WHITEPAPER

SALETTO® SALIVA COLLECTION DEVICE'S INTEGRATED FILTRATION IMPROVES ACCURACY IN THE LAB BY REDUCING AND STANDARDIZING SALIVA SAMPLES' VARIABLE VISCOSITY.

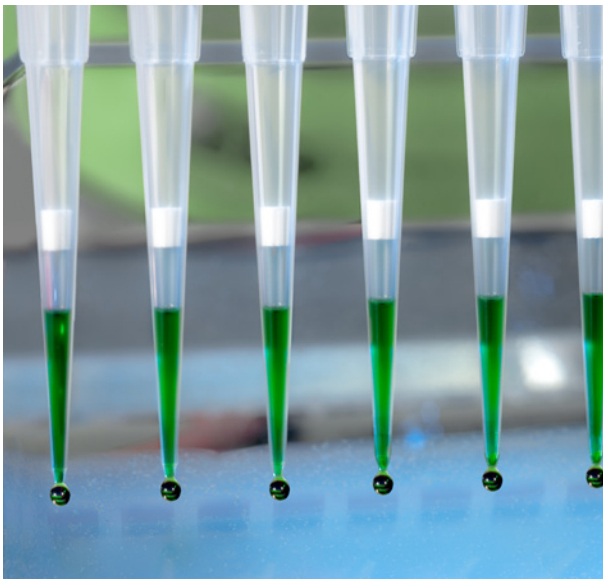
POREX
Life Sciences Institute™

Background Information

The COVID-19 pandemic revolutionized the global diagnostic landscape by shifting diagnostic sample collection towards an at-home environment, elevating the importance of safe and non-invasive sample collection methods, highlighting the need to streamline overstretched laboratory workflows, and made saliva an everyday sample type. To expand saliva-based diagnostics beyond COVID-19, resolving saliva sample variability is key. Saletto is the first saliva collection device to incorporate filtration that can reduce and standardize the viscosity of saliva samples, eliminating common preparation steps and sample dilution, producing a ready-to-use sample during the collection process.

Saliva is considered a viscoelastic fluid, and its viscosity is highly dependent on its secretion location within the mouth as well as the species and quantity of mucin that it contains. This inherent variability is present not only from patient to patient, but also within the same patient based on time of day. External factors, such as medicines, therapies, illness, exercise, and overall hydration levels can also cause changes in the viscosity of saliva[1].

Viscosity differences can cause inaccurate volume transfer in liquid handling equipment used in the laboratory. These inaccuracies can further lead to sample and analyte concentrations that are over or underrepresented, yielding erroneous results. In lateral flow assays, saliva viscosity may contribute



to the lack of test sensitivity. To combat viscosity, preparation steps such as centrifugation, and freeze-thaw occur prior to sample analysis. Or, the sample is coupled with enzymatic and chemical treatments to reduce viscosity, leading to dilution of the target analyte – which may be present in already low concentrations within saliva.

The Porex Life Sciences Institute believes that accessible, painless, and safe diagnostic testing must start from a high-quality sample. The Saletto® Saliva Collection Device is furthering saliva-based diagnostics by cleanly and securely collecting a sample while simultaneously removing impurities, saving valuable time and resources, and getting you a quicker and more reliable result.

Study Design

The purpose of this study was to determine how saliva viscosity could be reduced and standardized through filtration of the sample during the collection process, at room temperature. Standardization of saliva viscosity during the collection process could eliminate centrifugation or freeze-thaw prior to sample analysis and decrease the need to add liquid treatments to the sample which can cause analyte dilution.

The Test System

The VISCOlab 4000 was chosen to study the saliva viscosity because of its capability to measure small volume samples (e.g., 1 to 1.5 cc range), it's easy to use, clean, and maintain, and is widely demonstrated to be accurate and reliable. It contains a piston-style electromagnetic system to provide continuous viscosity and temperature readings. The low mass stainless steel piston inside the measurement chamber is magnetically forced back and forth in the fluid. The time required for the piston to move a fixed distance (about 0.2 inches) is related to the viscosity of the fluid in the chamber. Data is logged internally and can be output to a PC via the RS-232 serial port. The built-in display shows the in-situ data as well as the test status (e.g., temperature and viscosity stabilization, collection and qualification data, final viscosity, and temperature reporting, etc.).
A proper piston was selected to measure viscosity range from 1 to 20 cp which is suitable for typical saliva collected by the general population.

The Standards Used in the Study

A silicone-based fluid (Standard type: N2; ISO 17025/ ISO 17034 Viscosity and Density reference Standard) was used to check the instrument calibration within 1% of the measurement range, specifically, ±0.2 cp from target for our system.

Experimental Design, Control Articles and Sample Preparation

Individual saliva was collected into a polypropylene cup from each donor. The saliva sample was split in two portions: an unfiltered passive drool sample and a sample to be filtered through Saletto. The viscosity of the unfiltered saliva sample was measured. The other portion of the sample was pipetted onto the Saletto collection pad and pushed through the device's integrated filter. The purpose of this collection method was to ensure a uniform starting point for both the unfiltered and filtered Saletto samples for a direct comparison.

There were total of 8 unique donors included in the study, and samples were collected on random days and times spanning across approximately 4 weeks.

The viscosity was measured by a ViscoLab 4000 viscometer at room temperature without temperature control. The difference (temperature of unfiltered sample minus temperature of filtered sample) between the temperature of all sample pairs is shown in Figure 1. As seen in Figure 1, there is minimal temperature difference between the filtered and unfiltered samples and no clear trend, and thus the temperature effect on viscosity is considered negligible in this study.

An example of saliva viscosity data output from the serial port during measurement is shown in Figure 2. The average of the first two data points is reported as "initial viscosity" and the level-off region was reported as "final viscosity" which is shown on the viscometer display after measurement is complete.

From a rheological point of view, saliva can be considered a non-Newtonian fluid, meaning that its viscosity changes depending on the shear rate or stress applied to it. "Initial viscosity" refers to the viscosity of the saliva sample before any shearing is imposed on the sample. "Final viscosity," on the other hand, refers to the viscosity of a saliva sample during the shearing process. The difference between the initial viscosity and final viscosity can provide important information about the flow behavior of a Acompared to each other to determine the degree of change that occurred during the shearing process.

In this study, we focused only on the initial viscosity results since they are more akin to laboratory sample transfer workflows. The saliva sample is not exposed to any shearing or stress during the pipetting process, the sample is static.

Figure 1: Temperature Difference Between Unfiltered and Filtered Samples

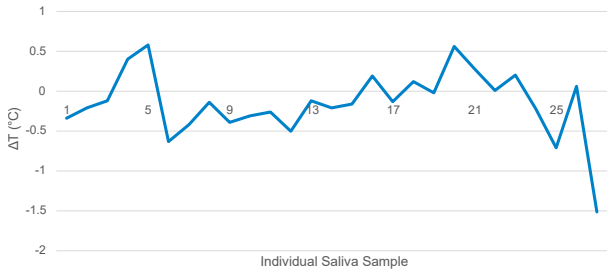
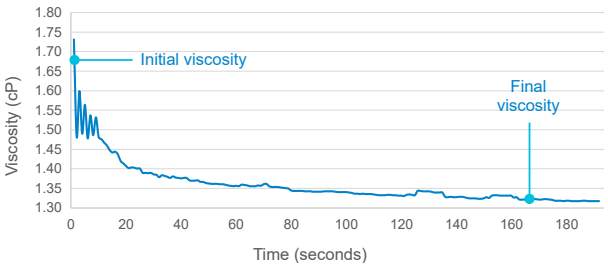


Figure 2: Example of Viscosity Data

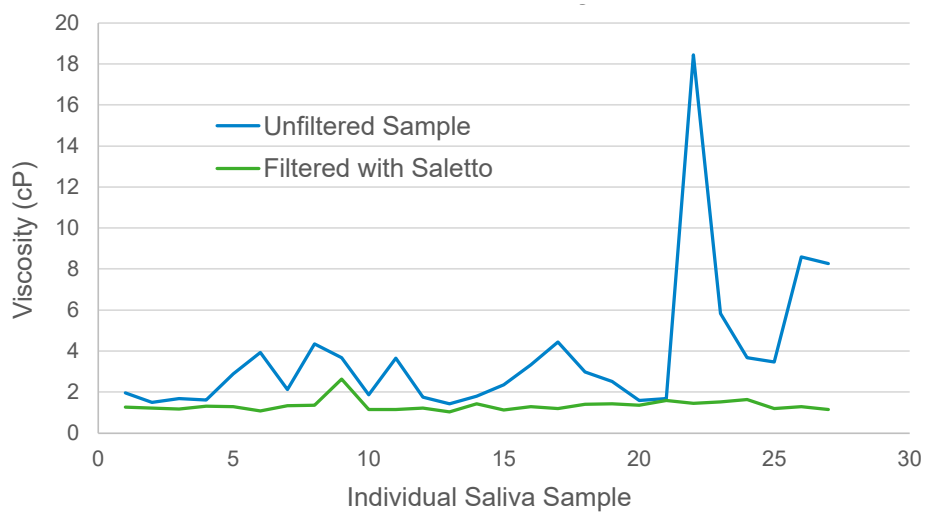


Summary of Results

Saliva samples were collected from eight participants across four weeks and analyzed at room temperature for a total of 27 data points.

We used centipoise (cP) as our unit to measure viscosity. Here, centipoise measures how easily a saliva sample flows. Lower centipoise indicate easier flow, and higher centipoise is more challenging, slower flow. For reference, water has a viscosity of about 1 cP at room temperature, while motor oil (SAE10) about 50 cp. Figure 3 shows the individual data points of initial viscosity, measured in centipoise, collected during the study from each donor.

Figure 3: Effect of Saliva Sample Filtration on Initial Viscosity



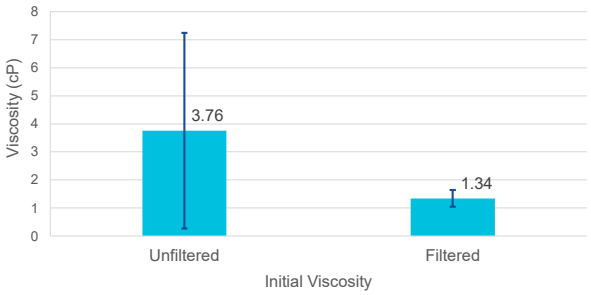
The average of initial viscosity from unfiltered samples was 3.76 cp. Based on this, we categorized the data into two groups, regular viscosity and high viscosity as follows:

- Regular viscosity: Samples < 3.76 cp (below the average)
- High viscosity: Samples ≥ 3.76 cp (above the average)

The initial viscosity measurements of all saliva samples filtered with Saletto are significantly more uniform than the viscosities of the unfiltered samples. This can be seen on the nearly horizontal line in Figure 3 and clearly demonstrates that viscosity is standardized by filtering the saliva sample with Saletto, regardless of the starting point of the sample (high or regular viscosity). Even if the sample is considered to be highly viscous, it is still reduced in a consistent way to resemble the filtered viscosity of samples considered to be in the regulary viscosity range. This is true across all donors in the study as well. Overall, the Saletto device produces much more uniform saliva samples with lower and less variable viscosities than unfiltered passive drool samples.

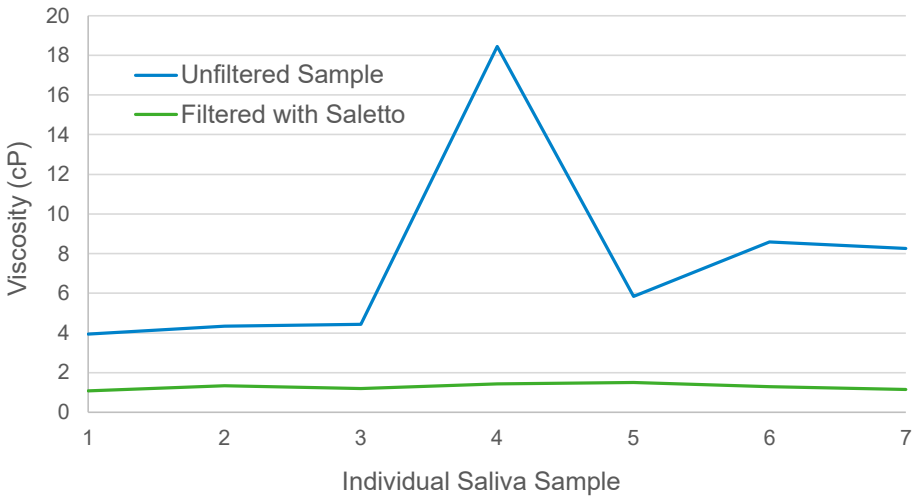
The reduction of viscosity of all samples across donors is summarized in Figure 4. Roughly 64% reduction in initial viscosity – 3.76 to 1.34 - was achieved when collecting and filtering the saliva samples with the Saletto device, as compared to passive drool samples. The standard deviation of the sample set was reduced by approximately 91% - from 3.49 to 0.30 - when collected and filtered with the Saletto device, as compared to passive drool samples.

Figure 4: Average Viscosities of All Samples Collected



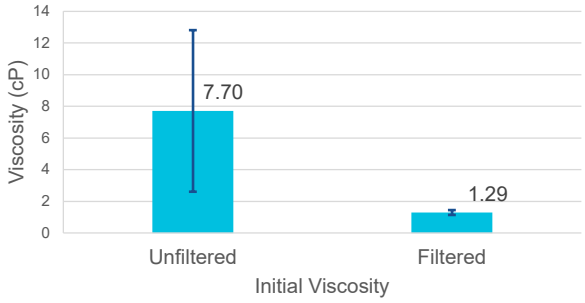
*Error bar represents standard deviation

Figure 5: Effect of Filtration on High Viscosity Samples



The initial viscosity results from the high viscosity category were plotted in Figure 5. The average initial viscosity from unfiltered saliva samples was measured as 7.70 cP with a standard deviation of 5.10. This was compared to the samples collected and filtered by the Saletto device which yielded an average initial viscosity of 1.29 cP with a standard deviation of 0.16. The Saletto device provided a dramatic reduction of 83% in average initial viscosity after filtration and reduction of 97% in standard deviation, in these high viscosity samples, as shown in Figure 6.

Figure 6: Average Viscosities of All High-Viscosity Samples



*Error bar represents standard deviation

Conclusion

Saletto's ability to not only reduce but standardize viscosity across both high and regular viscosity donors allow laboratories to avoid centrifugation and freeze-thaw protocols, eliminate the addition enzymatic and chemical reagents to the samples to lower viscosity, and should allow for easier automated pipetting calibration and more accurate sample transfer during the workflow.

References:

1. Eltze, L., Eltze, M., & Garcia, A. (2020). Variability of Saliva Viscosity - Potential Impact. In L. Ardelean, & L. C. Rusu (Eds.), Oral Health Care - An Important Issue of the Modern Society [Working Title]. IntechOpen. https://doi.org/10.5772/intechopen.93933

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