, using the SDNA-1000 device renders any infectious COVID-19 virus completely inactive offering not only a better, pain-free patient experience when compared to most all invasive swab sample collections but additionally provides for a safer laboratory experience as well. The ability of the SDNA-1000 device to deliver 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.

ABOUT SPECTRUM SOLUTIONS and SPECTRUM DNA

Headquartered in Salt Lake City, Utah, Spectrum Solutions and its medical device and services division, Spectrum DNA, focus their concentrated industry expertise on engineering innovative end-to-end solutions for both clinical diagnostic projects and commercial product plans. A single-source provider of on-site medical device development and manufacturing, custom packaging, kitting, and direct-to-consumer fulfillment. Its biosample collection devices, patented technologies, and dedicated services deliver measurable process optimization, unprecedented efficiency, and unmatched global scalability. For more information on the SDNA-1000, please visit spectrumsolution.com.

REFERENCES

Burton JE, et al., The effect of a non-denaturing detergent and a guanidinium-based inactivation agent on the viability of Ebola virus in mock clinical serum samples. J. Virol. Methods. 2017 Dec; 250:34-40.

TERMS

SARS-CoV-2/COVID-19: SARS-CoV-2 stands for: severe acute respiratory syndrome coronavirus 2. SARS-CoV-2 is the virus that causes COVID-19.

CPE: Cytopathic effect (CPE), structural changes in a host cell resulting from **viral** infection. CPE occurs when the infecting **virus** causes lysis (dissolution) of the host cell or when the cell dies without lysis because of its inability to reproduce.

RT-PCR: Viral detection via RNA extraction using bead-based nucleic acid extraction followed by quantitative PCR using dual labeled probe chemistry for the detection SARS-2 viral transcripts.

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