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3M Food Safety

Evaluation of nine surface ATP detection systems for hygiene monitoring.

Abstract

Food manufacturing businesses are increasingly under pressure to maintain and improve hygiene monitoring within the manufacturing environment to improve hygiene standards and ensure compliance with food safety management systems. The use of surface adenosine triphosphate (ATP) hygiene monitoring systems is widespread and provides food business operators with rapid, measurable and cost-effective tests. However, if ATP hygiene monitoring systems are not appropriately chosen and/or their capabilities and restrictions are not understood by users, a system that is not fit for purpose in the processing environment may be wrongly selected and lead to inaccurate monitoring of hygiene practices. In addition, the interpretation of inaccurate data may lead to management decisions that affect food safety and production efficiencies, which in turn may have a significant commercial impact on the business.

An independent study conducted by The Zero2Five Food Industry Centre at Cardiff Metropolitan University, Cardiff, Wales, UK evaluated the stability and repeatability of results generated by nine ATP hygiene monitoring systems.* The ATP signal kinetic decay rate over 2 minutes was determined. The 3M™ Clean-Trace™ Hygiene Monitoring and Management System used with the 3M™ Clean-Trace™ Surface ATP Test Swab was the only system that provided stable and consistent results over a span of 2 minutes at three temperatures of 10°C, 20°C and 35°C.

Introduction

In order for food and beverage manufacturers to comply with regulations, food safety professionals need to be confident that the hygiene monitoring strategies implemented within their organisations are reliable, accurate and fit for purpose.

The extensive use of ATP hygiene monitoring systems provides manufacturers with fast and quantifiable test results to ensure the safety and quality of the products they produce. However, this can only be achieved if the ATP hygiene monitoring system provides accurate, reliable, consistent and repeatable results. Many businesses use ATP hygiene monitoring tests to underpin the cleaning prerequisites on site to demonstrate best practice and release the area for use for hygiene management.

This study evaluated the stability of results generated by nine ATP hygiene monitoring systems. ATP signal kinetic decay rates over 2 minutes, at 20 or 30 second intervals (dependent on the device response rate), at variable environmental temperatures of 10°C, 20°C and 35°C were compared.

Materials and methods

Luminometers and ATP Test Swabs

Luminometer	ATP Test Swab
3M™ Clean-Trace™ LM1 Luminometer	3M™ Clean-Trace™ Surface ATP Test Swab
Biocontrol Lightning MVP ICON®	Biocontrol Lightning MVP ICON® Surface Sampling Device
Charm novaLUM II	Charm FieldSwab ATP Swab
Charm novaLUM II	Charm PocketSwab Plus ATP Swab
Hygiena EnSURE™	Hygiena SuperSnap Test
Hygiena EnSURE™	Hygiena UltraSnap Test
Kikkoman Lumitester	Kikkoman LuciPac™ Pen Test
Neogen™ Accupoint®	Neogen™ Accupoint® Advanced ATP Access Sampler
Neogen™ Accupoint®	Neogen™ Accupoint® Advanced ATP Surface Sampler

Preparation of ATP Standard

A frozen vial of 10^{-4}M ATP was defrosted then 10µL of 10^{-4}M ATP was added to 10 mL of sterile type 1 water in a universal container. This resulted in an ATP solution of 10^{-7}M ATP concentration. To achieve the solution with a 4 × 10^{-9}M ATP concentration, 400µL of the 10-7M ATP solution was mixed with 9.6mL of Milli-Q Type 1 water. Next, 900µL of the 4 × 10^{-9}M ATP was dispensed into sterile bijou bottles. The bottles were labeled for each temperature to be studied (at 10°C, 20°C and 35°C) and stored on ice in a polystyrene insulated container to ensure that ATP activity was preserved for the duration of the experiment.

Testing of the ATP standard signal

To ensure that the 4 × 10^{-9}M ATP concentration was stable throughout the experiment, it was tested at room temperature (20°C) using 3M™ Clean-Trace™ Biomass Detection Kit ULW10 and measured using a calibrated 3M Clean-Trace luminometer. One bijou bottle of 4 × 10^{-9}M ATP was removed from the ice container and left to stabilize at room temperature for 15 minutes before testing. Next, 100µLof Milli-Q Type 1 water, 100µL of the reconstituted luciferin/luciferase solution and 15µL of the 4 × 10^{-9}M ATP solution were pipetted into a plastic cuvette, one at a time, ensuring a new pipette tip was used for each of the solutions. The target range of the solution, when read in a 3M Clean-Trace luminometer, was 1,200 to 1,900 RLU (Relative Light Units). Once the stability and acceptability of the ATP standard was confirmed and the environmental test chamber acclimatised to the test temperature, as shown on the calibrated thermometer, the bijou of ATP was transferred to the environmental test chamber and acclimatized for 15 minutes before testing of the ATP devices proceeded.

Setting up the environmental chamber

A calibrated thermometer was placed into a universal container with 5mL of Milli-Q water then moved onto the cuvette rack inside the environmental chamber, ensuring it was in close proximity to instruments and swab test devices when held in the chamber during testing. The temperature of the thermometer in the universal container was monitored throughout the experiment. Testing was completed in the temperature order of 10°C, 20°C, and 35°C, followed by repeated testing at 20°C to assess for signs of irreversible enzyme loss at higher temperatures. The environmental chamber was tuned to the required temperature variable set point. Two different brands of luminometers, 45 surface ATP test devices for each brand, a digital stopwatch and a cuvette rack were placed in the environmental chamber, along with one bijou bottle of 4 × 10^{-9}M ATP solution. When the temperature on the calibrated thermometer, placed in the universal container of Milli-Q water, inside the environmental chamber, reached the variable temperature set point, the environmental chamber was left to stabilise at the given temperature for 15 minutes. Time was monitored using a digital clock timer.

Testing method

Using a calibrated pipette, 10µL of the 4 × 10-9M ATP solution was pipetted onto the midsection of the swab bud or swab contact area. The swab/sampler test device was immediately activated, following the manufacturer's instructions for use. The swab/sampler test device was inserted into the luminometer and the RLU reading was recorded immediately at Time Zero (T0). On test completion, the swab/sampler test device that had been read at T0 was removed from the luminometer, rotated and re-inserted into the luminometer chamber in a single lift and rotate movement.

A repeat reading of the same swab/sampler test device was taken at 20, 40, 60, 80, 100 and 120 seconds to assess the kinetic decay rate. For the Neogen and Biocontrol devices, repeat readings were performed at 30, 60, 90 and 120 seconds because the response time required by these systems to display RLU results was greater than 20 seconds. All swab/sampler test devices were discarded after the 120-second RLU value was recorded.

The same sequence of steps was repeated consecutively for a total of ten swab/sampler test devices for all manufacturers' systems. A new sterile pipette tip was used to dispense the ATP control solution for each of the ten swab/sampler test devices.

Following ten repeat tests on each luminometer, all used swab/sampler test devices, pipette tips and the ATP control solution were removed from the environmental chamber, which was then conditioned for the next temperature variable set point. During temperature conditioning of the environmental chamber, a new bijou of ATP, a new pair of luminometers to be tested, and the calibrated thermometer in the universal container of 5mL water were placed into and remained within the environmental chamber to ensure their acclimatisation.

Results

The results were expressed as follows for each competitor:

1 Graphical representation of RLU signal over time (mean RLU versus Time[s]). Shown in Graphs 1.1 to 1.9.
2 Bar chart to represent change in RLU values over time i.e., 0 minutes versus 1 minute and 2 minutes at 10°C, 20°C and 35°C. Shown in Graphs 1.10 to 1.27.
3 Percentage decay rate per minute at 10°C, 20°C and 35°C (Table 1.1).

3M™ Clean-Trace™ Surface ATP Test System
The results for the 3M Clean-Trace Surface ATP system indicate that the system had consistent and stable RLU readings over the 2-minute assessment period (Graphs 1.1, 1.10, 1.11) for all temperature variables. The mean RLU readings changed by a maximum of 8.66% (Table 1.1). The mean RLU readings at 35°C were lower than the RLU readings observed at 10°C and 20°C (Graphs 1.1, 1.10). Optimum signal intensity was observed at 20°C.

Biocontrol Lightning MVP ICON® ATP Test System
The mean RLU readings for the BioControl MVP ICON devices (Graphs 1.2, 1.12, 1.13) indicate that the RLU signal increased further after the point of activation, which suggests that the enzyme reaction had not fully completed. However, an overall decline was observed over the 2 minutes at 20°C and 35°C. At 10°C, the RLU signal was relatively stable with a maximum signal intensity change of 19.73% when read after 2 minutes. The signal was more unstable at 35°C with up to a 52% change in the signal intensity over 2 minutes. The optimum signal intensity was recorded at 35°C (Graph 1.12), however, at this temperature the signal showed rapid decline. The least percentage decay was observed at 20°C over the 2 minutes.

Charm novaLUM FieldSwab Plus ATP Test System
The RLU signal for the Charm novaLUM with FieldSwab was highly unstable at 35°C, shown by the signal decay of 96% at 1 minute, and nearly 100% at 2 minutes, after activation (Graphs 1.14, 1.15, Table 1.1). This indicates that the system was highly time and temperature dependent. The optimum signal intensity was 20°C, which supported the use instructions targeting room temperature stability. RLU values on either side of this 20°C optimum were considerably lower (Graphs 1.3, 1.14).

Charm novaLUM PocketSwab Plus ATP Test System
Mean RLU values for the Charm novaLUM PocketSwab Plus were considerably lower at 10°C and 35°C. At 35°C, the RLU signal decayed by 100% in less than 1 minute, showing the test device to be highly time and temperature dependent at that temperature. At 20°C, the signal decay dropped by 18% (Table 1.1) over the 2 minutes. The optimum signal intensity was observed at 20°C (Graphs 1.4, 1.16, 1.17), which is consistent with room temperature stability.

Hygiena EnSURE™ SuperSnap ATP Test System
The mean RLU results for the Hygiena SuperSnap (Graphs 1.5, 1.18, 1.19) indicate that the signal is highly time dependent. After 1 minute following activation, the signal had decreased by >53% at all temperatures studied (Table 1.1). The results also indicate that the optimum RLU signal for this device was observed at 35°C (Graph 1.5).

Hygiena EnSURE™ UltraSnap ATP Test System
The mean RLU observed for the UltraSnap test device showed an initial slight increase in signal at 10°C and 20°C, suggesting that the Luciferase/Luciferin reaction had not fully completed. However, a consistent decrease in RLU signal value was observed at 35°C (Graph 1.6). The percentage decay over time results (Table 1.1) indicate that at 20°C, the signal was relatively stable with <20% change over 2 minutes. The optimum RLU signal observed for this device was found at 35°C at time zero.

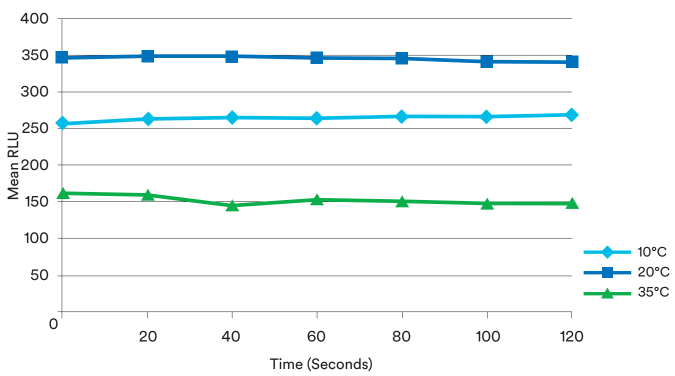
Neogen™ Accupoint® Advanced Access ATP Test System
The mean RLU results for the Neogen Accupoint Advanced Access ATP test devices (Graph 1.7, 1.22, 1.23) show that there is a decrease in signal under all temperature variables over the 2-minute period. The signal value at 10°C increased during the first 60 seconds with an off-scale reading of 134% change in signal (Graph 1.23) followed by a rapid signal decline of >50% (Graph 1.23) change in signal at 2 minutes. At 20°C, the signal declined by >50% over the first 60 seconds, and >90% by 2 minutes following activation of the device, indicating that the test device is time and temperature dependent. The difference in signal mean RLU values at each temperature set point was also observed to be notably different e.g., the mean RLU varied between 7 RLU (10°C), 71 RLU (20°C) and 205 RLU (35°C). These results indicate that this system is highly time and temperature dependent. The optimum signal was observed at 35°C (Graph 1.22); however, a decrease of more than 90% (Table 1.1) of signal value was also observed at this temperature over 2 minutes.

Neogen™ Accupoint® Advanced Surface ATP Test System
The results for the Neogen Accupoint Advanced Surface ATP test devices (Graphs 1.8, 1.24, 1.25) show a rapid decrease in signal value at 20°C over the 2-minute testing period. Table 1.1 shows a 93% decay in signal compared to time zero at 20°C. At 35°C, 100% of the signal has decayed within 30 seconds of the first RLU reading following activation. The RLU values at 10°C and 35°C are notably lower than at 20°C (89% and 87% lower respectively). The results indicate that this test device system is highly time and temperature dependent.

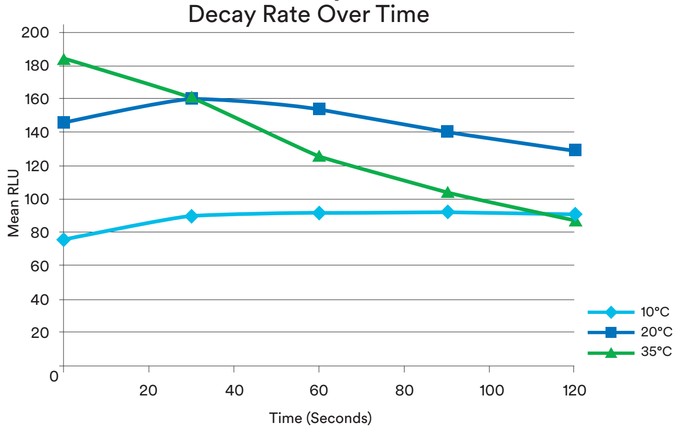
Kikkoman Lumitester PD-30 LuciPac™ ATP Test System
The results observed from the Kikkoman Lumitester PD-30 LuciPac ATP test system device showed an initial increase in the RLU signal value (Graph 1.9, 1.26) at both 10°C and 20°C, which indicated that the enzyme reaction may not have fully completed following activation and reading of the test device in the lumitester chamber. The RLU values shown at 10°C were much lower than those at 20°C and 35°C, which again is consistent with the user instructions. At all temperature variables, the RLU values were stable, with a maximum RLU signal decay percentage change of <15% when compared to the RLU value at time zero recorded at 35°C. The percentage RLU signal decay at 20°C was <4% when compared to time zero. Results suggested that the optimum operating temperature for this test device was 20°C (Graphs 1.9, 1.26, 1.27), which is consistent with the user instruction manual.

1. Mean RLU Signal over 2 minutes

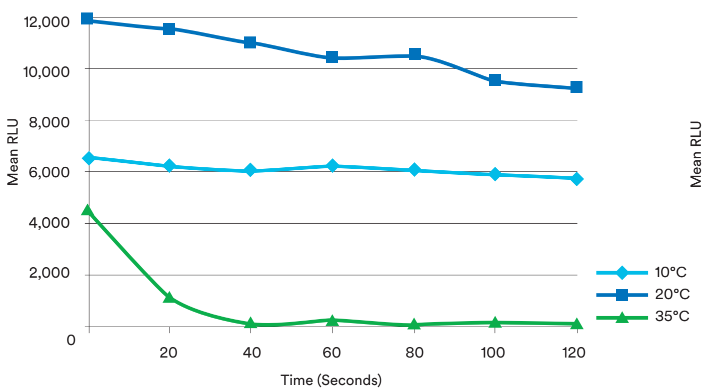
Graph 1.1: 3M Clean-Trace Surface ATP Test System
Decay Rate Over Time



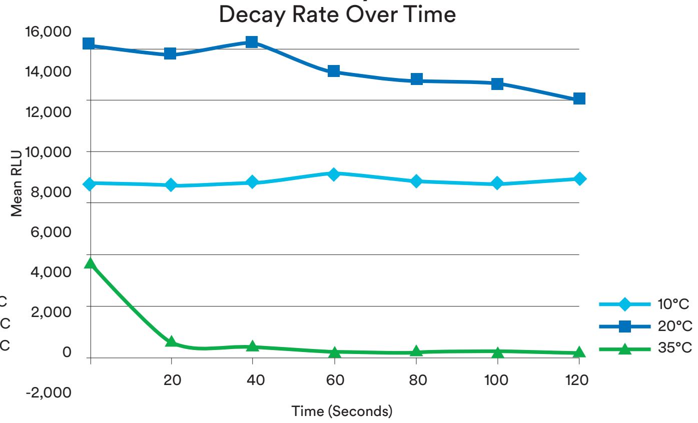
Graph 1.2: BioControl Lightning MVP ICON ATP Test System
Decay Rate Over Time



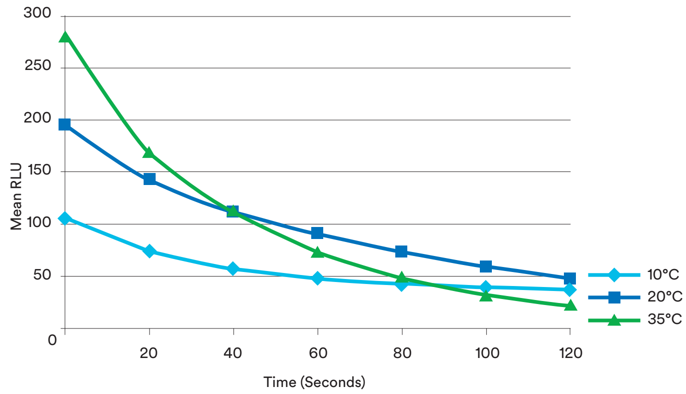
Graph 1.3: Charm novaLUM FieldSwab
ATP Test System
Decay Rate Over Time



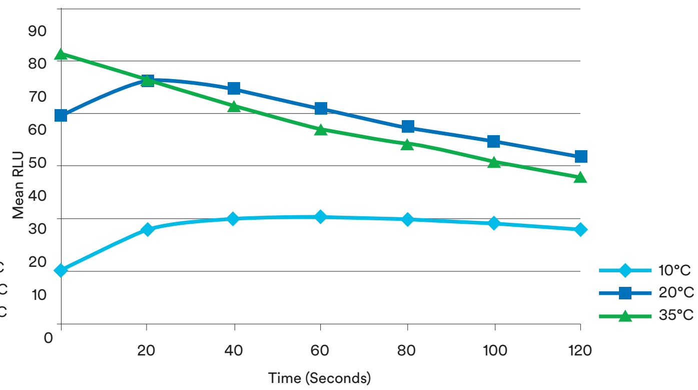
Graph 1.4: Charm novaLUM PocketSwab Plus
ATP Test System
Decay Rate Over Time



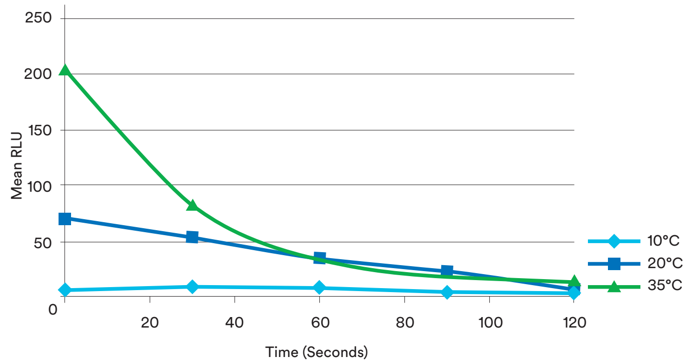
Graph 1.5: Hygiena EnSURE SuperSnap
ATP Test System
Decay Rate Over Time



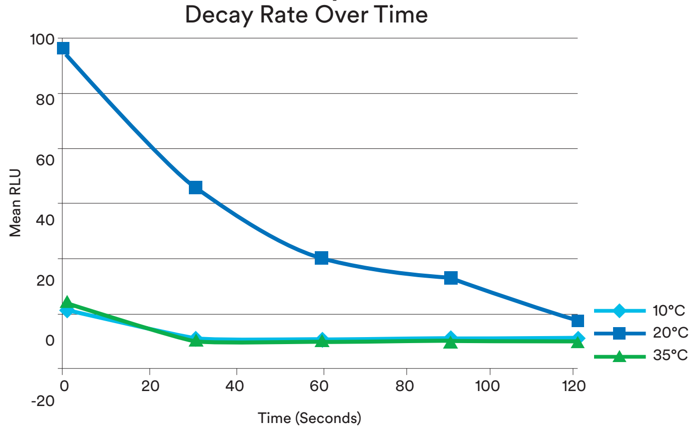
Graph 1.6: Hygiena EnSURE UltraSnap
ATP Test System
Decay Rate Over Time



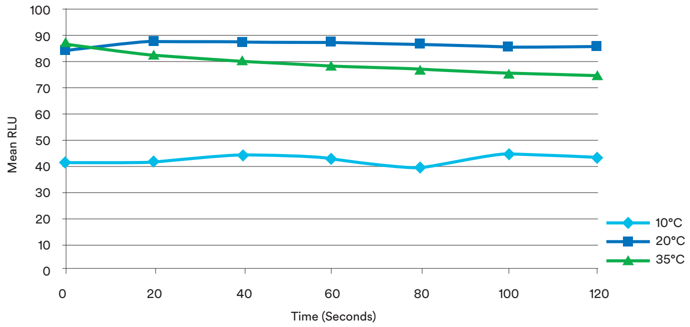
Graph 1.7: Neogen Accupoint Advanced Access
ATP Test System
Decay Rate Over Time



Graph 1.8: Neogen Accupoint Advanced Surface
ATP Test System
Decay Rate Over Time

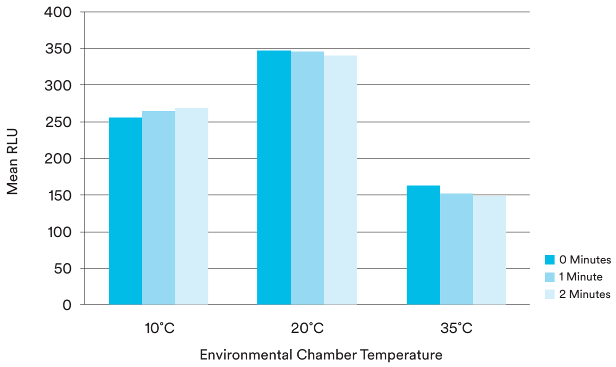


Graph 1.9: Kikkoman Lumitester PD-30 LuciPac
ATP Test System
Decay Rate Over Time

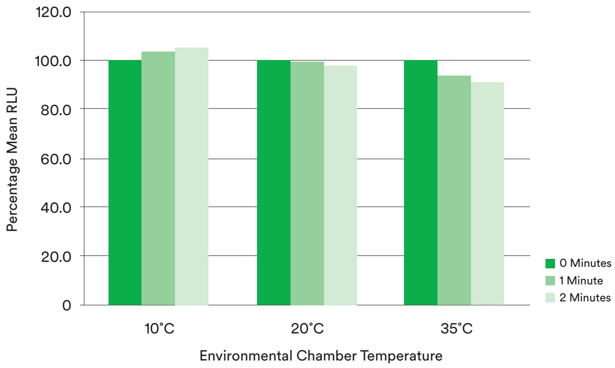


2. Mean RLU Change at Time 0, 1 minute and 2 minutes.

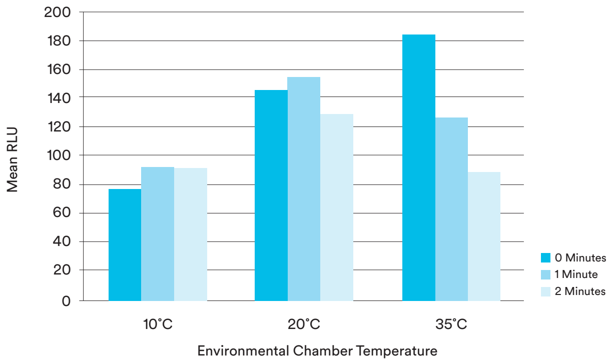
Graph 1.10: 3M Clean-Trace Surface
ATP Test System
Change in RLU Over Two Minutes



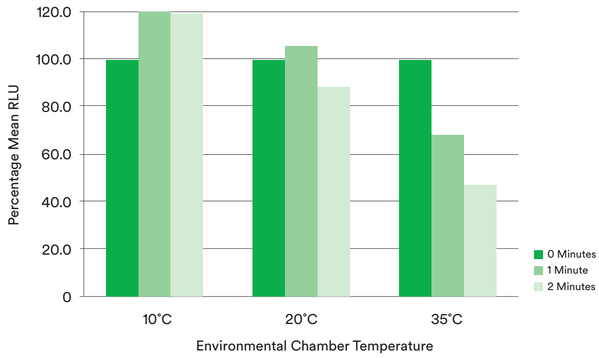
Graph 1.11: 3M Clean-Trace Surface
ATP Test System
Percentage Change in Signal Over Two Minutes



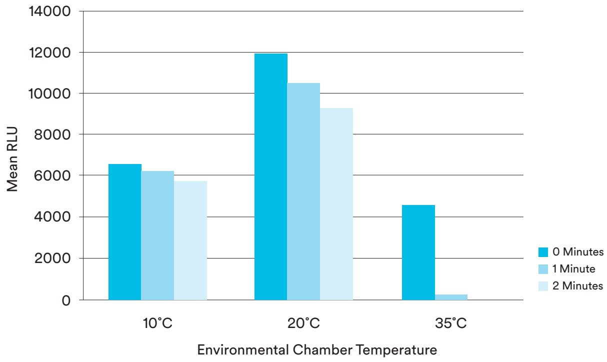
Graph 1.12: BioControl Lightning MVP ICON
ATP Test System
Change in RLU Over Two Minutes



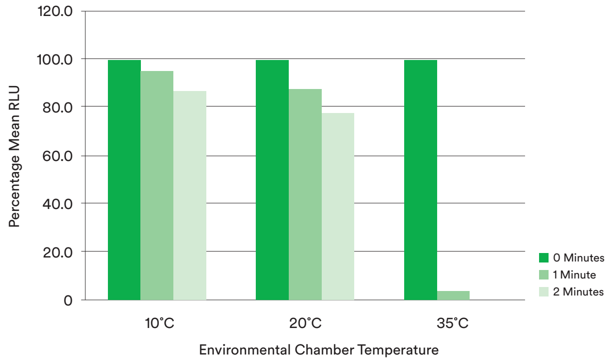
Graph 1.13: BioControl Lightning MVP ICON
ATP Test System
Percentage Change in Signal Over Two Minutes



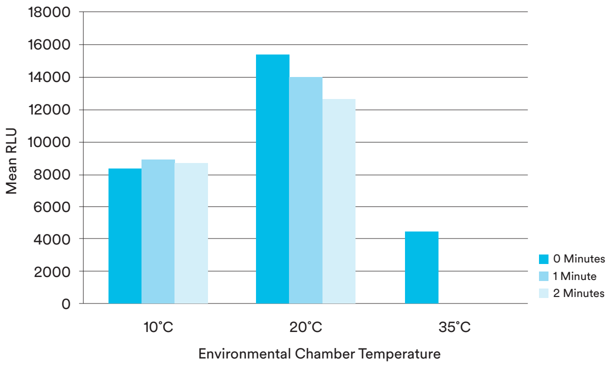
Graph 1.14: Charm novaLUM FieldSwab ATP Test System
Change in RLU Over Two Minutes



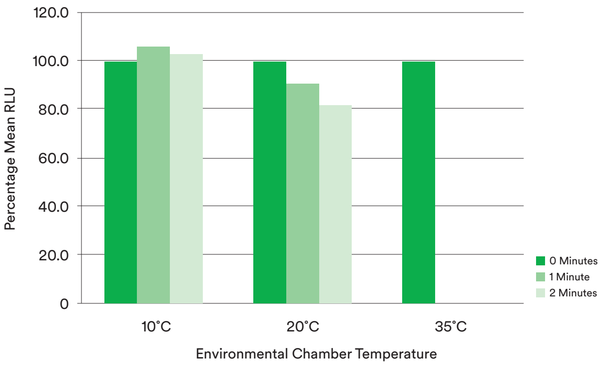
Graph 1.15: Charm novaLUM FieldSwab ATP Test System
Percentage Change in Signal Over Two Minutes



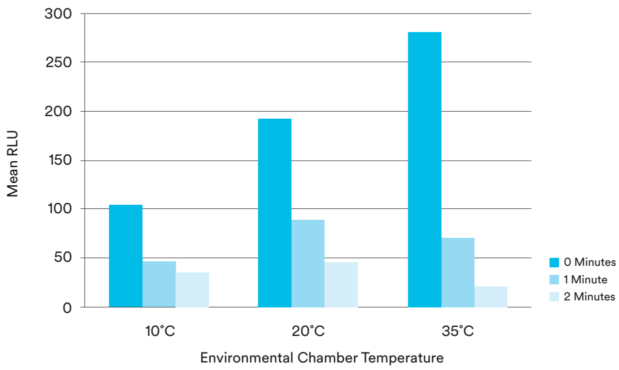
Graph 1.16: Charm novaLUM PocketSwab Plus ATP Test System
Change in RLU Over Two Minutes



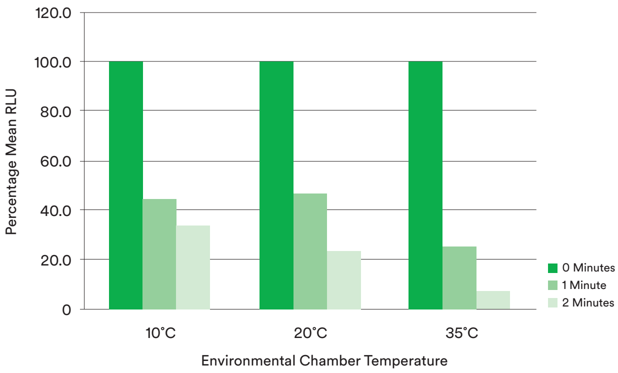
Graph 1.17: Charm novaLUM PocketSwab Plus ATP Test System
Percentage Change in Signal Over Two Minutes



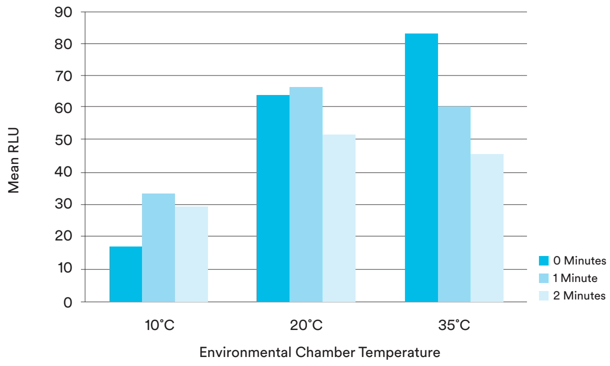
Graph 1.18: Hygiena EnSURE SuperSnap ATP Test System
Change in RLU Over Two Minutes



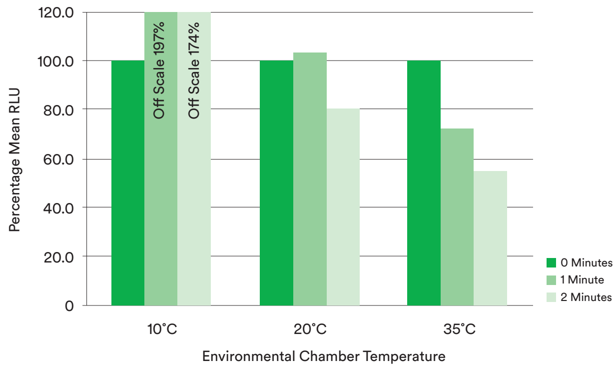
Graph 1.19: Hygiena EnSURE SuperSnap ATP Test System
Percentage Change in Signal Over Two Minutes



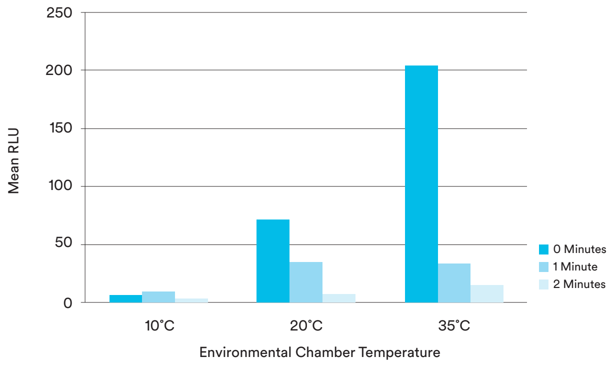
Graph 1.20: Hygiena EnSURE UltraSnap ATP Test System
Change in RLU Over Two Minutes



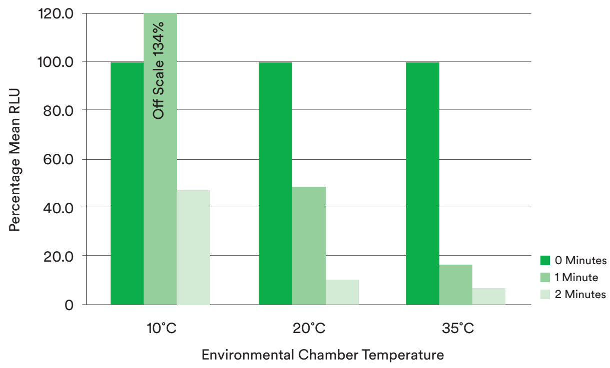
Graph 1.21: Hygiena EnSURE UltraSnap ATP Test System
Percentage Change in Signal Over Two Minutes



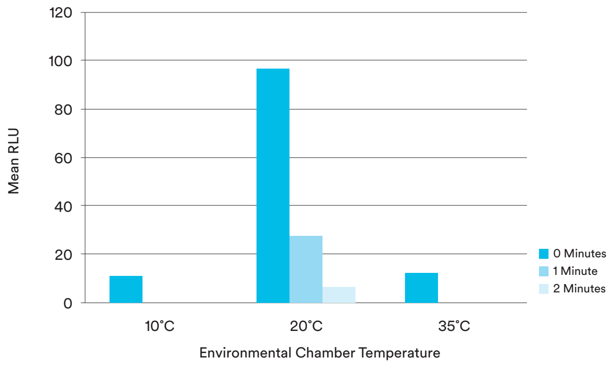
Graph 1.22: Neogen Accupoint Advanced Access ATP Test System
Change in RLU Over Two Minutes



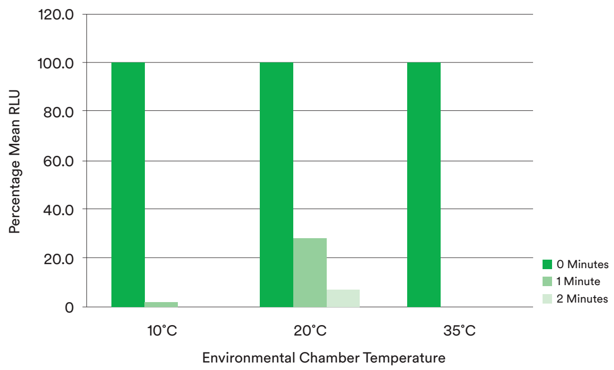
Graph 1.23: Neogen Accupoint Advanced Access ATP Test System
Percentage Change in Signal Over Two Minutes



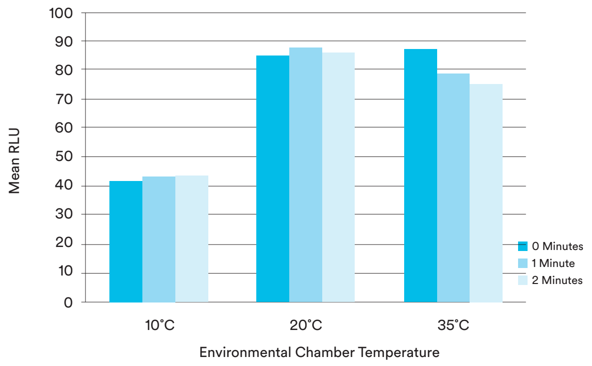
Graph 1.24: Neogen Accupoint Advanced Surface ATP Test System
Change in RLU Over Two Minutes



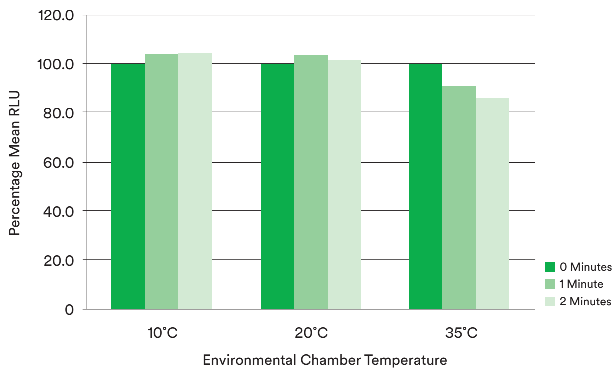
Graph 1.25: Neogen Accupoint Advanced Surface ATP Test System
Percentage Change in Signal Over Two Minutes



Graph 1.26: Kikkoman Lumitester PD-30 LuciPac ATP Test System
Change in RLU Over Two Minutes



Graph 1.27: Kikkoman Lumitester PD-30 LuciPac ATP Test System
Percentage Change in Signal Over Two Minutes



3. Percentage Decay Rate per Minute at 10°C, 20°C and 35°C

Table 1.1: Percentage (%) Signal Decay Per Minute						
ATP Test Device	Temperature Variable					
	10°C		20°C		35°C	
Time (Minutes)	1 min	2 min	1 min	2 min	1 min	2 min
3M Clean-Trace™ Surface ATP Test System	+3.56%	+5.13%	-0.40%	-1.93%	-6.02%	-8.66%
Biocontrol Lightning MVP ICON ATP Test System	+19.73%	+18.23%	+5.57%	-11.68%	-31.41%	-52.17%
Charm novaLUM FieldSwab ATP Test System	-4.84%	-11.19%	-12.18%	-22.22%	-96.55%	-99.43%
Charm novaLUM PocketSwab Plus ATP Test System	+6.12%	+3.30%	-9.20%	-18.11%	-100.00%	-100.00%
Hygiena EnSURE SuperSnap ATP Test System	-55.41%	-65.84%	-53.62%	-76.08%	-74.28%	-92.50%
Hygiena EnSURE UltraSnap ATP Test System	+97.04%	+73.96%	+3.60%	-19.41%	-27.50%	-45.24%
Neogen Accupoint Advanced Access ATP Test System	+33.85%	-52.31%	-51.34%	-89.53%	-83.49%	-92.97%
Neogen Accupoint Advanced Surface ATP Test System	-98.18%	-100.00%	-71.65%	-93.25%	-100.00%	-100.00%
Kikkoman Lumitester PD-30 LuciPac ATP Test System	+3.85%	+4.57%	+3.42%	+1.18%	-9.75%	-14.33%
Colour Key:	Less than 10% change in signal			Greater than 10% change in signal		

Discussion

Impact of testing at variable environmental temperatures:
Food manufacturers operate in a variety of temperatures, depending on the foods they produce. Surface sampling and data collection would be performed at environmental temperatures typically ranging from 15°C to 30°C. However, some ATP systems perform inconsistently, providing stable results at some temperatures but unstable results when operating temperatures increase or decrease. Therefore, the user should consider their process environment operating temperatures and select a testing system that ensures the validity and consistency of results.

The 3M Clean-Trace Surface ATP Test System demonstrated stable and consistent RLU signals at each of the temperature test conditions (10°C, 20°C and 35°C). This is essential for the reliability and repeatability of swab results, which enable the users to compare and trend data. This information can be used for compliance and due diligence as part of an effective hygiene management system.

Impact of time dependency
The time it takes to complete an ATP test can differ when testing the same site from day to day or even between shifts, due to time delays or differences in technique. Once a test is activated, time delays can occur when technicians are distracted, have unexpected conversations or need to navigate around equipment in the plant. Also, experienced technicians may work faster than newer personnel.

The following ATP test systems were highly time-dependent at 20°C: Charm NovaLUM FieldSwab, Neogen Accupoint Advanced Access, Neogen Accupoint Advanced Surface and Hygiena EnSure SuperSnap. The RLU signal decay was apparent at 20 seconds for the Hygiena EnSURE SuperSnap ATP test system and Charm novaLUM Fieldswab, and at 30 seconds for the Neogen Accupoint Advanced Access and Neogen Accupoint Advanced Surface ATP test systems. This rapid signal decay may lead to inaccurate and unreliable cleaning verification results and actions.

The system with the most stable time dependency results at 20°C was the 3M Clean-Trace Surface ATP test system with a 0.4% signal decay over 60 seconds and <2% decay over 2 minutes. The 3M Clean-Trace Surface ATP test system was the only system to consistently achieve achieve less than 10% signal decay values over 2 minutes post-activation.

Conclusions

Food manufacturing businesses are increasingly under pressure to maintain and improve hygiene monitoring within the manufacturing environment to ensure compliance with food safety management systems. Many manufacturers use ATP hygiene monitoring systems to reinforce their cleaning protocols.

User confidence in the reliability and repeatability of an ATP system is imperative, thereby an ATP system that is easily understood and capable of accurately monitoring hygiene in varying environments would support this. If a system is not reliable and repeatable it may lead to misinterpretation of data and lead to decisions that could affect food safety and production efficiencies.

It is important that the optimum operating temperature and time constraints for the ATP system are clearly communicated and understood by users. It is paramount that the hygiene monitoring system provides reliable, accurate and repeatable results to ensure consistency of data collection. This will provide the food manufacturing and hygiene teams with insight and information to manage hygiene processes and practices within the organisation effectively. Food manufacturers must implement effective and accurate hygiene assessments to optimise good hygiene control compliance, process efficiency and continual improvement of plant hygiene standards.

In this study, 3M Clean-Trace Hygiene Monitoring and Management System was the only ATP detection system to consistently and repeatedly perform across the varying time and temperatures. This system can provide confidence in results of ATP testing and help ensure hygienic conditions to produce safe food products.

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