Comparative Study of Commercial ATP Hygiene Monitoring Systems

hygiena

Scigiene Corporation

1295 Morningside Avenue, Unit 16
Scarborough, ON M1B 4Z4, Canada
Phone: 416-261-4865 Fax: 416-261-7879
www.scigiene.com

Overview

1. Scope of the study
2. Components of an ATP system
3. Performance results based on studies criteria
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Scope of the Study:
Design the most comprehensive ATP study done to date looking at 5 known 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 determine the hygienic status and potential risk of the object being sampled instantly.

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 the microbial test cannot provide.

ATP tests are not intended to replace microbial tests. However there is concurrent direct correlation between the results of the two methods because cleaning simultaneously removes both organic residues and microbes.

Purpose & Intent Defined the Performance Criteria of the Study

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

These performance criteria were determined by experimentation in controlled laboratory conditions using 3 sample types;

1.ATP dilutions pipetted direct to the swab bud
2.Dilutions of foodstuff in two states: pipetted directly to the swab bud & dried on stainless steel surfaces
3.Dilutions of microbial cultures typical of those of concern to food & beverage processors including: Escherichia coli, Lactobacillus plantarum, Pseudomonas aeruginosa, Salmonella typhimurium and Staphylococcus aureus and one yeast culture, Saccharomyces cerevisiea.

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The Systems:
Three key components make up an ATP system

* An ATP system consist 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 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 and accuracy, and more consistency. Simpler manufacturing has 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 luciferace/luciferin enzymes to generate light. The quality and quantity of the enzymes and the other components determine the performance of the chemistry.

† Background noise is light or electrical interference from the instrument or reagent swab device that causes a system to give a RLU reading in the absence of ATP. High background noise is commonly seen with photomultiplier tube machines. Some machines like the Charm Novalum deal with this by building in a background deduction formulation. This reduces sensitivity. Other systems convert RLU to log RLU units (or zones) to disguise the high background and variation. Signal – \Background Noise = True and Meaningful result

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 collect and release ATP from a sample.
   - Four of the test devices tested used swab tips and one used a sponge like tip (Neogen).

Five Commercial ATP Systems Used In Study

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

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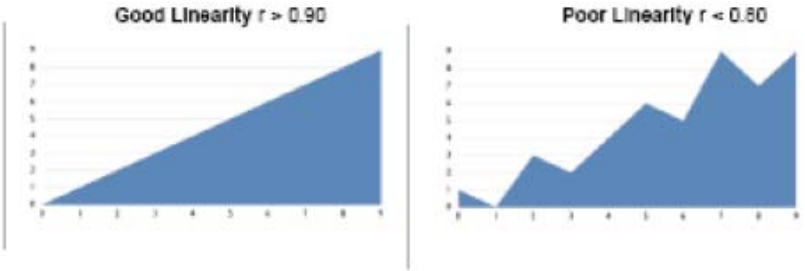
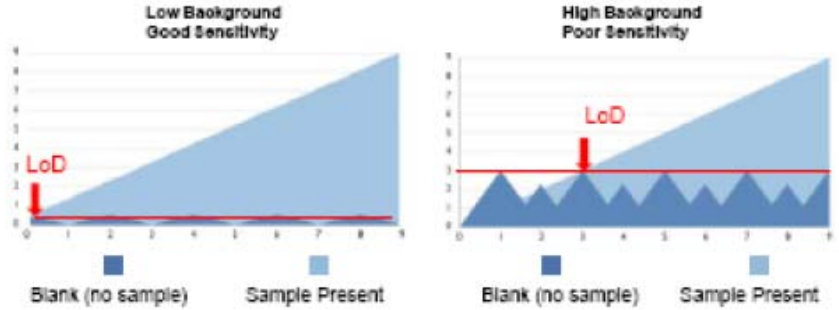
Performance Criteria:
Linearity, Sensitivity, Repeatability, and Accuracy

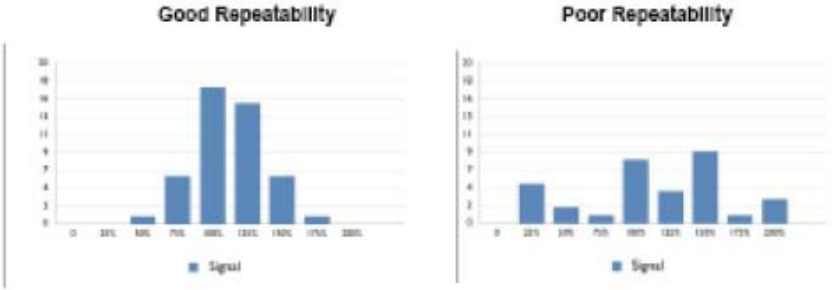
Linearity: Expression of predictability and reliability of the result.



Sensitivity: Smallest amount that can be detected above the background of the system.



Repeatability: Variation between measurements by the same operator using the same test sample. Expression of consistency and reliability of results.



Accuracy: Recovery and detection of all available ATP in the sample or on the swab.

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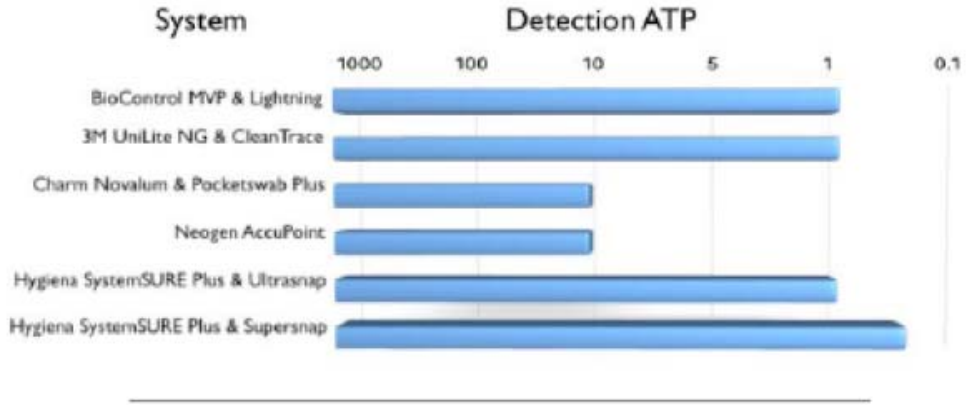
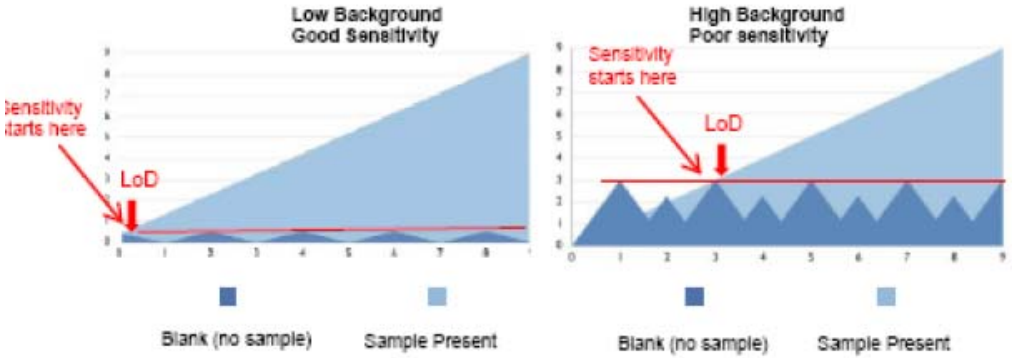
Sensitivity:
Smallest detectable amount





Background noise is signal detected by the system when a blank device is tested. Some systems like the Charm Science Novalum build in a background deduction formulation to compensate for the high background noise resulting in poor sensitivity.

Graph
Sensitivity is defined as the Limit of Detection. It is the smallest amount detectable above the background of 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 SystemsSURE Plus & Ultrasnap - LoD ~ 1.0
3M NG & CleanTrace - LoD ~ 1.0

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

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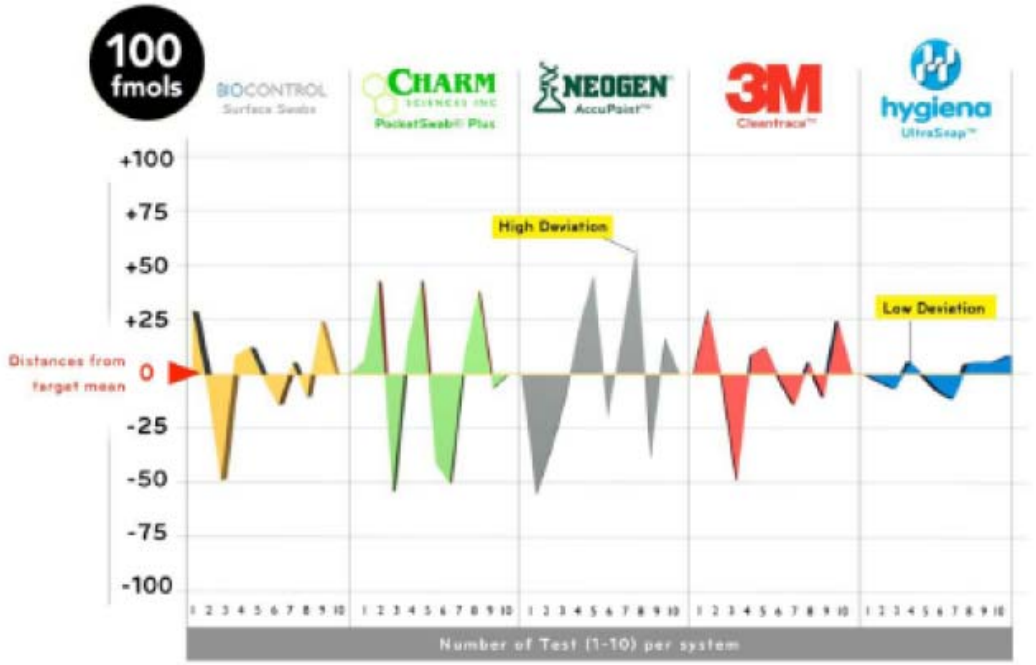
Repeatability:
Variation between measurement by the same operator using the same test sample



Graph
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

The graphs opposite from further detailed analysis reveals even greater differences between systems.

This is particularly important at critical values 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.

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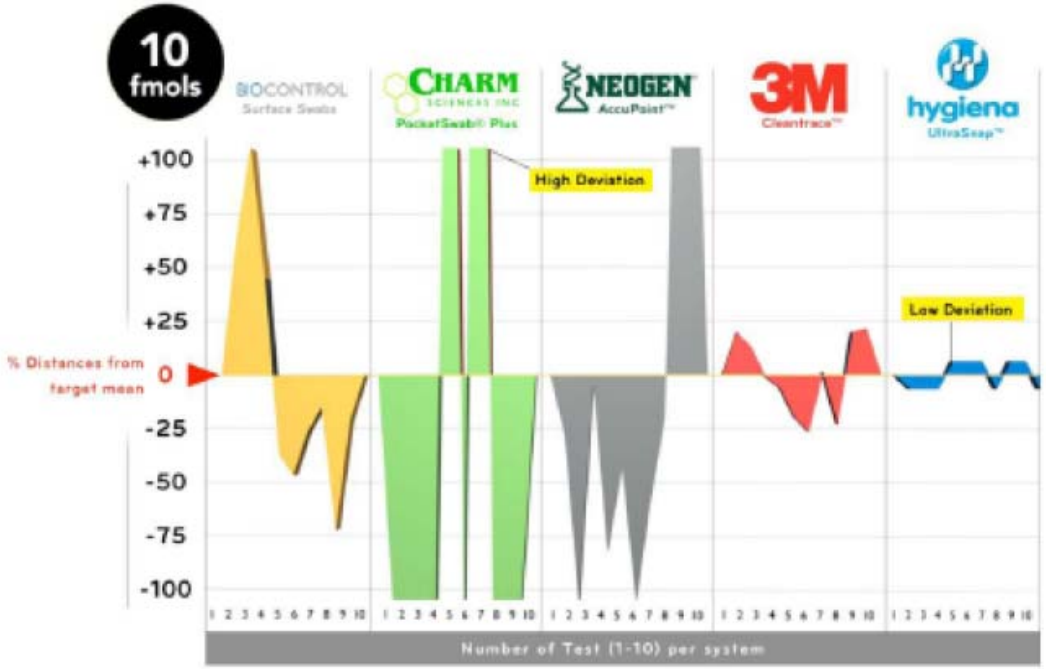
Repeatability:
At critical limits



Graph
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 graphs opposite reveals large variations and differences between systems at low ATP values (10fmols) that are essential for high quality cleaning.

Best Performance
Hygiena and Supersnap – CV = 7%CV

Average Performance
Hygiena and Ultrasnap = 10%CV
3M = 17% CV

Poor Performance
BioControl = 53% CV
Neogen = 116% CV
Charm Science = 214% CV

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.

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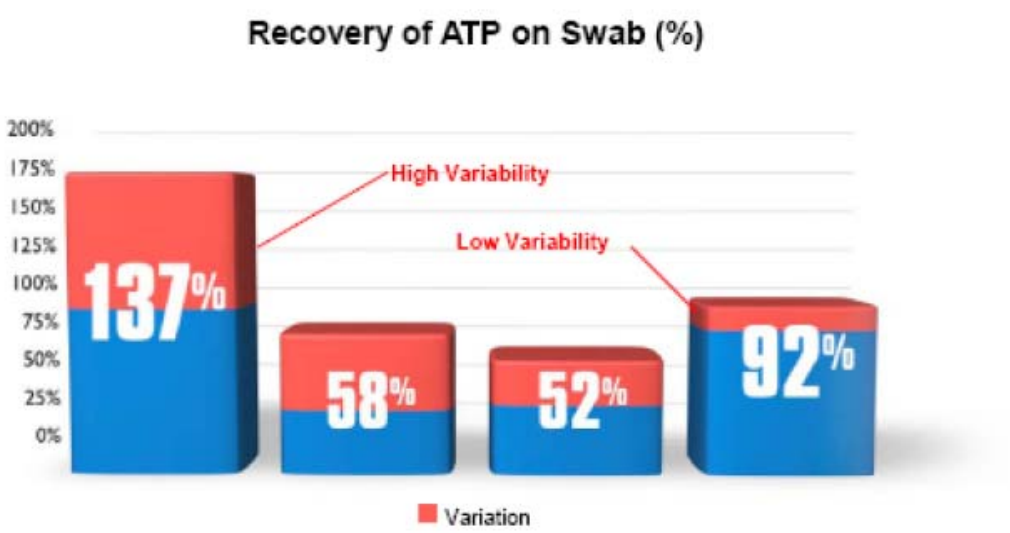
Accuracy:
Recovery and detection of all available ATP to reflect the true value of the sample



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.

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

Best Performance
Hygiena – 92% recovery

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

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

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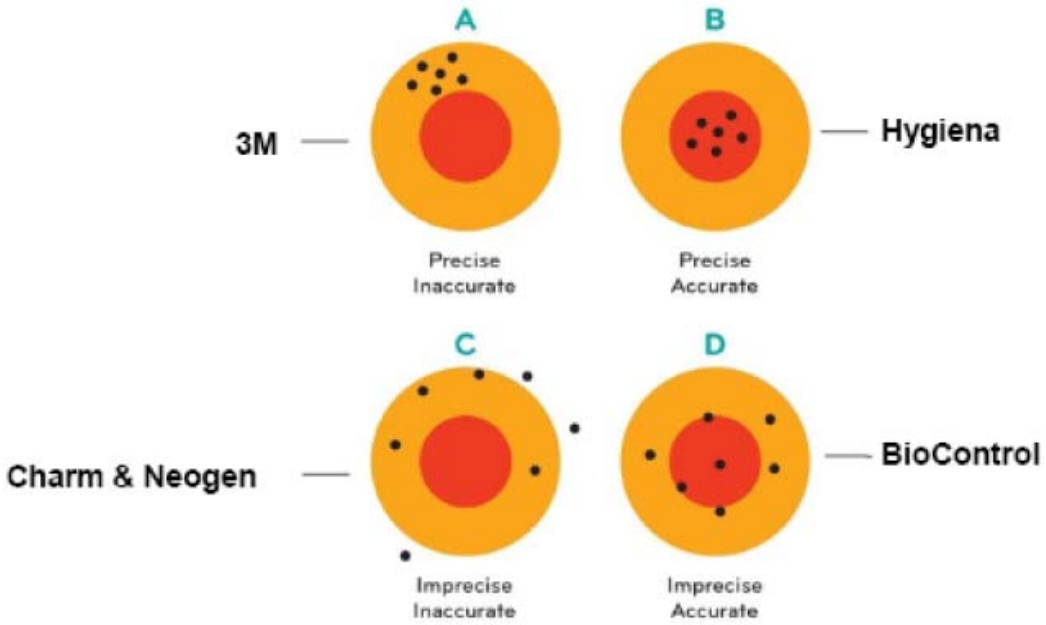
Precision and Accuracy:
Recovery and detection of all available ATP to give consistent reliable results closest to the true value



Graph
The target diagram opposite illustrates the meaning of precision and accuracy. This classification is used to describe the results generated by this comparative study. 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 detected 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

System	Linearity	Output (RLU)		Variability	Sensitivity
	(r)	Blank (Background at zero ATP)	Maximum (at 1000 fmols ATP)	Average (CV%)	Limit of detection (fmols ATP)
BioControl 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

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

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

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Section 2:
Detection of Foodstuffs

Food Type: Ground beef or Pasteurised Milk

Similar results obtained for orange juice and mixed green salad

Evaluation of ATP systems in a simulated Food & Beverage environment. This is just one industry that ATP systems are used to monitor hygiene and contact surfaces.

Background Information:

4 food items were chosen for this section of the study.

- Pasteurised milk
- Ground beef
- Orange juice
- Mixed green salad

4 food items were made into liquid suspensions and also dried on to a stainless steel surface to test for pickup and extraction capabilities.

Results:

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

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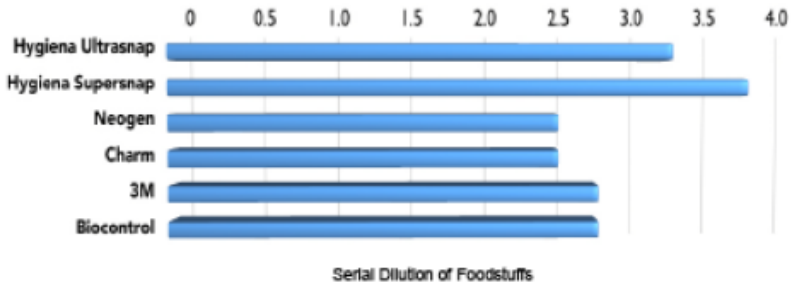
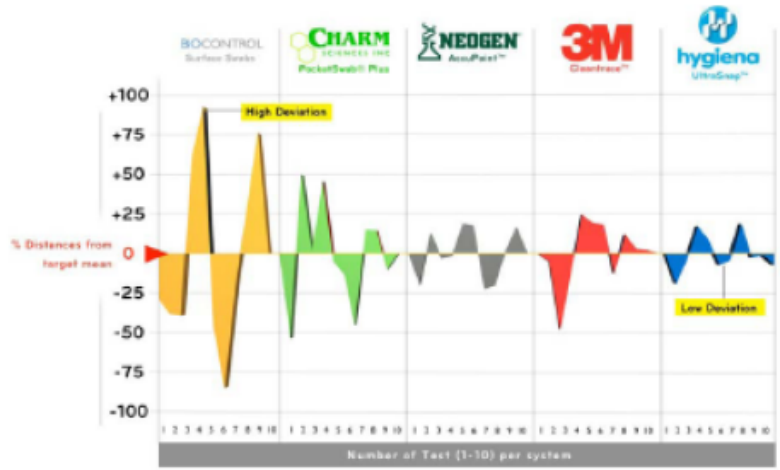
Detection of Foodstuffs:
Variation and extractability

Extraction Efficiency



Serial Dilution of Foodstuffs

Variation between systems with 1-10 dilution in Milk



Performance qualification:
The detection of reliably detect small amounts of food residues on surfaces after cleaning is dependent on the ability to detect all the available sample collected.
The extraction efficiency of the reagent swab device determines the smallest amount of sample that can be detected. The graph compares the extraction efficiency of each ATP system and shows that the larger the dilution factor then the more sensitive the systems.

Similarly the less variation in the system then the more consistent and reliable the result.

•Best Performance
•**Hygiena** SystemSURE Plus & Ultrasnap with x10 higher extraction efficiency and greatest consistency

•Average Performance
•**BioControl** Lightning MVP & MVP
•**3M** NG & CleanTrace.

•Worst Performance
•**Charm** Novalum & Pocketswab Plus
•**Neogen** Accupoint & Accupoint swabs

MILK: Coefficient of Variation / Dilution	CV% / Neat	CV% / 1:10	CV% / 1:100	CV% / 1:1,000	CV% / 1:10,000
BioControl	26%	57%	27%	61%	91%
3M	30%	20%	16%	10%	13%
Charm	24%	32%	39%	ND	ND
Hygiena / Ultrasnap	15%	11%	10%	15%	0%*
Hygiena / Supersnap	16%	11%	10%	43%	91%
Neogen	22%	15%	ND	ND	ND

*All RLU = 2 no CV%
ND = Not Detected

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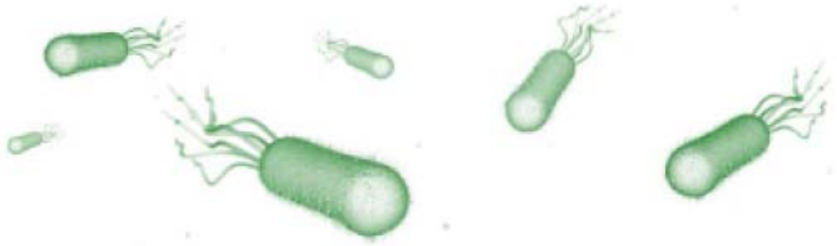
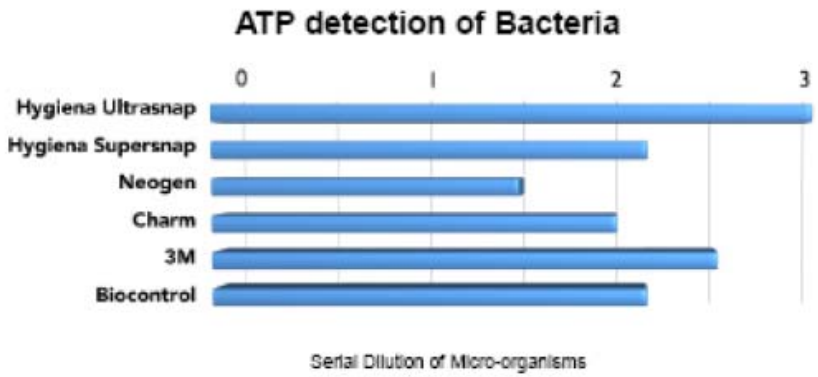
Section 3:
Detection of Micro-organisms





All organisms were detected and yeasts showed the highest amount of ATP because they were the largest. Staph aureus showed lowest amount of ATP. There was large variation in results from all organisms but good linearity was shown by all systems. ATP systems can detect these micro-organism in the range of 10,000 to 100,000 cfu/ml shown by this study. This is generally not sensitive enough for most cleaning or hygiene programs. Microbes multiply in organic matter and ATP systems verify that organic residues have been removed so reducing the risks. Therefore ATP testing is not a replacement for microbial testing, but an additional proactive step in support of a complete food safety or hygiene safety program.

Background :

ATP systems are intended to detect very small amounts of organic material on surfaces or in liquid samples. Micro-organisms are organic and contain ATP but at much smaller amounts than foodstuffs. Micro-organisms contain different amounts of ATP dependent on the type of microbes, size and state of health. The test cannot differentiate food ATP from microbial ATP.

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

Results from Section 3: Detection of Micro-organisms

Performance	Linearity	Sensitivity	Repeatability	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

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Snapshot:
Universal ATP test device that makes other systems better

Background:
The performance of Hygiena's liquid stable reagent technology in other luminometers was evaluated to see if Hygiena's new technology improved the performance of instruments of other systems .Snapshot was tested against all the same criteria using ATP, foodstuffs and microbes. Results from the study showed that Hygiena's test device technology is the most superior currently on the market.

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

Results from ATP, Foodstuffs & Micro-organism

Performance	Linearity	Sensitivity	Repeatability	Accuracy
Improvement in Performance	☑	☑	☑	☑
Same Performance				

In the all section of the study, Snapshot demonstrated:
- Improvement in linearity
- Improvement in accuracy
- Increased sensitivity (2x to 30x fold)
- Reduction in background noise
- Improved repeatability and consistency (lower c.v.)
- Increased extractability of ATP

Sensitivity Results

System	Sensitivity (Limit of Detection: fmols ATP)	
	Suppliers own swab	Hygiena Snapshot
BioControl MVP	1.1	0.04
3M UniLite NG	1.3	0.42
Charm Novalum	10.0	5.0

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Summary

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

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

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

➢The most precise, accurate and repeatable systems 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.

➢The Snapshot Universal swab improved the repeatability and sensitivity of the 3M UniLite NG, BioControl MVP and Charm NovaLum luminometers.

➢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 just as sensitive or more sensitive then the other commercial 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. It is clear that a larger RLU number does not mean a more sensitive reading. Charm displayed the highest RLU value result for each test and is one of the least sensitive systems.

➢All ATP systems can detect micro-organisms, but at a level of 10,000 – 100,000 CFU and this is generally a lot higher then cleaning standards allow. Detection of micro-organisms showed higher variation based on type and quantity.

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