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A Comparative Study of Commercial ATP Hygiene Monitoring Systems

The most comprehensive evaluation of ATP hygiene monitoring products has just been completed by one of the world's premier food & beverage reference laboratories. The purpose of the study was to look at the performance of 5 different ATP systems, the technologies used and how these systems worked with different samples. By the thorough application of good science, Hygiena hopes that this study becomes the foundation for further studies that evaluate ATP technology.

Overview
1. Scope of the study
2. Components of an ATP system
3. Performance results based on study criteria
4. Summary

Systems Evaluated

BioControl Lightning MVP   **Charm Science NovaLum**   **Hygiena SystemSURE Plus**   **Neogen Accupoint**   **3M Unilite NG Cleantrace**

Note:

This presentation was prepared by Hygiena and is based on data generated from the "Performance Evaluation of Various ATP Detecting Units" report done by Silliker Inc. Food Science Center Report RPN 13922 (2010). The conclusions presented in this literature piece are based on the data from the Performance Evaluation of Various ATP Detection Units report. Silliker, Inc. does not endorse or support the claims of this marketing information sheet, but does support all data and claims made in the Performance Evaluation of Various ATP Detecting Units report done by Silliker Inc. Food Science Center Report RPN 13922 (2010). The entire report is available from Hygiena upon request.

Scope of the Study
Determine the performance of leading ATP systems for
the detection of ATP, food residues and microorganisms

The purpose of ATP bioluminescence for hygiene monitoring is to provide a simple, rapid, direct, objective test for cleaning verification. It is an accurate, sensitive indicator test to instantly determine the hygienic status and potential risk of the object being sampled.

Unlike microbiological tests that take days to yield results, ATP testing provides valuable information in seconds. The results from ATP surface hygiene monitoring are different to those of microbial enumeration methods and give additional information that microbial tests cannot provide.

ATP tests are not intended to replace environmental microbial testing, but to be used in addition to such tests. By doing this you can reduce the amount of environmental microbial testing and verify the efficacy of cleaning programs in seconds versus days. There is concurrent direct correlation between the results of these two methods, because cleaning simultaneously removes both organic residues and microorganisms.

Performance Metrics
The key performance metrics evaluated:
1. Linearity
2. Repeatability
3. Sensitivity
4. Accuracy

Performance Criteria
3 different criteria were selected. This was considered by the reference lab and Hygiena to be a thorough analysis of the systems and the most thorough analyses done to date on ATP systems. All instruments and test devices were purchased by the reference lab from the original manufacturers to maintain the performance integrity of the system.

1. ATP Serial Dilution Study - Serial dilutions of ATP were pipetted directly onto the sample collection point of each test device. By using ATP as a control, user and sample variability are eliminated. In addition ATP was pipetted into the reading area of each device to measure how much of the ATP reaction is lost due of interference by the swab tip. The difference in readings from the two experiments shows accuracy of the system. Neogen was left out of the second part of this test because of the inability to access the reading area of the device.

2. Dilutions of Foodstuff - 4 food groups were selected and serial dilutions of each were prepared. For the first part, dilutions were pipetted directly onto the sample collection point of each test device, and in the second part, dried on stainless steel surfaces to replicate true environmental conditions.

3. Dilutions of Microbial Cultures - Serial dilutions of 6 microorganisms (Escherichia coli, Lactobacillus plantarum, Pseudomonas aeruginosa, Salmonella typhimurium and Staphylococcus aureus and one yeast culture, Saccharomyces cerevisiea) that are of concern to the food & beverage industry were cultured and dilutions were tested on each system.

Components of an ATP system

3 components make up an ATP system

Instrument + Bioluminescence Chemistry + Test Device Design = **OVERALL PERFORMANCE**
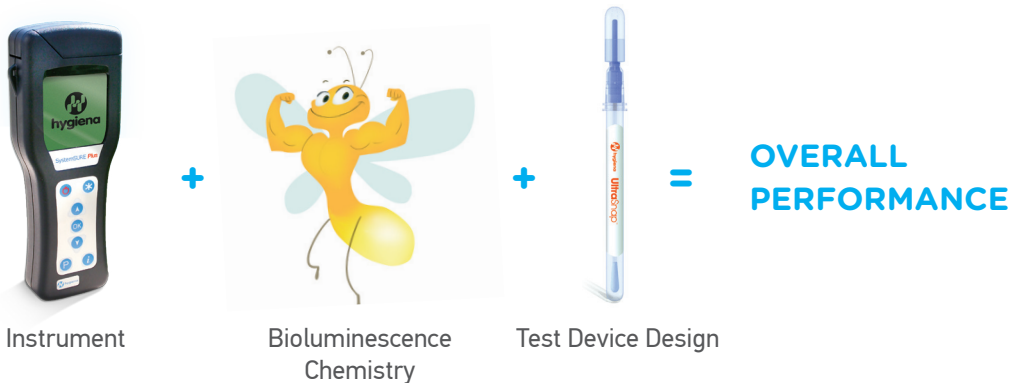
ATP System Overview

An ATP system consists of 3 components. Each component is a critical part in overall performance.

1. Instrument – Luminometer (2 types were evaluated)
- Photodiode: sensitive, robust, requires low voltage, does not come out of calibration over time (reducing maintenance costs and down time). It is low cost and has low background noise.
- Photomultiplier Tube: sensitive, fragile, requires high voltage, comes out of calibration over time (requiring yearly maintenance). It is expensive and has high background noise.

2. Bioluminescent Chemistry (2 variations evaluated)
- Liquid stable chemistry: new technology allows for immediate reaction with sample, gives greater precision, accuracy, and more consistency. Fewer manufacturing processes result in lower costs.
- Lyophilized chemistry: old technology (>30 years) requires complex expensive manufacturing, dry storage and rehydration at point of use that has larger variability.

All chemistry uses luciferase/luciferin enzymes to generate light. The quality and quantity of the enzymes and the other components determine the performance of the chemistry.

3. ATP Test Device Design
- ATP test device design is a critical part to system performance and usability. Good test device design will have excellent sample collection and containment of the sample, low background signal, easy device activation and mixing of sample with chemistry and is easy for the customer to use in their environment (flat surfaces, hard to reach areas and water/liquid samples).
- All test devices have a pre-moistened extractant on the swab bud to break through biofilms to collect and release ATP from the sample.
- Four of the test devices tested used a swab tip (BioControl, Charm Science, Hygiena, 3M) and one used a sponge-like tip (Neogen).
- The activation step when the mixing of sample and chemistry take place, also affects performance and is an important part of test device design. Two of the test devices evaluated required the swab tip to puncture through foil seals during activation (Charm, 3M); three test devices had chemistry react with the sample without having to puncture through foil seals (BioControl, Hygiena, Neogen).

5 Systems and Components
Breakdown of each system

BioControl Lightning MVP and MVP Surface sampling device
Type of Sensor: Photomultiplier tube based system
Chemistry Format: Lyophilized pellet
Collection Point: Woven swab
Activation Step: Swab bud does not puncture foil seals to mix sample and chemistry

1.

Charm Science Novalum and Pocketswab Plus
Type of Sensor: Photomultiplier tube based system
Chemistry Format: Lyophilized pellet
Collection Point: Foam swab
Activation Step: Requires puncturing of foil seals to mix sample and chemistry

2.

Hygiena SystemSURE Plus with Ultrasnap and Supersnap devices
Type of Sensor: Photodiode based system
Chemistry Format: Liquid stable
Collection Point: Woven swab
Activation Step: Swab bud does not puncture foil seals to mix sample and chemistry

3.

Neogen AccuPoint and surface sampler
Type of Sensor: Photodiode based system
Chemistry Format: Lyophilized pellet
Collection Point: Sponge swab
Activation Step: Swab bud does not puncture foil seals to mix sample and chemistry

4.

3M UniLite NG CleanTrace and CleanTrace device
Type of Sensor: Photomultiplier based system
Chemistry Format: Liquid stable
Collection Point: Woven swab
Activation Step: Requires puncturing of foil seals to mix sample and chemistry

5.

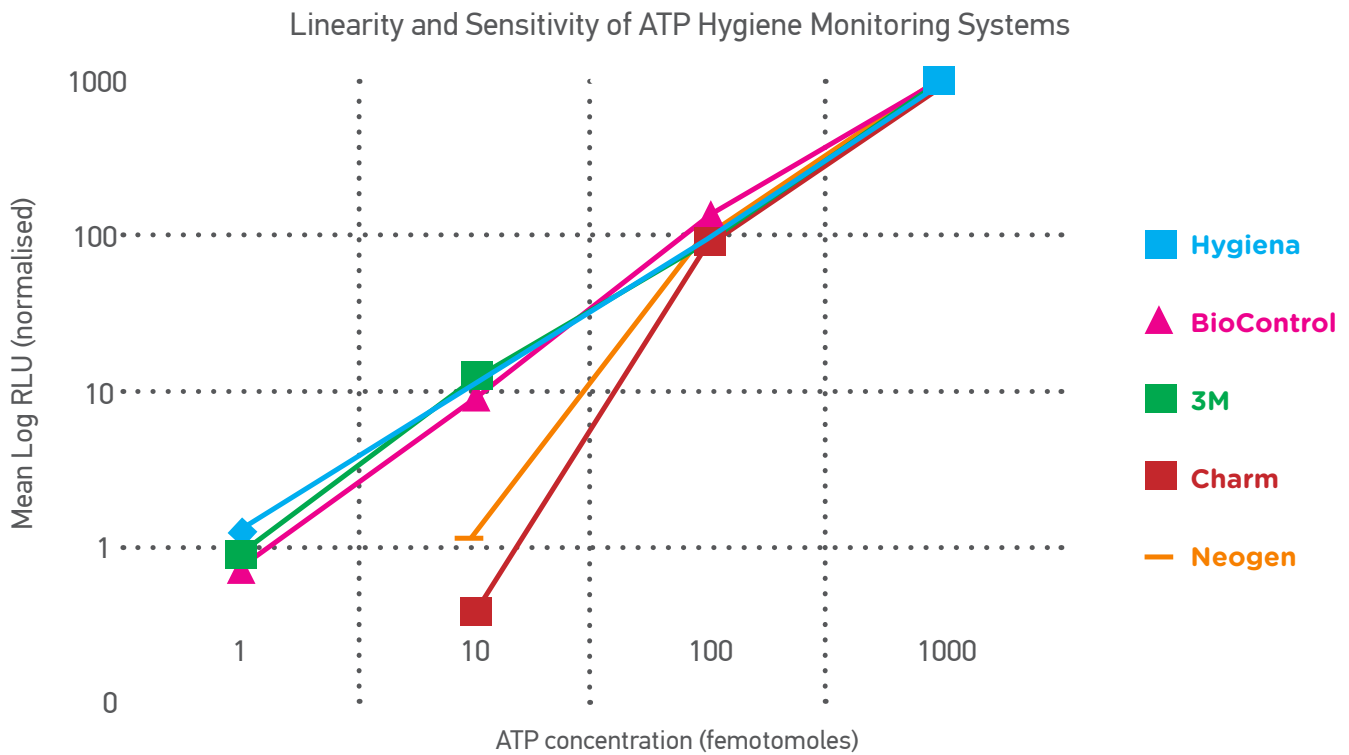# Section 1 - ATP Serial Dilutions Performance Results
Evaluation of Linearity

Section Overview:

Serial dilutions of ATP were pipetted directly onto the sample collection point of each test device. By using ATP as a control, user and sample variability are eliminated and true performance of the system can be evaluated.

In addition ATP was pipetted into the reading area of each device to measure how much of the ATP reaction is lost due to interference by the swab tip. The difference in readings from the two experiments shows accuracy of the system. Neogen was left out of the second part of this test because of the design of the test device (see page 8).

Linearity: Expression of predictability and reliability of the result

Linearity and Sensitivity of ATP Hygiene Monitoring Systems

Legend:
- Hygiena
- BioControl
- 3M
- Charm
- Neogen

X-axis: ATP concentration (femotomoles)
Y-axis: Mean Log RLU (normalised)

Graph

The linearity graph shows a direct, proportional, straight-line relationship between RLU and ATP. Linearity is described by the term Correlation Coefficient (r) which shows how well the data approaches the perfect relationship (r = 1.0) between ATP and RLU. If a perfect relationship exists, r = 1.0 implies that the linear equation describes the relationship between X and Y perfectly, with all data points lying on a line for which Y (RLU) increases as X (ATP) increases in a constant predictable way. The closer to r = 1.0 a system can get, the more precise and reliable the results will be. The further away from r = 1.0 a system gets, the less reliable the results.

Best Performer(s)

BioControl, Hygiena & 3M all showed good linearity for the detection of ATP (r = 0.98)

Evaluation of Repeatability

Repeatability: Variability in results with the same sample
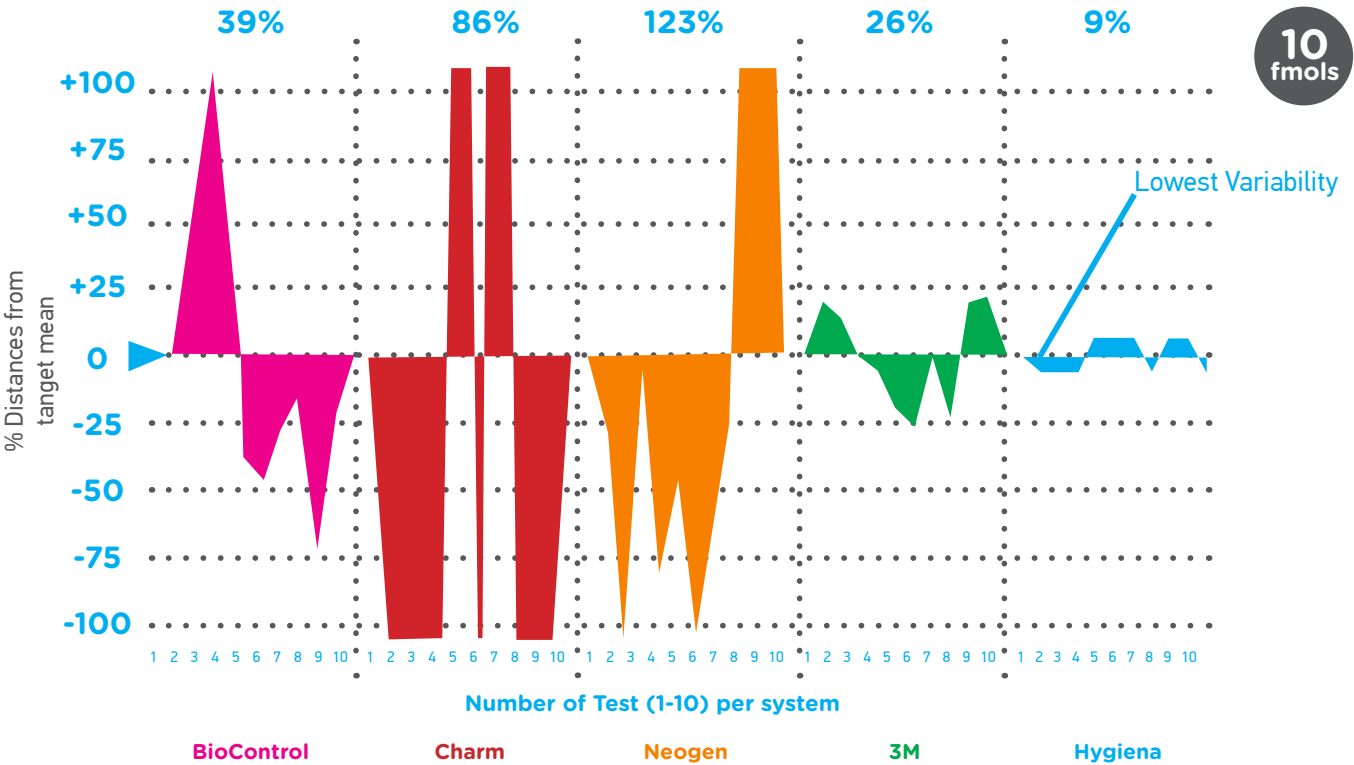
Graph

Repeatability is described by the term Coefficient of Variation (CV%). The higher the CV%, then the greater the variability between each result with the same sample. More variability means the results are less consistent and more unreliable. Further detailed analysis reveals even greater differences between systems at critical levels of 10 and 100 fmols ATP where recommended Pass / Fail limits are usually set. The graphs show that only Hygiena can consistently deliver dependable results at low ATP levels that are required for the maintenance of high standards of quality and safety.

Best Performer(s)

Hygiena and Supersnap: CV = 9%

Sensitivity: Smallest detectable amount of sample

System	ATP Detection (fmoles)					
	1000	100	10	5	1	0.1
BioControl MVP & Lighting	● ● ● ● ●	● ● ● ●	● ● ● ●	● ●		
Charm Novalum & Pocketswab Plus	● ● ●	● ● ●	● ●			
3M UniLite NG & CleanTrace	● ● ● ●	● ● ● ●	● ● ●	● ●		
Hygiena SystemSURE Plus & Ultrasnap	● ● ● ●	● ● ● ●	● ● ● ●	● ● ●	●	
Hygiena SystemSURE Plus & Supersnap	● ● ● ●	● ● ●	● ● ● ●	● ● ●	● ● ●	●
Neogen AccuPoint	● ● ●	● ● ●	● ●			
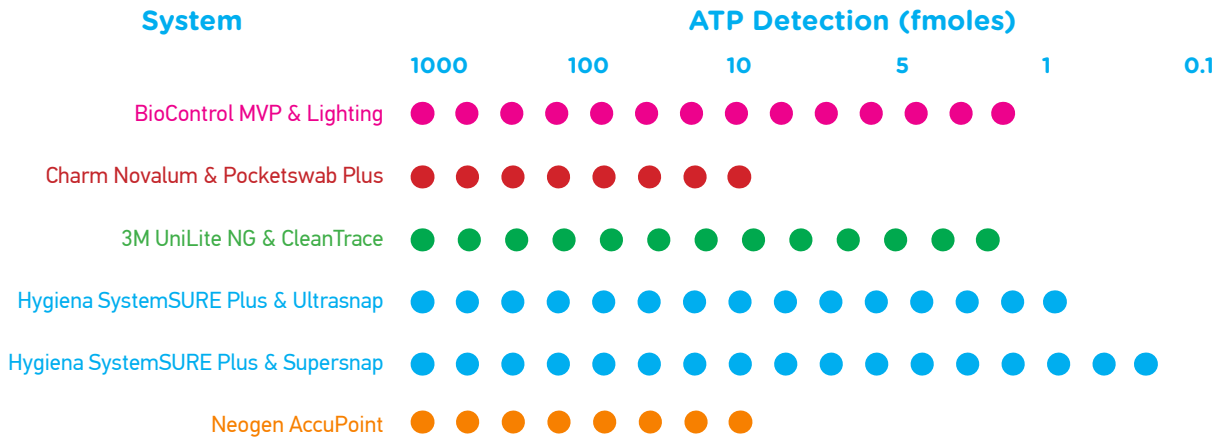
Low Background Good Sensitivity

Blank (no sample) Sample Present
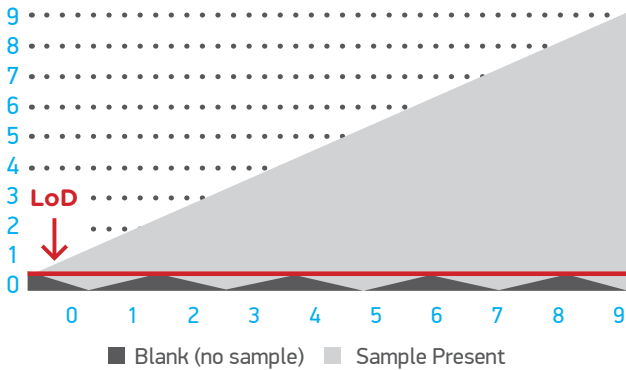
High Background Poor Sensitivity

Blank (no sample) Sample Present
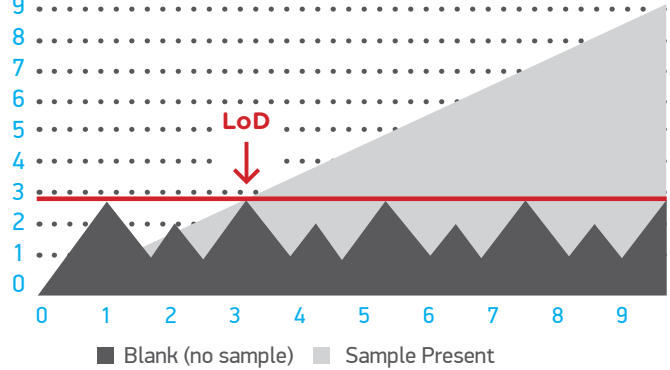
Graph

Sensitivity is defined as the Limit of Detection (LoD). It is the smallest amount of ATP detectable above the background noise of the system. Background noise is the signal (RLU value) detected by the system in the absence of ATP that can come from both the instruments (as electrical interference) and the reagent swab device (as chemical interference from impurities).

Best Performer(s)

Hygiena SystemSURE Plus & Supersnap: LoD = 0.17 fmoles

Accuracy: Recovery and detection of all available ATP to reflect the true value of the sample

Recovery of ATP on Swab (%)
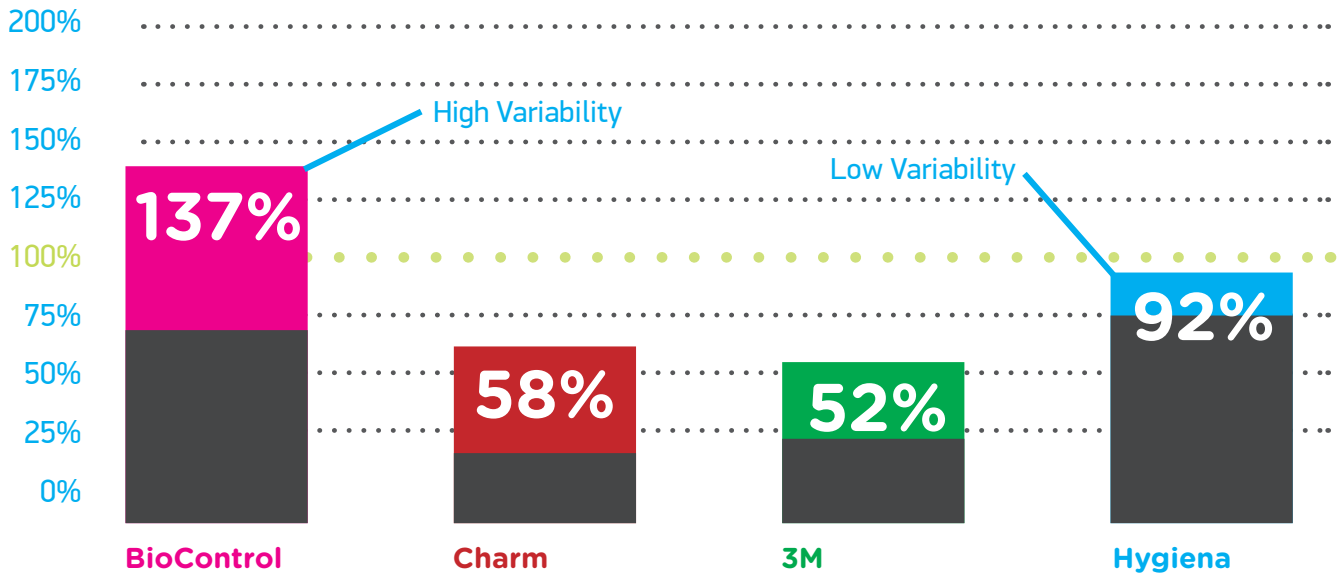
Graph

ATP (at 100 fmols) was added to each test device and measurements were made (using 10 replicates) to determine how much of the available sample was actually detected. If 100% of the ATP was detected, then the system is accurate and gives a true meaningful result. Less than 100% means that only part of the sample was detected due to some interference within the system. This means that the system does not give a true result and is not 100% accurate. Over 100% indicates extra signal coming from the test device.

Best Performer(s)

Hygiena: 92% recovery

Section 1 - Summary Performance Results

Evaluation of Linearity, Repeatability, Sensitivity and Accuracy

Summary of ATP Detection

System	Linearity	Output (RLU)		Variability	Sensitivity
	(r)	Blank (Background at zero ATP)	Maximum (at 1000 fmols ATP)	Overall Average (CV%)	Limit of detection (fmols ATP)
BioControl Lightning MVP with Lightning swab	0.982	283	975,941	39%	1.1
3M UniLite NG with CleanTrace swab	0.988	4.3	7386	26%	1.3
Charm Novalum with Pocketswab Plus	0.949	0 *	418,517	86%	10.0
Hygiena SystemSURE Plus with Ultrasnap swab	0.988	0 †	1589	28%	1.0
Hygiena SystemSURE Plus with Supersnap swab	0.987	0 †	4949	9%	0.17
Neogen Accupoint with Accupoint swab	0.976	0 *	15,649	123%	10.0

This chart shows the overall mean average results of all the systems for the controlled ATP section of the study. Results show that all ATP system have a linear reaction. As more ATP is added to the test the RLU result will increase proportionally. Further analysis of the data shows that variability and sensitivity vary from system to system in a controlled environment. In the field, more variability would be introduced by the users and surfaces, resulting in more variability. Systems with less variability and better sensitivity therefore will give more accurate and consistent results.

Performance Observations

All instruments have different RLU values for a given amount of ATP. High RLU output does not equate to better sensitivity or performance. Charm system had highest RLU ouput and poorest sensitivity.

* in the Output column denotes that the system has background signal that it must compensate for and that a zero reading on these systems is not a true zero. These systems were the least sensitive.

† in the Output column denotes that the system has a true zero background on blank swabs. There is no background signal created by the system or test device, which means any signal detected comes from the sample.

Section 1 - Summary
Performance Results

Evaluation of Linearity, Repeatability, Sensitivity and Accuracy

Performance	Linearity	Sensitivity	Reapeatability	Accuracy
Best Performance	Hygiena BioControl 3M	Hygiena	Hygiena	Hygiena
Average Performance	Charm Neogen	BioControl 3M	BioControl 3M	BioControl
Worst Performance		Charm Neogen	Charm Neogen	Charm Neogen 3M

"The Hygiena swabs collectively are more sensitive to ATP and better at detecting low level food and cultures than all other systems."

Page 3 - Executive Summary, Performance Evaluation of Various ATP Detecting Units.

Section 2 - Food Residue Detection Performance Results

Section Overview:

ATP systems are generally used as a cleaning verification test. In order to replicate an environment where the systems are typically used, 4 food items (pasteurized milk, ground beef, orange juice, mixed green salad)were diluted down to low concentrations and 10μL samples were tested. In addition, dilutions were added to stainless steel surfaces and tested wet and dried. Liquid food samples (orange juice and milk) were diluted using ATP-free sterile water (v/v). Solid food samples (ground beef and salad greens) were first stomached using 10g of sample in 90 ml ATP-free sterile water and then diluted using ATP-free sterile water

(w/w). All test samples were shaken by hand for 2 minutes for homogenization. For testing food samples on surfaces, 500μL of ground beef suspension at 1:10 and 1:1,000 dilutions and 500μL of milk at 1:1 and 1:1,000 dilutions were spread evenly onto individual 4x4 inch stainless steel surfaces and immediately tested after preparation. An additional set of stainless steel surfaces were prepared as described above and allowed to dry at room temperature for 18-24 hours. After drying, each individual stainless steel piece was swabbed and analyzed by each ATP unit. Ten replicates of each dilution were tested.

Food Residue Detection

Dilution of all foodstuff detected
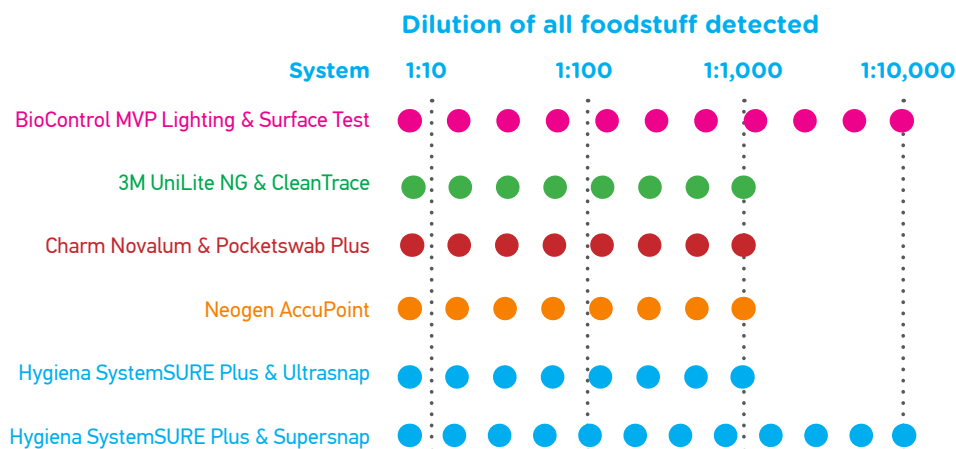
All systems showed the ability to detect foodstuff dilutions between 1:100 to 1:1,000 at 10μL. Only the Hygiena and BioControl systems detected dilutions at 1:10,000. Detection levels were very dependent on the food item tested (see graphs on page 12). Hygiena and BioControl were the only systems able to detect the lowest levels of all foods tested.

Dried dilutions of foodstuff detetcted on a surface
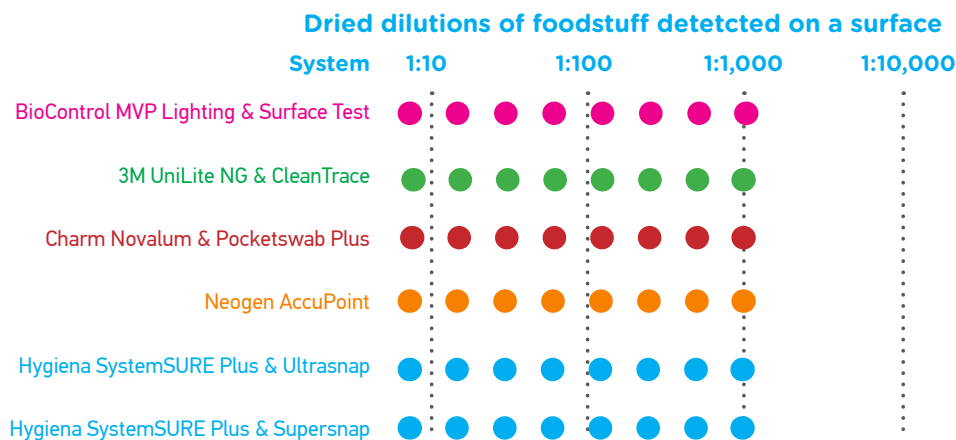
All systems were able to detect 500μL at 1:1,000 dilution of ground beef and milk dried on a surface.

10µL of Liquid Suspension

Dilution of ground beef

System	1:10	1:100	1:1,000	1:10,000
BioControl MVP Lighting & Surface Test				
3M UniLite NG & CleanTrace				
Charm Novalum & Pocketswab Plus				
Neogen AccuPoint				
Hygiena SystemSURE Plus & Ultrasnap				
Hygiena SystemSURE Plus & Supersnap				

Dilution of pasteurized milk

System	1:10	1:100	1:1,000	1:10,000
BioControl MVP Lighting & Surface Test				
3M UniLite NG & CleanTrace				
Charm Novalum & Pocketswab Plus				
Neogen AccuPoint				
Hygiena SystemSURE Plus & Ultrasnap				
Hygiena SystemSURE Plus & Supersnap				

Dilution of orange juice

System	1:10	1:100	1:1,000	1:10,000
BioControl MVP Lighting & Surface Test				
3M UniLite NG & CleanTrace				
Charm Novalum & Pocketswab Plus				
Neogen AccuPoint				
Hygiena SystemSURE Plus & Ultrasnap				
Hygiena SystemSURE Plus & Supersnap				

Dilution of mixed green salad

System	1:10	1:100	1:1,000	1:10,000
BioControl MVP Lighting & Surface Test				
3M UniLite NG & CleanTrace				
Charm Novalum & Pocketswab Plus				
Neogen AccuPoint				
Hygiena SystemSURE Plus & Ultrasnap				
Hygiena SystemSURE Plus & Supersnap				

Summary of Food Residue Detection

Performance	Linearity	Sensitivity	Reapeatability	Accuracy
Best Performance	Hygiena BioControl 3M Charm Neogen	Hygiena BioControl	Hygiena 3M BioControl	Hygiena BioControl
Average Performance		Hygiena 3M	Charm	3M Charm
Worst Performance		Charm Neogen	Neogen	Neogen

Section 3 - Microorganism Detection Performance Results

Section Overview:

Serial dilutions of 5 bacteria and 1 yeast culture were prepared and tested in each system to determine the limits of detection.

ATP systems are intended to detect very small amounts of organic material on surfaces or in liquid samples. Microorganisms are organic and contain ATP but at much lower levels than foodstuffs. Microorganisms contain different amounts of ATP depending on the type of microbe, its size and state of health. The test cannot differentiate food ATP from microbial ATP.

All organisms were detected and yeasts showed the highest amount of ATP because they have the greatest mass. There was large variation in results from all organisms, but good linearity was shown by all systems. ATP systems can detect these microorganism, but the limit of detection was shown to be 10,000 to 100,000 cfu/ml. This is generally not sensitive enough for most environmental microbial programs.

Microorganism Detection

Serial Dilution of Microorganisms

| System | Neat | 1:10 | 1:100 | 1:1,000 |

BioControl Lighting MVP & Surface Test
3M UniLite NG & CleanTrace
Charm Novalum & Pocketswab Plus
Neogen AccuPoint
Hygiena SystemSURE Plus & Ultrasnap
Hygiena SystemSURE Plus & Supersnap

Summary of Microorganism Detection

Performance	Linearity	Sensitivity	Reapeatability	Accuracy
Best Performance	Hygiena BioControl 3M Charm Neogen	Hygiena	Hygiena 3M	Hygiena
Average Performance		Hygiena BioControl 3M Charm	Hygiena BioControl Charm	BioControl 3M Charm
Worst Performance		Neogen	Neogen	Neogen

Conclusions

- 5 commercial ATP systems were compared for the detection and measurement of ATP, foodstuffs and microorganisms.

- All systems were shown to give a good linear response to all 3 sample types, however there was a difference in the sensitivity, repeatability and accuracy between systems.

- The most precise, accurate and repeatable system was the Hygiena SystemSURE Plus with either Ultrasnap or Supersnap test devices.

- The least sensitive and most variable systems were the Neogen AccuPoint and Charm Science Novalum systems with their respective test devices.

- ATP hygiene monitoring is a cleaning verification test, so accuracy and consistency at low ATP levels is critical. Study showed that Charm and Neogen have the poorest sensitivity and highest variability.

- Neogen's sponge sample collection tip did not collect a better sample then other test devices. Neogen was one of the worst performers in sensitivity, repeatabiltiy and accuracy when looking at ATP, food residues and microbial detection.

- SystemSURE Plus photodiode based system is equal to or better than photomultiplier tube based system.

- Data shows that each system displays a different RLU for any given sample. This could be confusing to users comparing systems. A larger RLU number does not mean a more sensitive reading. Charm displayed the highest RLU value result for each sample type and is one of the least sensitive systems.

- All ATP systems can detect microorganisms in the absence of other organic matter, but at a level of detection of 10,000 – 100,000 cfu/ml. This is generally a lot higher then cleaning standards allow. Detection of microorganisms showed a large variation based on species and quantity. Hygiena Supersnap had the best extraction efficiency for the recovery and detection of microbes.

- The properties of the ATP assay are well suited for the determination of cleanliness, with cleanliness being defined as the absence of organic (derived from life) material. Clean surfaces have little to no ATP, while dirty surfaces have ATP and perhaps live microbial cells. The result of an ATP test that is available in seconds permits the immediate assessment of the sampled area condition and whether additional cleaning action is required. Traditional method of determining cleanliness is the aerobic plate count, which requires two days to complete. This procedure is limited in the type of microorganisms it can detect and does not detect organic residue.

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**To receive the full Performance Evaluation of
Various ATP Detection Units report, please email or call us.**

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