

Hygiena® Response to Neogen's Report: Third Party Study Demonstrates Not All ATP Systems Produce Stable, Consistent Results

Aim

To examine and analyze the data shown in the Neogen® (formerly 3M™) report with reference to both sampling protocol and results.

The Initial Report Claim

The Neogen report summary states that repeat readings from the same sample over the course of 2 minutes produced stable results using Neogen Clean-Trace® devices (Figure 1) compared to other manufacturers' devices, which do not produce stable results. Each chart in the Neogen report showed 7 data points spaced 20 seconds apart at 3 temperatures (low, normal/recommended, high).

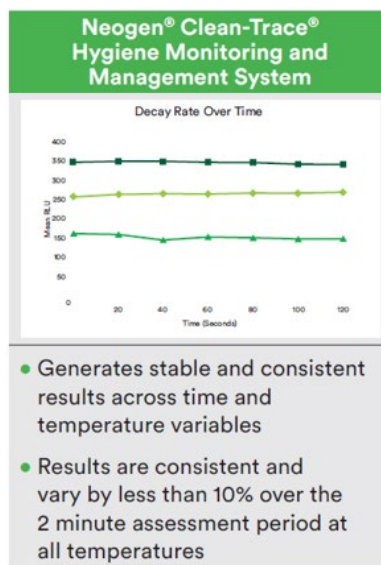


Figure 1. Clean-Trace Results from the Neogen Report.

ATP was applied to swabs, and 7 measurements were taken 20 seconds apart at 3 temperatures: 20 °C (dark green, normal), 10 °C (light green, low) and 35 °C (green, high).

Comment 1: Stability Over Time is Not Relevant for Real-World Testing

This data from the Neogen report shows that their chemistry produces a steady-state light output over a 2-minute time interval when ATP was added directly to the sampling swabs. However, in real-world testing, detergent and sanitizer residue may affect test results over time in unpredictable ways. In addition, steady-state results do not help the end user and contradict their own kit insert (Figure 2). The statement “only activate when you are about to measure the test device” renders the steady-state readings over 2 minutes irrelevant.

All manufacturers of ATP hygiene swabs, including Neogen, state that the swabs must be activated and read quickly to obtain the best results. They do not advise activating and waiting to read the results. Most users of any ATP hygiene monitoring swab will perform the testing at the site in question or transport unactivated swabs to a central location and activate sequentially to gain the most accurate results.

How to best utilize Neogen® Clean-Trace® Surface ATP	
Do	Do Not
Hold the Neogen Clean-Trace Surface ATP test device in the VERTICAL position on activation and shake rapidly from SIDE TO SIDE for at least 5 seconds and read immediately in the Neogen Clean-Trace LM1 Luminometer.	Activate when not in the vertical position, do not shake up and down, do not shake like a thermometer.
Replace the swab in the tube but do NOT activate the Neogen Clean-Trace Surface ATP test device if test devices are to be taken back to another location for testing. Only activate when you are about to measure the test device.	Activate all Neogen Clean-Trace Surface ATP test devices then take them to another location for testing or activate and leave on a surface to test one at a time.
Activate and measure Neogen Clean-Trace Surface ATP tests ONE AT A TIME when a number of samples are to be measured.	Activate all Neogen Clean-Trace Surface ATP test devices and then test one at a time.
Place the Neogen Clean-Trace LM1 Luminometer in the vertical position when a measurement is made.	Hold the Neogen Clean-Trace LM1 Luminometer horizontally or lay down on the bench to read a Neogen Clean-Trace Surface ATP test device.
Always remove the last Neogen Clean-Trace Surface ATP test device from the Neogen Clean-Trace LM1 Luminometer chamber when the measurement is completed.	Leave a Neogen Clean-Trace Surface ATP test device in the chamber when the measurement is completed.

Figure 2. Excerpt from Product Instructions UXL100 for the Neogen Clean-Trace Device (Issue date 2024-01). The red boxes highlight instructions emphasizing that measurements should be taken after activation without delay.

Comment 2: Higher RLUs at Higher Temperatures Provide More Sensitive Results at the Recommended Measurement Timepoint

The Neogen report concluded that its system “generates stable and consistent results across time and temperature variables.” However, further data analysis shows that this conclusion is only partly true: *If a user follows the instructions by reading the Neogen Clean-Trace results immediately after activation, the temperature at which the device is being measured will affect the result.*

In their study, the temperature range was 10 °C (low), 20 °C (normal, recommended) and 35 °C (high). As discussed above, only the first reading should be analyzed when following the test instructions. When the first readings from the Neogen chart are plotted against temperature, the results show that their chemistry does not obey the Michaelis-Menten enzyme kinetics model. The expected Michaelis-Menten effect of temperature on enzyme activity means that the hotter the temperature, the faster the reaction will occur (i.e., higher RLUs with ATP tests). However, the Neogen Clean-Trace chemistry is very unstable at elevated temperatures and produces lower RLUs compared to the results from the more normal/ambient temperature of 20 °C (Figure 3), which could lead to more false negative results.

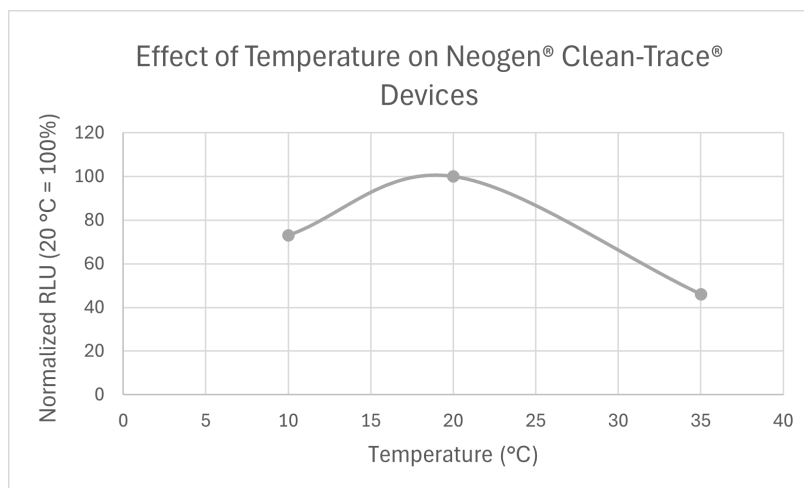


Figure 3. At Higher Temperatures, the Neogen Clean-Trace Device May Not Detect ATP Accurately.



User Scenarios: What Does This Mean for Users Testing in Hot Environments?

In practical applications, testing may take place in environments with varying temperatures, so the temperature-related performance of devices, such as the Neogen Clean-Trace, can result in inconsistent readings across different locations. This high-temperature effect could mean that users may obtain passing results from surfaces that would fail at “normal” temperatures.

The other problem is that there is a greater likelihood of contamination flourishing at elevated temperatures. Therefore, at higher temperatures, users need a more sensitive/higher detection level than at lower temperatures.

This bell-shaped temperature-dependent effect observed in the Neogen chemistry is also seen with other testing devices (Figure 4).

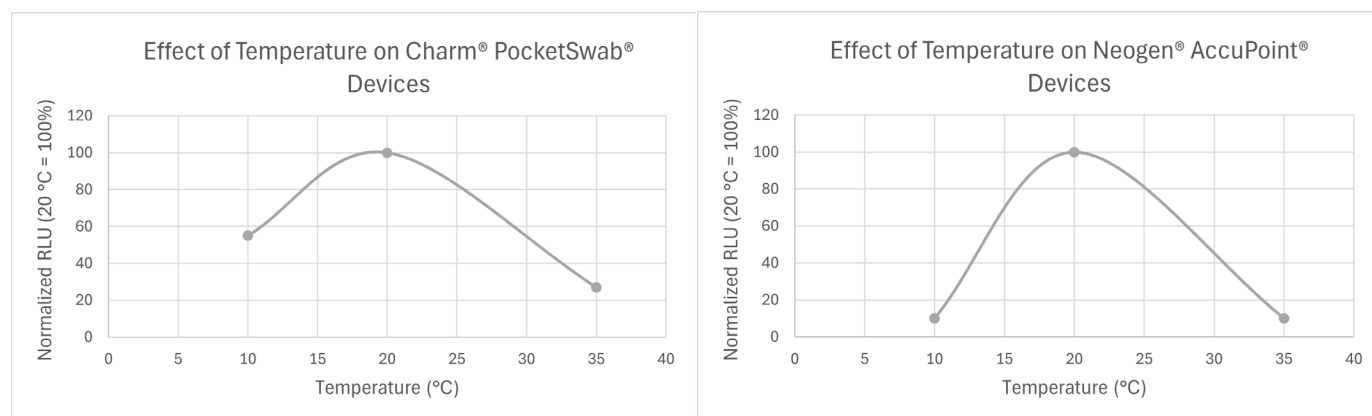


Figure 4. Additional Devices that Exhibit Low Sensitivity at High Temperatures.

Devices that do not have this temperature dependency but follow Michaelis-Menten kinetics normally include Hygiena® UltraSnap® and SuperSnap® (Figure 5; Product No. US2020 and SUS3000, respectively).

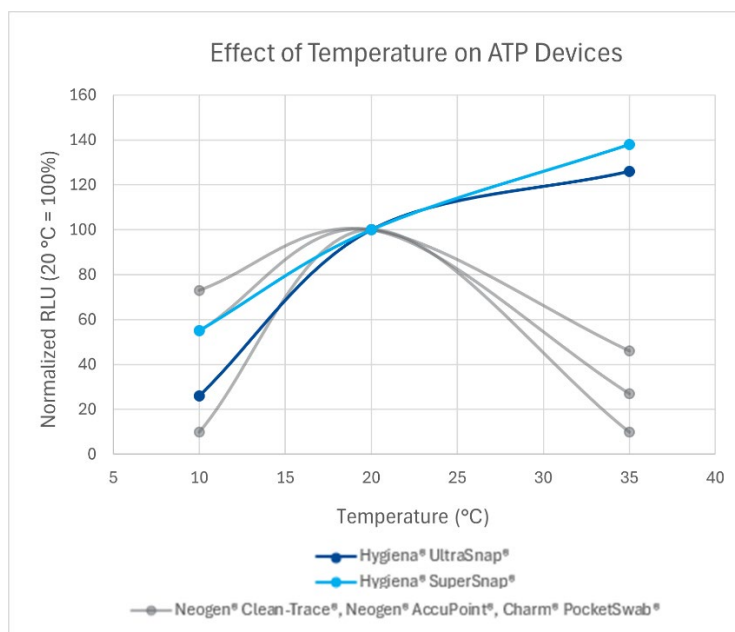


Figure 5. Results from Hygiena UltraSnap and SuperSnap Devices Increase with Increasing Temperatures (dark and light blue). For comparison, Neogen and Charm data (gray) show decreased RLUs at higher temperatures.



For Hygiena's systems, as the temperature increases, the system becomes more sensitive, leading to higher RLUs up to 35 °C. In testing environments, increasing the temperature range from normal usage to higher temperatures (e.g., during summer or in tropical/temperate countries) will reduce the likelihood of observing false negatives from uncleaned surfaces and will increase the likelihood of a positive result from uncleaned surfaces, leading to overall cleaner environments.

At colder temperatures, the likelihood of finding contamination decreases similarly for all devices, because as the temperature decreases, enzymatic reactions occur more slowly, resulting in lower RLUs.

Summary

- The stability of repeated measurements is not a useful metric for users.
 - Each device should be used as recommended: after device activation, measurements should be taken once and not repeated or delayed.
 - To run swabs as efficiently as possible, users typically follow the recommended protocol; hence, a 2-minute, delayed read, no matter how stable, will not be used in real-world testing scenarios.
- Neogen and Charm devices tend to become less active at higher temperatures (e.g., 35 °C), instead of following steady-state kinetics.
 - In hot environments, there is a greater likelihood of obtaining passing results for surface tests due to the instability of the Neogen and Charm formulations at higher temperatures. The decreased RLU values can lead to misleading results regarding surface contamination.
- Hygiena's ATP bioluminescence chemistry is better for use in the field because it obeys Michaelis-Menten kinetics and shows increased activity as the temperature increases.
 - These devices provide higher RLUs that decay faster over time, but this still leads to better and more sensitive results at the early timepoint that is recommended by all manufacturers.