A Comparative Study of Commercial ATP Hygiene Monitoring Systems

Based on data generated by Silliker Group Inc

Abstract

The performance of 5 leading commercially available of ATP hygiene monitoring systems were compared in the largest independent study of its kind. Linearity, Sensitivity, Repeatability, Precision and Accuracy are the major requirements of an effective ATP system.

The findings indicate that some of the systems show large variations and background interference which significantly affects their ability to meet the performance criteria.

The best system was revealed to be Hygiena SystemSURE Plus and the poorest systems were Charm Novalum and Neogen Accupoint.

Scope of the Study:
Determine the performance of ATP detection systems

Purpose & Intent for ATP Hygiene Monitoring

The purpose of ATP bioluminescence for hygiene monitoring is to provide a simple, rapid, direct, objective test for cleaning verification. It is a sophisticated, 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

Performance Criteria

The key performance criteria evaluated were:
1. Linearity
2. Sensitivity
3. Repeatability
4. Accuracy

These performance criteria were determined by pipetting ATP dilutions on to the swab bud under controlled laboratory conditions.

ATP Detection Systems:
Three key components make up a system

An ATP system consist of 3 components.
Each component is a critical aspect of overall performance.

1. **Instrument – Luminometer (2 types were evaluated)**
   - Photodiode: sensitive, robust, requires low voltage, does not drift with time. It is low cost and has low background noise.
   - Photomultiplier Tube: Sensitive, fragile, requires high voltage, drifts with time. It is expensive and has high background noise.

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

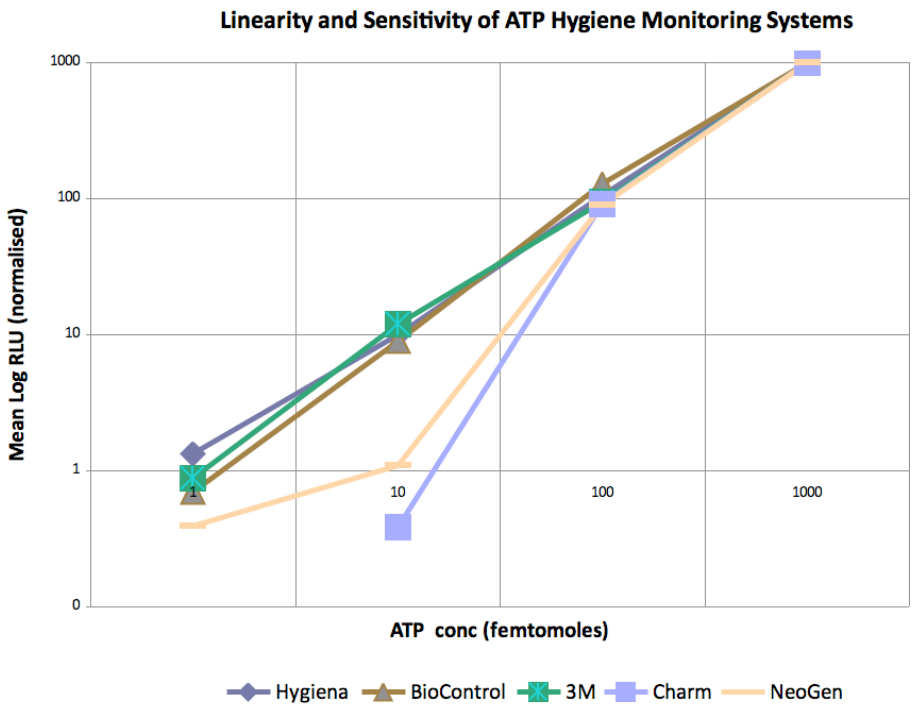
   All chemistry uses luciferase/luciferin enzymes to generate light. The quality and quantity of these enzymes used help to determine the performance of the chemistry.

3. **Reagent Swab Device – Design & Wetting Agent**
   - ATP test device design and components play important roles in performance and cost per test.
   - ATP test devices are pre-wetted with an extractant to break up biofilms, collect and release ATP from a sample.

Five Commercial ATP Systems Used In Study

- **BioControl Lightning MVP & Surface sample devices**
  Photomultiplier tube based system / lyophilized chemistry / woven swab
- **Charm Science Novalum & Pocketswab Plus**
  Photomultiplier tube based system / lyophilized chemistry / woven swab
- **Hygiena SystemSURE Plus with Ultrasnap & Supersnap devices**
  Photodiode based system / liquid stable chemistry / woven swab
- **Neogen AccuPoint instrument and surface sampler**
  Photodiode based system /lyophilized chemistry / sponge swab
- **3M UniLite NG CleanTrace and CleanTrace swabs**
  Photomultiplier based system / liquid stable chemistry / woven swab

Linearity:
Expression of predictability and reliability of the result



Graph

The linearity graph shows a straight-line relationship between RLU and ATP.

$$y = mx + c$$

where 'y' = RLU and 'x' = ATP, and both increase in a constant predictable way.

The straighter the line the better the linearity, the better the precision and more reliable the detection. This is particularly important at low ATP levels at low RLU values.

Linearity is described by the term Correlation Coefficient (r) which shows how well the data approaches the perfect fit i.e. r = 1.000

Best Performance

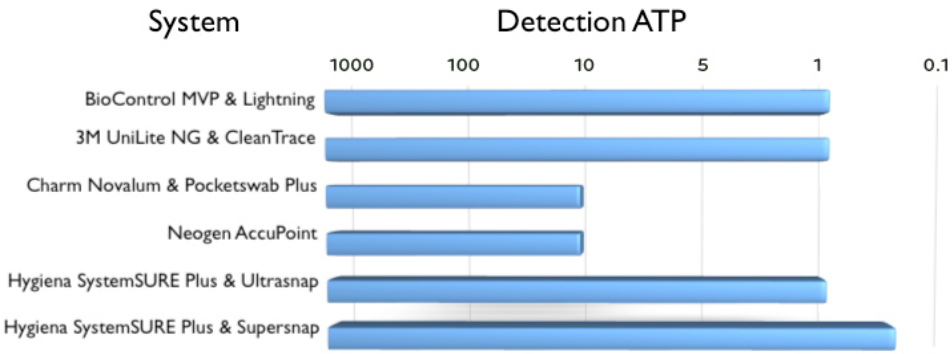
BioControl, Hygiena & 3M all showed good linearity for the detection of ATP r = 0.94 – 0.98

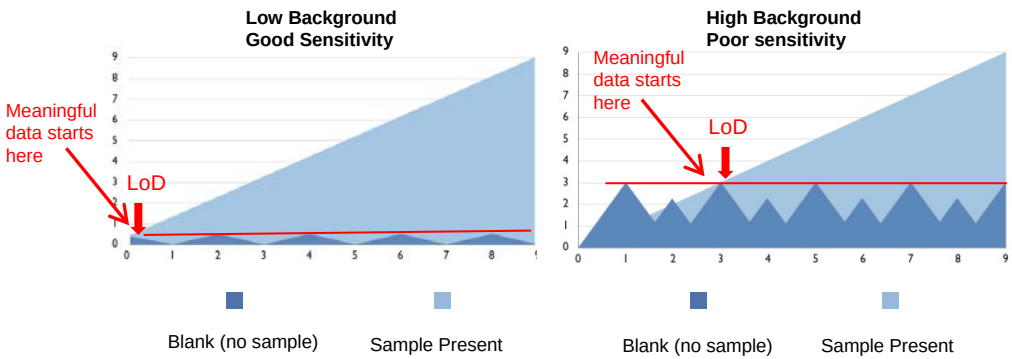
Poor Performance

Neogen & Charm systems were not linear at low ATP levels; both systems displayed 0 RLU in the presence of ATP detected by other systems

Data located in Table 7 and 13 of Silliker report and Appendixes A, B, C

Sensitivity:
Smallest detectable amount of sample





Background noise is interference from the instrument and swab device that gives a positive RLU reading when there is no ATP present - sometimes referred to as false positives.
High background noise is commonly seen with photomultiplier tubes

Graph

Sensitivity is defined as the Limit of Detection (LoD). It is the smallest amount detectable above the background noise of the system. The smaller the LoD the more sensitive the system.

Background noise is the signal detected by the systems in the absence of ATP that can come from both the instruments (as electrical interference) and the reagent swab devices (as chemical interference from impurities).

Signal – Background Noise = True meaningful result
A low background noise means a clear signal with little interference that enables the detection of the lowest amount of sample i.e. maximum sensitivity.
The graph shows the limit of detection (LoD) for each ATP test system

Best Performance

Hygiena SystemSURE Plus & Supersnap – LoD = .017 fmols

Average Performance

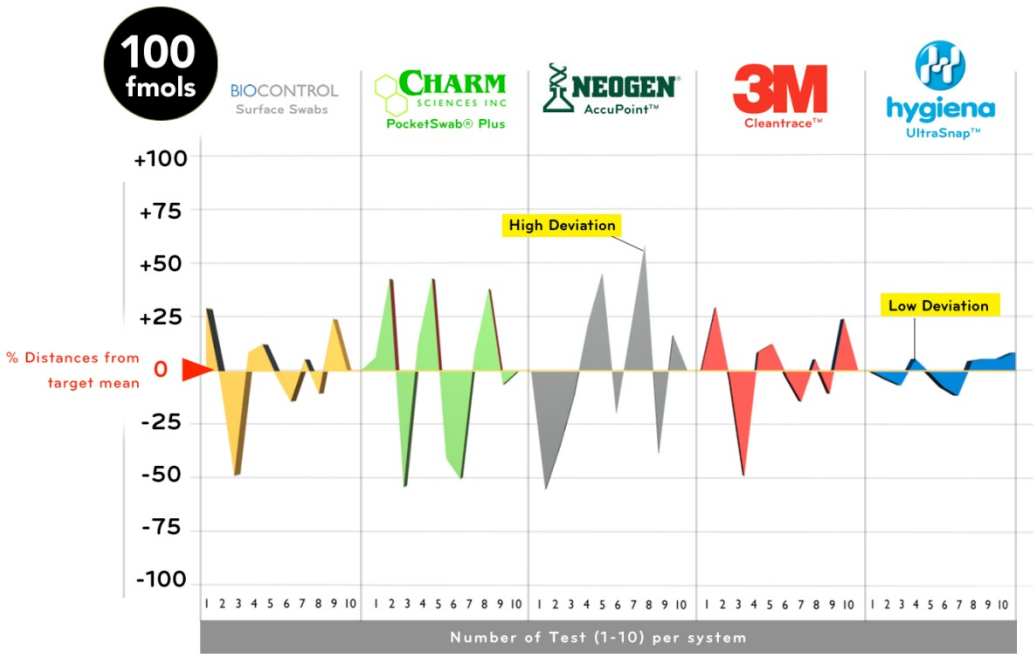
BioControl Lightning MVP & MVP swabs - LoD ~ 1.0
Hygiena SystemSURE Plus & Ultrasnap - LoD ~ 1.0
3M NG & CleanTrace - LoD ~ 1.0

Poor Performance

Charm Novalum & Pocketswab Plus - LoD = 10.0
Neogen Accupoint & Accupoint swabs - LoD = 10.0

Data located in Table 11, 12 and 13 of Silliker report

Repeatability:
Variation between measurement by the same operator using the same test sample



Variation is described by the term Coefficient of Variation (CV%). The higher the CV% then the greater the variability which means the results is less consistent and more unreliable.

The overall variation across the whole range of ATP measurements show;

Best Performance
Hygiena and Supersnap – CV = 9

Average Performance
3M = 26% CV
Hygiena and Ultrasnap = 28%CV
BioControl = 39% CV

Poor Performance
Charm Science = 86% CV
Neogen = 123% CV

Recommended Pass / Fail limits are usually set close to100 fmols ATP so good repeatability is essential at this critical value.

The graphs opposite show that Hygiena is the only system with low deviation which means it delivers the most reliable, consistent and dependable results.

This is particularly important where the highest standards of quality and safety requires the detection of low levels of ATP.

Data located in table 9 and 10 of Silliker report

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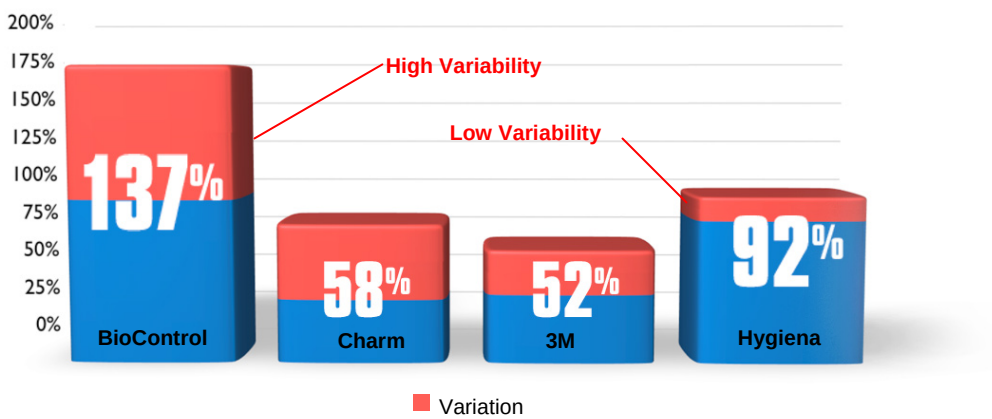
Accuracy:
Recovery and detection of all available ATP to reflect the true value of the sample

Recovery of ATP on Swab (%)



Note: Neogen Accupoint was not a part of this section of the study because of the design of the test device. Performance in the other sections of the study indicate that recovery of sample would be poor.

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 results.

The best system will be closest to 100%

Less than 100% means that only part of the sample was detected due to some interference within the system. This means that the result and is not true and accurate.

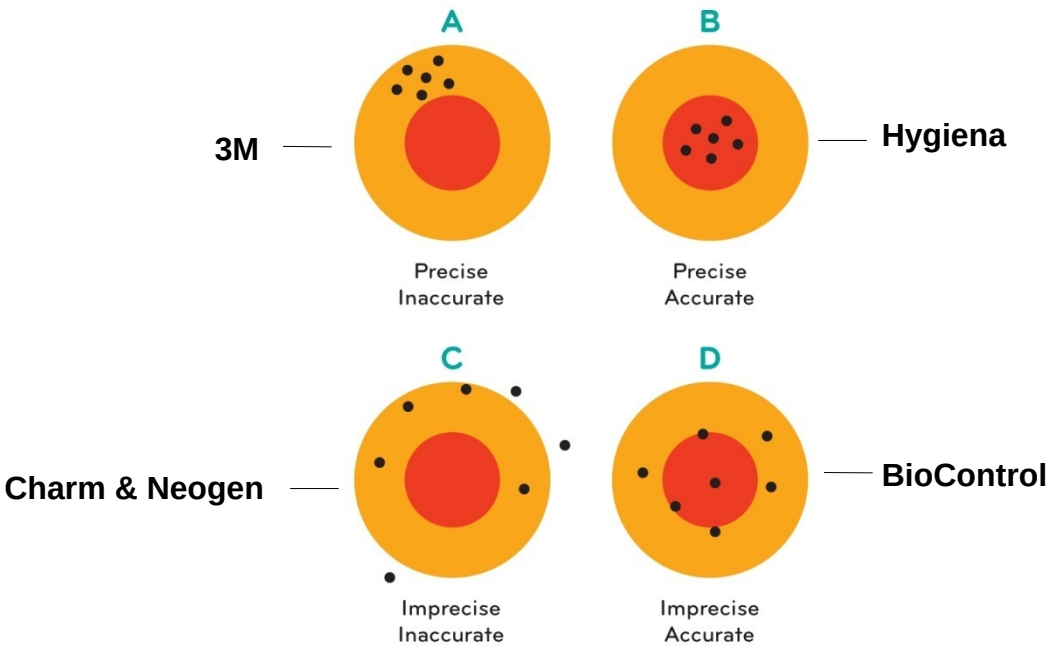
Best Performance
Hygiena – 92% recovery

Average Performance
BioControl – 71 to 137% recovery (highly variable)

Worst Performance
3M – 52% recovery
Charm Science – 58% recovery

Data located in figure 2 page ‘26’ of the Silliker report

Precision and Accuracy:
Recovery and detection of all available ATP to give consistent reliable results closest to the true value



Graph

The illustration opposite is used to describe the performance of a test and it shows how precision and accuracy are linked. It is important that they are considered together.
Better precision and accuracy means more consistent and reliable results that are closest to the true value.

The Hygiena system was the only system to be both precise and accurate.

Best Performance

Hygiena SystemSURE Plus & Ultrasnap displays precise, accurate results.

Average Performance

BioControl Lightning MVP & MVP swab displays accurate results, but is not precise.

3M NG & CleanTrace system is precise but only recovered 52% of the sample and is not accurate.

Worst Performance

Charm Novalum & Pocketswab Plus display imprecise and inaccurate results.

Neogen Accupoint & Accupoint swabs display imprecise and inaccurate results.

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Results Summary:
ATP detection looking at linearity, variability, repeatability & sensitivity

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

High RLU output does not give better sensitivity or performance

* does not detect below 10 fmols at which level the instrument shows 0 RLU.

** not a genuine zero reading (limited instrument output)

† SystemSURE Plus is the only system with genuine low background that is linear to zero RLU

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

New Technology, better system design = BETTER PERFORMANCE

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Summary

- 5 commercial ATP detection systems were compared for the detection and measurement of ATP
- Most systems were shown to give a good linear response to ATP, however there was a difference in the sensitivity, repeatability , precision and accuracy between systems.
- The most precise, accurate and repeatable system was the Hygiena SystemSURE Plus with either Ultrasnap or Supersnap swabs.
- The least sensitive and most variable systems were the Neogen AccuPoint and Charm Science Novalum systems with their respective swabs.
- ATP hygiene monitoring is a cleaning verification test so accuracy and consistency at low ATP levels is critical. Study shows that Charm and Neogen have the poorest sensitivity and highest variability.
- Data confirms that the SystemSURE Plus photodiode based system is better than or at least equal to the other commercial system.
- Data shows that each system displays a different RLU for any given sample. This could be confusing to users comparing systems. It is clear that 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.

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Disclaimer:
This presentation was prepared by Hygiena and is solely based on data generated from the comparative study of ATP hygiene monitoring systems by Silliker Inc. Food Science Center Report RPN 13922 (2010).This presentation contains all the conclusions drawn by Silliker and further detailed analysis of the raw data. Silliker Inc supports all claims made in its report RPN 13922 however the additional data analysis has not been verified by Silliker Inc.