Transforming Nucleic Acid Collection Efficacy with Proprietary Technology

The Benefits of the Rhinostics® Anterior Nasal, Vaginal, and Blood Collection Devices in RT-PCR Testing

The COVID-19 Pandemic as a Catalyst for Innovation in Molecular Testing Methodologies

The emergence of the novel coronavirus (SARS-CoV-2) in the United States presented a number of challenges in the clinical laboratory industry. It revealed deficiencies in affordable, high-throughput molecular testing methologies, limitations in commercially available testing supplies and personal protective equipment (PPE), and challenges in achieving operational efficiency amidst overwhelming sample volume. The COVID-19 pandemic however, also served as a catalyst for critical, timely innovation in molecular testing across the entire laboratory life cycle – from collection to result.

More specifically, access to traditional cotton tipped and polyester flocked swabs was undermined by the overwhelming demand from drive-thru testing sites, home-testing, clinics, hospitals, and public health facilities, desperate to serve their communities and quell the panic and transmission. Despite manufacturer's attempts to scale production, the addition of viral transport medium, absorbent coatings and sterile packaging requirements, along with the pressure of seemingly endless demand, only served to exacerbate the problem.

Additionally, while these conventional swab (cotton, flocked, polyester) types are effective in collecting adequate amount of viral material, their design often inhibits the release of this viral material during processing, unless the swab is immediately suspended and transported in viral transport media (VTM). The lack of viral material in the eluent led to an increased incidence of false negative test results and potentially resulted in unknowing spread of COVID-19 in households, workplaces, and communities. And the required VTM presented a significant costly, logistical roadblock.

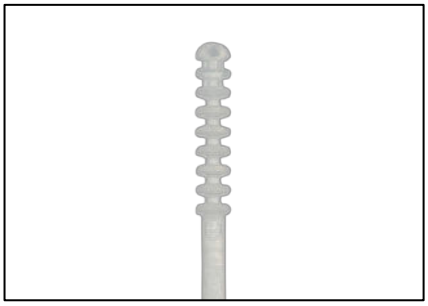
Despite all of these challenges, a number of innovations emerged during the COVID-19 pandemic, including a industry disrupting, proprietary nasopharyngeal swab followed quickly by an automated anterior nares swab designed to enhance, not impede, laboratory workflow. The founders of Rhinostics, desperate to address the needs of clinical laboratories, partnered with researchers at Harvard University and the Wyss Institute to develop a swab that was cost effective, could be manufactured quickly and in high quantities, was not subject to the constraints of the traditional supply chain, and that was effective at both collecting and releasing viral material without the use of VTM. The result of these efforts was the GrooveSwab® Nasopharyngeal Swab. A first of it's kind, polypropylene swab with a grooved surface, specifically designed for the collection and release of viral material from nasopharyngeal specimens, without the need for VTM or any other transport media (including normal saline).

The GrooveSwab® Nasopharyngeal Swab

Inspired by nature, researchers designed the GrooveSwab® to mimic the way papillae on a cat's tongue utilize surface tension forces to trap and wick liquid, replacing spike-like papillae with a smooth, conical shape. The unique stacked ring structure, composed of polypropylene, did not require any absorbant material often featured on traditional cotton or polyester nasopharygneal swabs.

Tiny papillae or "spikes" on a cat's tongue help to trap liquids.

Polypropylene, already a popular choice in life science consumables annd medical devices, is a versatile, hydrophobic plastic that can be readily manufactured using single injection molding techniques. Additionally, polypropylene is both chemically and biologically resistant, sterilizable, and has the ability to retain its shape even after bending or flexing.



The head of the GrooveSwab Nasopharyngeal Swab features a stacked ring structure that helps to capture copious sample while ensuring a comfortable patient experience.

Easy to manufacture, and scalable, the GrooveSwab® also offerred a more comfortable patient experience. In lieu of rough, dry bristles, the slim, smooth design of the GrooveSwab® head means less irritation of the nasopharyngeal mucosa, while also offering superior performance. Not only does the stacked ring structure aid in capturing copies amounts of sample, the hydrophobic nature of polypropylene ensures superior recovery of sample by fully eluting into solution. Furthermore, the GrooveSwab® eliminates

the need for viral transport media (VTM) or saline, enhancing sample stability, reducing shipping costs, and increasing the likelihood of a viable, testable sample reaching the laboratory.

See "GrooveSwab White Paper V.051222" for detailed infromation related to research studies performed on the GrooveSwab® nasopharyngeal swab.

From Manual to Automated: The Emergence of the RHINOstics® Automated Swab

Once the team at Rhinostics had perfected the design and manufacture of the GrooveSwab® nasopharyngeal swab, they turned their attention to improving internal laboratory workflow and began investigating a collection device solution for automation. With decades of experience in laboratory automation, the team quickly re-tooled their polypropylene design, and landed on a truly innovative, automation solution – the RHINOstics® Automated Swab.

The RHINOstic® automated swab combines effective, easy-to-manufacture materials with automation to allow for home collection, increase laboratory throughput, and lower costs. The RHINOstic® Automated Swab collection device consists of a hydrophobic polymer swab with a threaded lid attached for transport in a 1 ml transport tube (cryotube) and can be used for anterior nares (nasal), buccal cells, vaginal, penile, anal, wound, and other types of biological swab samples.. Once the sample is collected, the swab is screwed securely into the transport tube, so the threaded lid aligns with the threading on the open portion of the transport tube. The thread swab cap is screwed shut onto the transport tube and can then be transported to the testing laboratory under ambient temperatures.

The RHINOstic® Automated Swab is intended to collect a swab sample at home or in any healthcare setting by patients or medical professionals.

Efficiency in Action: Evaluation of the RHINOstics® Automated Swab

To demonstrate the effectiveness of the RHINOstics® automated swab, a team of clinical consultants prepared a series of studies to evaluate recovery of DNA/RNA material from anterior nasal, vaginal, and penile samples. Volunteers self-collected various sample types and these samples were run on a QuantStudio Flex 7 RT-PCR instrument.

In the first study, 20 anterior nasal samples were self-collected by 4 different volunteers. Volunteers were instructed to insert the swab end of the RHINOstics® automated swab in each nostril and swirl around the interior surface of the nares 3-4 times. The samples were inserted into the transport tube and stored at ambient temperature to await processing. Once received, laboratory personnel rehydrated the anterior nasal samples with 1 mL of NOVA Scientific extraction buffer and vortexed the samples for approximately 30 seconds to ensure complete elution of DNA/RNA material into solution.

The samples were then prepared, using standard operating procedures, to be run using the VIASURE Respiratory (SARS-CoV-2, RSV, Flu A/B) RT-PCR kit. This study was designed to evaluate the presence of endogenous control (VIC), the primary indicator of adequate recovery and extraction of DNA/RNA from patient samples collected using the RHINOstics® automated swab.

Sample	Ct Value	Target
Patient 1	29.392	Endogenous Control (VIC)
	27.505	
	28.280	
	28.311	
	29.618	
Patient 2	29.00	Endogenous Control (VIC)
	30.01	
	30.98	
	26.59	
	29.85	
Patient 3	36.72	Endogenous Control (VIC)
	33.97	
	34.59	
	32.17	
	32.70	
Patient 4	35.28	Endogenous Control (VIC)
	35.70	
	33.01	
	34.36	
	32.24	

Figure 1. Endogenous control results (Ct values) for patient samples collected using the RHINOstic® Automated Swab and evaluated using the VIASURE Respiratory (SARS-CoV-2, RSV, Flu A/B) RT-PCR kit. Endogenous control positive if Ct value <40.

Results were evaluated using the result interpretation guidelines featured in the VIASURE Respiratory (SARS-CoV-2, RSV, Flu A/B) IFU. All samples were positive for endogenous control (amplification in the VIC channel with Ct values < 40), indicating adequate recovery and extraction of RNA from

patient anterior nasal samples collected using the RHINOstics® automated swab.

In a second study, 20 vaginal swab samples were self-collected by 4 different volunteers. Volunteers were instructed to insert the tip of the RHINOstics® automated swab gently into the vaginal canal, and swirl for approximately 60 seconds. The samples were inserted into the transport tube and stored at ambient temperature to await processing. Once received, laboratory personnel rehydrated the vaginal swab samples with 1 mL of NOVA Scientific extraction buffer and vortexed the samples for approximately 30 seconds to ensure complete elution of DNA/RNA material into solution.

The samples were then prepared, using standard operating procedures, for testing, using the ThemoFisher TruMark™ STI Select Panel, a real-time PCR assay for the detection of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma genitalium (MG), and Trichomonas vaginalis (TV). This study was designed to evaluate the presence of the internal process control (RNase P), the primary indicator of adequate recovery and extraction of DNA/RNA from patient samples collected using the RHINOstics® automated swab.

Sample	Ct Value	Target
Patient 1	25.82	RNase P (IPC)
	25.53	
	26.13	
	25.82	
	26.40	
Patient 2	25.66	RNase P (IPC)
	26.95	
	26.29	
	25.32	
	28.55	
Patient 3	25.48	RNase P (IPC)
	23.85	
	25.55	
	24.22	
	24.81	
Patient 4	24.20	RNase P (IPC)
	24.44	
	23.90	
	19.26	
	23.82	

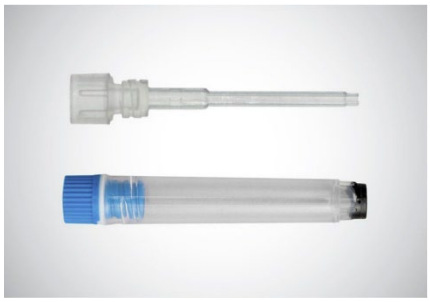
Figure 2. Internal process control (RNase P) rresults (Ct values) for patient samples collected using the RHINOstic® Automated Swab and evaluated using the TruMark™ STI Select Panel. Internal process control (RNase P) positive if Ct value <40.

Results were evaluated using the result interpretation guidelines featured in the TruMark™ STI Select Panel IFU. All samples were positive for internal processing control (amplification of RNase P with Ct values < 40), indicating superior recovery and extraction of RNA from vaginal samples collected using the RHINOstics® automated swab.

Continued Innovation: The VERIstic® Blood Collection Device

Following development and refinement of their polypropylene-tipped RHINOstic® automated swab, the team at Rhinostics turned their sights towards another opportunity for innovation – blood collection devices. With the emergence of at-home testing kits during the COVID-19 pandemic, researchers began exploring a solution for at-home blood collection. Using well understood fluid mechanics and well-established technology, the Rhinostics team developed a novel, capillary action mediated blood collection device, known as the VERIstic®.

The VERIstic® is a novel capillary device developed to bring improved materials and remove laboratory workflow bottlenecks for small volume blood and other biological samples when they reach the laboratory. With a simple lancet blood prick in the home or clinic, the purpose-built open design rapidly and efficiently wicks 50 µL of blood from the finger while also enabling full elution in the lab. By combining a capillary with a cap that is enabled for automated, robotic decapping, the VERIstic® lowers the time and cost significantly for diagnostic assays that need small volume blood samples.



The VERIstic® is intended to collect a blood sample at home or in any healthcare setting by patients or medical professionals.

To demonstrate the effectiveness of the VERistic® blood collection devices, a team of clinical consultants prepared a series of studies to evaluate recovery of DNA material from blood samples. Volunteers self-collected 20 blood samples using a single-use lancet and the VERistic® blood collection device, and these samples were run on a QuantStudio Flex 7 RT-PCR instrument.

The samples were then processed by laboratory personnel by first adding 300 uL of 10x PBS buffer (pH = 7.4) to facilitate the release of the blood sample into the transport tube. This solution was then aliquoted into a separate microcentrifuge tube for extraction. Extraction was performed using the Qiagen Mini DNA kit's spin protocol.

Once processed, the samples were prepared for testing, in accordance with the NOVA Scientific HCV/HBV lyophilized assay, a real-time PCR solution for evaluating blood samples for presence of Hepatitis B (HBV) and Hepatitis C (HCV) virus. This study was designed to evaluate the presence of the endogenous control (VIC), the primary indicator of adequate recovery and extraction of DNA from patient blood samples collected using the VERistic® blood collection device.

Sample	Ct Value	Target
Patient 1	26.95	Endogenous Control (VIC)
	24.74	
	24.69	
	24.48	
	23.75	
Patient 2	24.34	Endogenous Control (VIC)
	24.33	
	23.89	
	23.98	
	23.54	
Patient 3	25.59	Endogenous Control (VIC)
	26.39	
	24.71	
	23.77	
	25.58	
Patient 4	24.26	Endogenous Control (VIC)
	24.81	
	23.99	
	25.17	
	24.71	

Figure 1. Endogenous control results (Ct values) for patient samples collected using the VERIstic® blood collection device and evaluated using the NOVA Scientific HCV/HBV RT-PCR Assay. Endogenous control positive if Ct value <40.

Results were evaluated using the result interpretation guidelines featured in the NOVA Scientific HCV/HBV RT-PCR Assay IFU. All samples were positive for endogenous control (amplification in the VIC channel with Ct values < 40), indicating superior recovery and extraction of DNA from blood samples collected using the VERIstic® blood collection device.

Conclusion

By designing products that can be readily manufactured, using single-injection molds and polypropylene, the RHINOstics® suite of collection devices offers a truly efficient, innovative, and cost-effective solution for any laboratory workflow. Studies indicate superior DNA and RNA recovery from anterior nasal, vaginal, and blood samples collected using the RHINOstics® automated swab and the VERIstic® blood collection device.