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Performance Evaluation of Various ATP Detecting Units

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Executive Summary

In early 2009 Silliker Group Corporation was commissioned to perform an extremely comprehensive study to examine the performance of several commercially available ATP (Adenosine Triphosphate) bioluminescence hygiene / sanitation monitoring systems. The properties of the ATP assay are well suited to determinations 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 minutes permits the immediate assessment of the sample condition and whether additional cleaning action is required. The traditional method of determining cleanliness is the aerobic plate count. This test requires 2 days to complete. This procedure is limited in the type of microorganisms it can detect and does not detect organic residue.

The study was designed to assess system performance when challenged with varying levels of pure ATP, food residues and microbes. There were 4 key study phases. Each phase of the study was designed to measure system performance under specific conditions against parameters chosen to approximate real world environmental situations and are vital technical measurements. It is important to understand that the combination of these factors and no single factor alone can clearly define system overall performance. Not all study phases include all systems.

1. **Detection of pure ATP**

   Swabs and solution

2. **Detection of ATP from pure microbial cultures**

   Escherichia coli, Lactobacillus plantarum, Pseudomonas aeruginosa, Salmonella Typhimurium and *Staphylococcus aureus* and one yeast culture- *Saccharomyces cerevisiea*

3. **Detection of ATP from food**

   Ground beef, Milk (pasteurized 2% low fat, Orange Juice (pasteurized without pulp),Salad (bagged mixed salad greens)

4. **Detection of ATP from food soiled stainless steel surfaces**

   Ground Beef, Milk

At each stage of the study and for each combination of factors, 10 replicate sub-samples were tested at each test parameter. A total of >5000 data points were generated and these were analyzed mathematically to describe the performance of the tests in terms of Linearity, Repeatability, Sensitivity, and Accuracy. A key factor in the study of performance was the use of clone reagent swabs. These are reagent swabs that are formatted to operate with multiple instruments but are not proprietary to the instrument manufacturer. The study data indicates that the use of clone swabs is acceptable. Due to differences in RLU scales, data output (pass / fail settings based on RLU scale) and

instrument linearity, the user must understand individual system nuances to successfully convert from a proprietary reagent swab to a clone.

The key findings of the study are

1. 1. The linear correlation coefficient values for the ATP solution, microorganisms, and foods showed that the Log10 RLU readings and Log10 dilutions were linearly correlated. All Linear coefficient calculations can be found in tables 7 and 8 of the final report and were generally >0.9. However at low ATP levels , the Neogen and Charm systems lost linearity and could not detect <10 fmols ATP.
2. To quantify repeatability, the coefficient of variation (CV%) for each dilution of the ATP solution, microorganisms and foods were calculated. A high number of the average CV% values lies over the 10% to 35% range, which is reasonable for this type of assay and these types of studies. However the CV of individual systems for ATP detection ranged from 9% to 123%. The Hygiena SystemSURE with the Hygiena Supersnap swab had 9% CV whereas Neogen Accupoint and Charm Pocketswab had 123% and 86% CV respectively.
3. High RLU values do not confer a greater sensitivity to a system. Sensitivity depends greatly on several factors within the each system including both instrument and reagent output as well as the background of the system. The systems with large RLU scales such as Neogen and Charm systems also showed limit of detection of 10.0 fmols compared to other systems with a limit of detection of 1 fmol.
4. Clone swabs (Hygiena Snapshot) consistently improved the instrument performance by reducing the background noise and improving both sensitivity and repeatability of 3M CleanTrace, Charm Novalum and BioControl MVP systems.
5. The mean calculated sensitivity (or limit of detection) of the most and least sensitive systems differed by approximately 60 fold. The Hygiena SystemSure with the Hygiena Supersnap swab was the most sensitive reagent / instrument combination with a limit of detection of 0.17fmols. (data in tables 11 and 12)
6. When tested against the microbial cultures, the overall best extraction index included Hygiena Supersnap swab and Hygiena Snapshot swab. Several systems were included but no significant difference was observed.(tables 16, 17 and 18) The limit of detection was typically 10,000 to 100,000 bacteria / ml.
7. When tested against the food samples, the overall best extraction index included Hygiena Snapshot swab. Several systems were included but no significant difference in extraction rates was observed. (tables 16, 17 and 18). However there was a difference in the sensitivity of the systems when testing foodstuffs. This was independent of the type of foodstuff and was similar to the sensitivities determined for ATP. The most sensitive system for the detection of food residues was Hygiena SystemSURE Plus with Supersnap swab and the least sensitive systems were the Neogen Accupoint and Charm Pocketswab.

The report that follows this summary contains a large amount of detailed experimental data. It should be thoroughly reviewed to fully understand the depth of the experiments and the conclusions drawn from that data.

Objective

The purpose of this study was to evaluate commercial ATP units targeted for use in the food industry. The study was intended to provide comparative data and not as a comprehensive evaluation or review.

Background

ATP is a molecule that is essential and common to all plant, animal and microbial cells. ATP may persist long after the cells have died. Measurement of ATP requires only a few minutes and is based upon the firefly luciferase bioluminescence assay.

The properties of the ATP assay are well suited to determinations of cleanliness, with cleanliness being defined as the absence of organic (derived from life) material. Clean surfaces have no ATP, while dirty surfaces have ATP and perhaps live microbial cells. The result of an ATP test that is available in minutes permits the immediate assessment of the sample condition and whether additional cleaning action is required. The traditional method of determining cleanliness is the aerobic plate count. This test requires 2 days to complete. This procedure is limited in the type of microorganisms it can detect and does not detect organic residue.

ATP bioluminescence systems are available from a number of commercial companies. Measures of how these systems perform under controlled conditions will be helpful to customers as well as manufacturers that must make informed decisions.

Materials and Methods

ATP Monitoring Systems and Devices

The study was conducted using two sets of ATP monitoring systems and swabs. In the first set (Set 1), the performance of five different commercially available ATP monitoring systems was evaluated using eight different commercially available swabs (Table 1). In the second set (Set 2), the performance of three different commercially available ATP monitoring systems of Set 1 was evaluated using four different commercially available swabs (Table 2).

Table 1. First set of ATP monitoring system and swab combinations used in the sensitivity studies

ATP monitoring system	Swab
Biocontrol Lightning MVP Unit	Hygiena Snapshot SBC 1575
	Lightning
3M Clean Trace NG Luminometer	Clean Trace
	Hygiena Snapshot SPXL 1333
Charm Sciences novaLUM Unit	Hygiena Snapshot CH 1616
	Pocketswab Plus
Hygiena SystemSURE Unit	Hygiena Ultrasnap
Neogen Accupoint Unit	Neogen Accupoint

Table 2. Second set of ATP monitoring system and swab combinations used in the sensitivity studies

ATP monitoring system	Swab
Biocontrol Lightning MVP Unit	Hygiena Snapshot SBC 1575
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333
Hygiena SystemSURE Unit	Hygiena Supersnap
	Ultrasnap

Test Matrices

Test samples included water spiked with ATP as a measure of system sensitivity, microbial cultures and food soil representing organic residue likely to be present in environmental samples. The test samples, as described below, were prepared and analyzed by the ATP monitoring systems and swabs listed in Tables 1 and 2.

Part A: Detection of Pure ATP

The sensitivity of the ATP monitoring systems was first compared using water samples spiked at various levels of ATP (Table 3). In order to evaluate the influence of swab materials on ATP, the spiked water samples were tested either by depositing a pre-determined amount of samples directly onto the swab bud or into the activated reagent in the swab device. For the blank (control) samples, a commercially available ultra pure sterile water product was used (Rockland Inc., Gilbertsville, PA).

Table 3. ATP spiked water used in the sensitivity studies

Recovery method	ATP level (femtomole)
ATP recovery from swab	0
	0.1
	1
	5
	10
	100
	1,000
ATP recovery in solution	0
	100

1. ATP recovery from swab
    a. 10 µL of sample containing ATP levels at 0 (blank), 0.1, 1, 5, 10, 100, 1000 femtomoles (fmoles) was pipetted directly onto the appropriate swab bud.
    b. The swab device was activated.
    c. The swab was placed in the ATP unit.
    d. The measurement was started and the relative light units (RLU) result was read and recorded.
    e. This procedure was repeated with 10 replicates of each dilution on each swab device using each ATP system and swab combination of Set 1[1] and Set 2.
    f. Test results were reported as follows:

	ATP recovery from swab						
	1000 fmoles	100 fmoles	10 fmoles	5 fmoles	1 fmoles	0.1 fmoles	0 fmoles
Replicates 1-10							

2. ATP recovery in solution
    a. The swab device was activated without adding the sample.
    b. The swab was removed from the activated device.
    c. 10 µL of sample containing ATP at the level of 0 (control blank) and 100 fmol was added into the reagent in the swab device.
    d. The swab was placed in the device.
    e. The swab device was placed in the ATP unit.
    f. The measurement was started and the RLU result was read and recorded.
    g. The ATP in solution tests were only performed on the following ATP system and swab combinations of Set1.
        i. Biocontrol Lightning MVP unit using Lightning swab,
        ii. Clean Trace NG Luminometer using Clean Trace swab,
        iii. Charm Science unit using Pocketswab Plus swab,
        iv. Hygiena SystemSURE unit using Hygiena Ultrasnap,
        v. Neogen Accupoint was omitted per client's request
    h. The ATP in solution tests were performed on all the ATP systems and swabs of Set 2.
    i. This procedure was repeated with 10 replicates of each dilution on each swab device using each ATP system and swab combination of Set 1[1] and Set 2.
    j. Test results were reported as follows:

	ATP recovery in solution	
	100 fmoles	0 fmoles (blank)
Replicates 1-10		

[1] ATP systems and swabs of Set 1 were tested three times for each replicate, providing 30 readings

Part B: Detection of ATP from pure microbial cultures grown in broth

Five bacterial cultures, *Escherichia coli, Lactobacillus plantarum, Pseudomonas aeruginosa, Salmonella* Typhimurium and *Staphylococcus aureus* and one yeast culture-*Saccharomyces cerevisiea* were obtained from the Silliker Inc., Food Science Center culture collection (FSC-CC) (Table 4). The bacterial cultures were cultivated in 10 mL of tryptic soy broth (TSB) and incubated at 35°C for 18-24 h. *S. cerevisiea* was cultivated in 10 mL of sabourand dextrose broth and incubated at 30°C for 48 h. After incubation, each culture was washed once with sterile deionized water by centrifugation at 8000 rpm for 20 min and reconstitution with sterile deionized water. The cell level in each dilution of the bacterial cultures was determined by plating serial dilutions on tryptic soy agar (TSA) incubated 35°C for 24 h. The cell level in each dilution of the yeast cultures was determined by plating serial dilutions on potato dextrose agar (PDA) incubated 25°C for 5 d.

Table 4. Bacterial strains used in the sensitivity studies conducted with pure cultures

Culture	Source	FSC-CC Number
Escherichia coli	Chicken broth	1809
Lactobacillus plantarum	Juice drink	998
Pseudomonas aeruginosa	Soil	2606
Saccharomyces cerevisiea	ATCC MYA 658	2847
Salmonella Typhimurium	USDA culture	1860
Staphylococcus aureus	Potatoes	1561

Each culture was serially diluted 10-fold up to 10^{-6} with sterile deionized water and analyzed by each ATP system and swab combination of Set 1 and Set 2 by pipetting 10 µL of culture dilution directly onto each swab bud, placing the swab device into the ATP unit and reading the RLU result. After testing, the cell level in each dilution was determined by plating serial dilutions of the bacterial cultures on tryptic soy agar (TSA) and the yeast culture on potato dextrose agar PDA). The TSA plates were incubated at 35 ± 1°C for 24 ± 2 h and the PDA plates were incubated at 30 ± 1°C for 48 h prior to enumeration. Test results were reported as follows:

	ATP from pure culture						
	0 dilution (undiluted)	1:10 dilution	1:100 dilution	1:1,000 dilution	1:10,000 dilution	1:100,000 dilution	1:1,000,000 dilution
Replicates 1-10							

Part C: Detection of ATP from Food

Food samples that are commonly available from commercial supermarkets were used to represent a range of product groups for this portion of the study (Table 5). Liquid food samples (orange juice and milk) were diluted using ATP-free sterile water (v/v) in the following ratios: full strength liquid (0 dilution); 1:10; 1:100; 1:1000; and 1:10,000. Solid food samples (ground beef and salad greens) were first stomached using 10 g of sample in 90 ml ATP-free sterile water and then diluted using ATP-free sterile water (w/w) in the following ratios: 1:10 (stomached samples); 1:100; 1:1,000; and 1:10,000. All test samples were shaken by hand for 2 min for homogenization.

Table 5. Food samples used in the sensitivity studies

Food
Ground beef
Milk (pasteurized 2% low-fat)
Orange juice (pasteurized without pulp)
Salad (bagged mixed salad greens)

Ten replicates of each food suspension were analyzed by each ATP system and swab combination of Set 1 and Set 2 by pipetting 10 µL of food suspension dilution directly onto each swab bud, placing the swab device into the ATP unit and reading the RLU result. Test results were reported as follows:

	ATP from Food				
	1:1 (full strength liquid; 0 dilution)	1:10	1:100	1:1,000	1:10,000
Replicates 1-10					

Part D: Detection of ATP from Food Soiled Stainless Steel Surfaces

Some brands of swabs are wet, and stay wet, during the intended shelf life while other brands of swabs are dry. Test results may vary due to wet and dry swabs, and wet and dry surfaces. Therefore, in addition to the food suspension dilutions tested, stainless steel surfaces soiled with ground beef and milk were tested (Table 6).

Table 6. Food suspension dilutions tested on stainless steel surface in the sensitivity studies.

Food	Dilution
Ground beef	1:10
	1:1,000
Milk	1:1
	1:1,000

Five hundred (500) µL of food suspension from the 1:10 and 1:1,000 dilutions of the ground beef and 500 µL of food suspension from the 1:1 (full strength liquid; 0 dilution) and 1:1,000 dilutions of the milk and were spread evenly onto individual 4x4 in^2 stainless steel surfaces and immediately tested after preparation by swabbing each swab bud over the stainless steel surface, placing the swab device in the ATP unit and reading the RLU result. An additional set of stainless steel surfaces were prepared as described above and allowed to dry at room temperature for 18-24 h. After drying, each individual stainless steel piece was swabbed by each swab bud and analyzed by each ATP unit and reading the RLU result. Ten replicates of each dilution were tested. Test results were reported as follows:

	ATP from Food Soiled Stainless Steel Surface		
	1:1 (full strength liquid; 0 dilution)	1:10	1:1,000
Replicates 1-10			

Data Analysis

Linearity:

The linear correlation coefficient (**r**) measures the strength and the direction of a linear relationship between the sample variables. The value of **r** for samples at varying ATP levels and food samples at varying dilution levels against the corresponding RLU readings was calculated to determine the linear relationship.

Repeatability

Repeatability means the level of agreement between successive results obtained with the same method on the same test sample. The ATP measurement repeatability was expressed as a coefficient of variation (CV%), which is the standard deviation (SD) expressed as percentage of the mean (i.e. CV % = 100 × SD/mean).

Sensitivity

Limit of detection (LOD) in this study was calculated as the ratio of the mean of a true blank with three standard deviations to RLU per fmoles (mean +3 sd/RLU per fmoles). Some systems such as the SystemSure, the blanks can run at 0, the next significant RLU is used as the lowest detection limit. Charm and Neogen instruments have a built in algorithm, which discounts some of the RLU measured and therefore they do not display an RLU value for less than 10 fmoles. For these systems an LOD value of 10 fmoles is stated.

Relative Light Unit (RLU) per femtomole

The RLU per femtomole values were calculated by dividing RLU readings to corresponding ATP levels. In order to minimize the variability, the average RLU per femtomole was calculated using the first three ATP dilutions (i.e. 1,000, 100 and 10 fmoles).

Comparison of index (extraction index) for microorganisms and food samples

The lowest detectable concentration levels for microbial and food samples are presented as the lowest dilution at which ATP could be detected. The extracted fmole per dilution was calculated as the ratio of the RLU reading without background to the RLU per fmole value. The extracted fmole per dilution values that show greater than 1 ATP fmole are counted as extracted. The comparison using the extracted ATP rather than RLU normalizes the data making analysis comparable and extraction levels more relevant to each system.

Results and Discussion

Test results are reported in relative light unit (RLU) readings in this study. The sensitivity of each of the 12 ATP monitoring systems of Set 1 and Set 2 was tested using aqueous ATP solutions, cell suspensions and exudates of food samples. Each ATP monitoring system uses a different measurement scale. The test results of ten replicates of seven dilutions of the ATP solution, seven dilutions of cell suspensions, five dilutions of liquid food samples (i.e. milk and orange juice), four dilutions of solid food samples (ground beef and salad) and two dilutions of food exudates on stainless steel surfaces are shown in Appendix A.

The formulation Hygiena swab products used in Set 1 and Set 2 differ in order to show the effects of extractant on subsequent detection and test performance. Hygiena products used in Set 2 are those supplied routinely on a commercially basis.

Linearity

Log10 RLU values of ten replicates were plotted against Log10 dilutions of the test matrices (Appendix B). The best-fit line is represented by the solid line and the 95% confidence limits presented as dashed lines. The linear correlation coefficient (***r***) of the best-fit line was determined to measure the linearity of ATP monitoring systems. A value of 1.0 represents a perfect fit of the regression line to the data. Values greater than 0.8 indicate the curve fits the data very well.

The linear correlation coefficient values of the ATP monitoring systems tested for RLU over the range of dilutions of the ATP solution, microorganisms, and foods are summarized in Tables 7 and 8. All regressions were significant. A total of 132 correlation coefficients were calculated. All correlation coefficient values with the exception of an outlier value of 0.643 determined by the Neogen Accupoint with Neogen Accupoint swab for *E. coli*, were greater than 0.8 and provided strong evidence that the Log10 RLU readings and Log10 dilutions were linearly correlated.

Repeatability

To quantify repeatability, the coefficient of variation (CV%) for each dilution of the ATP solution, microorganisms and foods were calculated (Appendix C). The CV% values indicate the amount of variation. The higher CV% values represent greater variation and hence less repeatability. The CV% values increased as the limits of detection were approached. This is expected because closer to the detection limit there is much less ATP to measure and there is more variability in the measurement. The CV% were erratic between dilutions of the test matrices and ranged from 2% to 316%.

For comparative purposes, the average CV% of the ATP solution data, microbial cultures and food samples was calculated. All CV% were then averaged for each ATP monitoring system (Table 9-10). The average coefficient of variation values ranged from a low of 6% by the BioControl Lightning MVP with Hygiena Snapshot SBC 1575 swab to a high of 186% by the Neogen Accupoint with Neogen Accupoint swab. The relative frequency distribution of the average CV% values is presented in Figure 1. A high number of the average CV% values lies over the 10% to 35% range, which is reasonable for this type of assay and these types of studies.

The natural variation of biological assays such as ATP bioluminescence combined with the variation from sample collection and operator handling during hygiene monitoring applications means that the test results do not have the same precision as other analytical methods. The results from ATP hygiene measurements are used as a rapid qualitative

assessment of cleaning and results are typically expressed in board bands of Pass , Caution or Fail that typically equate to 10 – 100fmols of ATP. The ATP hygiene monitoring application is not intended to be used as a precise determination of ATP content. The trending of RLU or Pass / Fail results are much more meaningful in routine manufacturing operations.

Sensitivity

ATP Solution

The average RLU readings, standard deviations and CV% values for dilution of the ATP solution, microorganisms and food samples are summarized in Appendix C. The average background readings from the system (i.e. reagents outputs in the 0.0% ATP blank solution) for the 10 replicate test samples were 0.0 RLU for the swab devices of Charm Science with Pocketswab Plus swab (Set 1), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1) (Appendix C-Table C1) and the Hygiena SystemSure with Hygiena Supersnap swab (Set 2) and Hygiena SystemSure with Hygiena Ultrasnap swab (Set 2) (Appendix C Table C2).

The average background reading for the Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575 swab, BioControl Lightning MVP with Lightning swab, Clean Trace NG Luminometer with Clean Trace swab, Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab, and Hygiena SystemSure with Hygiena Ultrasnap swab of Set 1 were 142.17, 283.17, 4.0, 0.83, and 0.67 RLU, respectively (Appendix C-Table C1). The average background reading for the Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575swab and Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab were 199 and 1.90 and RLU, respectively (Appendix C-Table C2). ATP analysis of the ATP solutions showed that the calculated LODs ranged from 0.17 fmoles to 10 fmoles (Tables 11 and 12). The mean calculated LODs of the most and least sensitive systems differed by approximately 60 fold. The Hygiena SystemSure with Hygiena Supersnap swab was the most sensitive, while the Charm Science with Pocketswab Plus swab and the Neogen Accupoint with Neogen Accupoint swab were the least sensitive systems.

The RLU output and range shown of different systems varies considerably because the RLU is not a standard unit of measurement and is unique to each test system. High RLU values do not confer a greater sensitivity to a system and this is shown in Table 13 that summarizes the performance characteristics of the tests systems as supplied commercially.

ATP detection and recovery was variable between systems (Figure 2). BioControl had a high recovery of ATP but it was also highly variable (+/- 37%). Hygiena ATP recovery was high (92%) with good repeatability (9% CV). The Charm Science with Pocketswab recovered only 57% ATP with 20% variability, and Clean Trace NG Luminometer systems recovered only 52% ATP with a variability of 10%. A reduced recovery of ATP means that the accuracy of the system is also reduced.

Hygiena SnapShot is designed to be used with other luminometers such as 3M Clean Trace NG Luminometer, BioControl MVP and Charm Sciences novaLUM. Table 14 shows snapshot performance for ATP detection compared to other systems and their corresponding swabs.

Snapshot increases the performance of other luminometers and detects lower levels of ATP by;

- Increasing the linearity of ATP response
- Reducing the background and giving similar or greater RLU output per unit of ATP
- Reducing the variation and thereby increasing repeatability and consistency of ATP detection
- Increasing the extractability of ATP and thereby increasing the accuracy of the measurement
- Improving the sensitivity of the system
- Similar results were also obtained with the detection of foodstuffs.

Microorganisms

Data for six different microbial cultures using various ATP systems and swab devices are presented in Appendix C-Table C3 through C14. During the course of testing the dilutions, the lower detection limit was observed; hence not all 10-fold dilutions were analyzed by each swab device. The analysis of each culture used the RLU per femtomole calculation to normalize the RLU measured by each system to femtomoles. This normalization is required to bring all measured RLUs onto a similar scale; this scale can then be easily compared device to device and instrument to instrument. Comparisons using RLUs is difficult due to the differing scales used and the variable machine and reagent background RLUs which do not contribute to the measured signal. Hence, the normalization of the data to RLU per femtomole is required for accurate comparative.

Escherichia coli

E. coli had a culture level of 9.59 log10 CFU/ml for the first set of swab devices (Set 1) analyzed and 9.08 log10 CFU/ml for the second set of swab devices (Set 2) tested. The Biocontrol Lightning MVP with Hygiena Snapshot 1575 swab (Set 1), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1), Hygiena SystemSure with Hygiena Ultrasnap swab (Set 1 and 2), and Neogen Accupoint with Neogen Accupoint swab (Set 1) were the least sensitive swab devices analyzed for the detection of ATP from *E. coli* as these swab devices were able to detect the *E. coli* at the 1:100 diluted culture level, and not when the culture was subsequently diluted (Appendix C-Table C3, Appendix D Table D2). The Biocontrol Lightning MVP with Lightning swab (Set 1), Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 2), Clean Trace NG Luminometer with Cleantrace swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot 1333 swab (Set 1 and 2), Charm Science with Pocketswab (Set 1) and Hygiena SystemSure with Hygiena Supersnap (Set 2) appeared to be the most sensitive swab devices analyzed as these were able to detect ATP from *E. coli* at the next dilution (1:1,000) tested (Appendix C-Tables C3 and C4, Appendix D Table D2).

Lactobacillus plantarum

L. plantarum had a culture level of 6.45 log10 CFU/ml for the first set of swab devices (Set 1) analyzed and 9.48 log10 CFU/ml for the second set of swab devices (Set 2) tested. The Biocontrol Lightning MVP with Lightning swab (Set 1), Hygiena SystemSure with Hygiena Ultrasnap swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1) were the least sensitive swab devices analyzed for the detection of ATP from *L. plantarum,* as these swab devices were able to detect *L. plantarum* at the 1:10 diluted culture level (Appendix C-Table C5, Appendix D Table D2). The Biocontrol MVP with Hygiena Snapshot 1575 swab (Set 1), Clean Trace NG Luminometer with Cleantrace

swabs (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot 1333 swab (Set 1), Charm Science with Hygiena Snapshot 1616N swab (Set 1), Charm Science with Pocketswab (Set 1), and Hygiena SystemSure with Hygiena UltraSnap swab (Set 2) could extract ATP from the next dilution (1:100). The most sensitive swabs were Biocontrol Lightning MVP with Hygiena Snapshot 1575 swab (Set 2), Clean Trace NG Luminometer with Hygiena Snapshot 1333 swab (Set 2) and Hygiena SystemSure with Hygiena Supersnap swab (Set 2), as these were able to detect ATP from *L. plantarum* at the 1:1,000 dilution level. (Appendix C-Tables C5 and C6, Appendix D Table D2).

Pseudomonas aeruginosa

P. aeruginosa had a culture level of 8.32 log10 CFU/ml for the first set of swab devices (Set 1) analyzed and 8.52 log10 CFU/ml for the second set of swab devices (Set 2) tested. The Clean Trace NG Luminometer with Cleantrace swab (Set 1), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1), Charm Science with Pocketswab Plus swab (Set 1), Hygiena SystemSure with Hygiena Ultrasnap swab (Set 1 and 2), Neogen Accupoint with Neogen Accupoint swab (Set 1) and Hygiena SystemSure with Hygiena Supersnap swab (Set 2) were the least sensitive swab devices analyzed for the detection ATP from *P. aeruginosa,* as these swab devices were able to detect *P. aeruginosa* at the 1:100 diluted culture level, and not when the culture was subsequently diluted (Appendix C-Tables C7 and C8, Appendix D Table D2). The Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 1 and Set 2), Biocontrol Lightning MVP with Lightning swab (Set 1) and Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 1 and 2) appeared to be the most sensitive swab devices analyzed as these were able to detect ATP from *P. aeruginosa* at the 1:1,000 diluted culture level (Appendix C-Tables C7 and C8, Appendix D Table D2).

Saccharomyces cerevisiae

S. cerevisiae had a culture level of 7.49 log10 CFU/ml for the first set of swab devices (Set 1) analyzed and 7.74 log10 CFU/ml for the second set of swab devices (Set 2) tested. The Charm Science with Hygiena Snapshot CH 1616 swab (Set 1), Charm Science with Pocketswab Plus swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1) were the least sensitive swab devices analyzed for the detection of ATP from *S. cerevisiae* as these swab devices only were able to detect *S. cerevisiae* at the 1:1,000 diluted culture level, while all other systems could detect ATP from *S. cerevisiae* at the 1:10,000 diluted culture level (Appendix C-Tables C9 and C10, Appendix D Table D2).

Salmonella Typhimurium

S. Typhimurium had a culture level of 7.96 log10 CFU/ml for the first set of swab devices (Set 1) analyzed and 9.08 log10 CFU/ml for the second set of swab devices (Set 2) tested. The Biocontrol Lightning MVP with Hygiena Snapshot 1575 swab (Set 1), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1) were the least sensitive swab devices analyzed for the detection of ATP from *S.* Typhimurium as these swab devices were able to detect ATP from *S.* Typhimurium at the 1:10 diluted culture level, while the Biocontrol Lightning MVP with Lightning swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 1), Charm Science with Pocketswab (Set 1) and Hygiena SystemSure with Hygiena Ultrasnap swab (Set 1 and 2) could detect ATP from *S.* Typhimurium at the 1:100 diluted culture level. The most sensitive systems were Clean Trace NG Luminometer with Cleantrace swab (Set 1), Biocontrol Lightning MVP with Hygiena Snapshot 1575 swab (Set 2), Clean Trace NG Luminometer with Hygiena

Snapshot SPXL 1333 swab (Set 2) and Hygiena SystemSure with Hygiena Supersnap swab (Set 2) as these swab devices were able to detect ATP from *S.* Typhimurium at the 1:1,000 diluted culture level (Appendix C-Tables C11 and C12, Appendix D Table D2).

Staphylococcus aureus

S. aureus had a culture level of 8.23 log10 CFU/ml for the first set of swab devices (Set 1) analyzed and 8.76 log10 CFU/ml for the second set of swab devices (Set 2) tested. The Biocontrol Lightning MVP with Snapshot 1575 swab (Set 1), Biocontrol Lightning MVP with Lightning swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 1), Charm Science with Pocketswab (Set 1), Hygiena SystemSure with Hygiena UltraSnap swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1) were the least sensitive swab devices analyzed for the detection of ATP from *S. aureus*, as these swab devices were able to detect ATP from *S. aureus* at the pure culture level, and no detection was observed at any lower levels of *S. aureus* (Appendix C-Table C13, Appendix D Table D2). The Clean Trace NG Luminometer with Cleantrace swab (Set 1) and Hygiena SystemSure with Hygiena UltraSnap swab (Set 2) could detect ATP from *S. aureus* at the 1:10 diluted culture level, while Charm Science with Hygiena Snapshot CH 1616 swab (Set 1), Biocontrol Lightning MVP with Hygiena Snapshot 1575 swab (Set 2), Clean Trace NG Luminometer with Hygiena Snapshot 1333 swab (Set 2) were able to detect ATP from *S. aureus* at the 1:100 diluted culture level. The most sensitive swab device analyzed for the detection of ATP from *S. aureus* was the Hygiena SystemSure with Hygiena Supersnap swab (Set 2) as it was able to detect ATP from *S. aureus* at the 1:1,000 diluted culture level (Appendix C-Tables C13 and C14, Appendix D Table D2).

Compendium Extraction Index

To fully evaluate how the systems perform across the range of bacteria, the lowest level from each system for each bacterium can be assessed by analyzing at which dilution level in each dilution series 1 femtomole of ATP can be extracted above the blank values. This relationship is then tabulated in Table 15.

The systems with the overall best extraction index include Hygiena SystemSURE with Hygiena Supersnap swab (Set 2), Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 2) and Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 2). The overall extraction index is -3.00 (which is a mean extraction level of 1:1,000) across all bacteria measured. The next best systems are in the -3.00 to -2.00 range (i.e. 1:100 to 1:1000 dilution region). These systems include Clean Trace NG Luminometer with Clean Trace swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 1), Hygiena SystemSURE with Hygiena Ultrasnap swab (Set 2), Biocontrol Lightning MVP with Lightning swab (Set 1), Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 1), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1) and Charm Science with Pocketswab Plus swab (Set 1). With the other systems with extractions below the 1:100 dilution across all bacteria includes Hygiena SystemSURE with Hygiena Ultrasnap swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1).

Large differences were observed in the ATP results from different species of microorganism and these were lower than expected. This may be a reflection of species difference and size or the effect of the culture preparation and sample storage during testing. The limit of detection for most systems was $10^5 – 10^6$ bacteria CFU/ml which equates to $10^3 – 10^4$ bacterial per swab. Similarly $10^3 – 10^4$ yeast/ml which equates to 10^1

– 10^2 yeasts per swab. However the prime purpose of the ATP hygiene monitoring application is to detect product residue after cleaning because residues are a direct objective measurement of cleaning efficiency, and the level of ATP in foodstuffs is far greater than that of microbes. The ATP test is not intended to be a replacement for microbiological tests. The post-cleaning standard for bacteria on surfaces is typically 100 – 500 CFU per 100 cm^2 which is equivalent to 100 – 500 CFU per swab that is clearly not detectable by the ATP test as shown above.

Food Samples

Raw Ground Beef

The Biocontrol Lightning MVP with Lightning swab (Set 1) was the least sensitive swab device analyzed for the detection of ATP for raw ground beef as this swab device only was able to detect this food suspension at the 1:10 dilution level, while Biocontrol Lightning with Hygiena Snapshot 1575swab (Set 1), Clean Trace NG Luminometer with Cleantrace swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshots 1333 swab (Set 1 and Set 2), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1), Charm Science with Pocketswab Plus swab (Set 1), and Neogen Accupoint with Neogen Accupoint swab (Set 1) could detect ATP at the 1:100 dilution level. Hygiena SystemSure with Hygiena Ultrasnap swab (Set 1 and Set 2) and Hygiena SystemSure with Hygiena Supersnap swab (Set 2) were able to detect ATP for raw ground beef at the 1:1,000 dilution level. The most sensitive swab device was BioControl Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 2) as this swab device was able to detect ATP from raw ground beef at the lowest (1:10,000) dilution tested (Appendix C-Tables C15 and C16, Appendix D Table D1).

Milk

The Neogen Accupoint with Neogen Accupoint swab (Set 1) was the least sensitive swab device analyzed for the detection of ATP from pasteurized 2% low fat milk as this swab device only was able to detect this food suspension at the 1:10 dilution level, while the BioControl Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 1), BioControl Lightning MVP with Lightning swab (Set 1), Clean Trace NG Luminometer with Clean Trace swab (Set 1), Charm Science with Hygiena Snapshot CH 1616 swab (Set 1), Charm Science with Pocketswab Plus swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 2) and Hygiena SystemSURE with Hygiena Supersnap swab (Set 2) could detect ATP from this food suspension at the 1:100 dilution level. Hygiena SystemSure with Ultrasnap swab (Set 1) and BioControl Lightning MVP with Hygiena Snapshot 1575 swab (Set 2) were able to detect ATP at the 1:1,000 dilution level. The most sensitive swabs were Clean Trace NG Luminometer with Hygiena Snapshot 1333 swab (Set 1) and Hygiena SystemSure with Hygiena Ultrasnap swab (Set 2) as these devices were able to detect ATP from pasteurized 2% low fat milk at the 1:10,000 dilution level (Appendix C-Tables C17 and C18, Appendix D Table D1).

Orange Juice

The Charm Science with Pocketswab Plus swab (Set 1), Hygiena SystemSure with Ultrasnap swab (Set 1) and Neogen Accupoint with Neogen Accupoint swab (Set 1) were the least sensitive swab devices analyzed for the detection of ATP from orange juice containing no pulp as these swab devices only were able to detect this food suspension at the 1:1,000 dilution level, while all other systems analyzed were able to detect ATP for orange juice at the lowest dilution (1:10,000) tested (Appendix C-Tables C19 and C20, Appendix D Table D1).

Mixed Salad Greens

The Charm Science with Hygiena Snapshot CH 1616 swab (Set 1) was the least sensitive swab device analyzed for the detection of ATP from orange as this swab device only was able to detect this food suspension at the 1:100 dilution level, while Clean Trace NG Luminometer with Cleantrace swab (Set 1), Charm Science with Pocketswab Plus swab (Set 1), Hygiena SystemSure with Hygiena Ultrasnap swab (Set 1), Neogen Accupoint with Neogen Accupoint swab (Set 1), and Clean Trace NG Luminometer with Snapshot 1333 (Set 2) could ATP from bagged mixed salad greens at the 1:1,000 dilution level (Appendix C-Table C21, Appendix D Table D1). The most sensitive swab devices analyzed for the detection of ATP for bagged mixed salad greens were the BioControl Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 1), BioControl Lightning MVP with Lightning swab (Set 1), Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 1), Biocontrol Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 2), Hygiena SystemSURE with Hygiena Supersnap swab (Set 2) and Hygiena SystemSURE with Hygiena Ultrasnap swab (Set 2) as these were able to detect ATP from salad greens at the lowest dilution (1:10,000) tested (Appendix C-Tables C21 and C22, Appendix D Table D1).

Wet versus Dry Food Soil

All swab devices analyzed were able to detect ATP from the wet and dry soiled stainless steel surfaces from the different food suspensions of raw ground beef and pasteurized 2% low fat milk (Appendix C-Tables C23-C26). The RLU reading of the wet and dry soiled stainless steel surfaces were higher than that of food suspension at the same dilution levels. This may be attributed to the difference in volumes used for food suspensions (i.e. 10 μL) and stainless steel coupons (i.e. 500 μL).

Snapshot improved sample recovery from dry surfaces (Figure 3). This is attributed to snapshot's saturated swab bud and extractant that ensures good recovery of sample and increase RLU output compared to suppliers own swab.

Overall Comparison

For overall comparative purposes, the average extraction index of each microbial and food sample, and the ATP monitoring system were calculated. When tested against the microbial cultures, the Hygiena SystemSure system with Hygiena Supersnap swab (Set 2), BioControl Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 2) and Clean Trace NG Luminometer with Hygiena Snapshot SPXL 1333 swab (Set 2) appeared to be the most sensitive ATP monitoring system analyzed as they were able to detect ATP from the microbial cultures at higher dilution levels compare to all other systems (Table 15). Most RLU output due to ATP derived from microbial cultures was highest in *S. cerevisiae* and lowest in *S. aureus.*

When tested against the food samples, the Hygiena SystemSure with Hygiena Ultrasnap swab (Set 2) and the BioControl Lightning MVP with Hygiena Snapshot SBC 1575 swab (Set 2) appeared to be the most sensitive ATP monitoring systems analyzed as they were able to detect ATP from the food samples at higher dilution levels compare to all other systems (Table 16). Most RLU output due to ATP derived from food residues was highest in orange juice and lowest in ground beef.

Each system with generic or clone swabs can be tabulated and graded according to performance from each section, aqueous ATP detection, ATP recovery from swab, extraction of ATP from microbial cultures and extraction of ATP from food stuffs. The

comparison is shown in Table 17, each category ranks the systems using the most current versions of each system either swab or instrument.

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

Table 7. Correlation coefficient of ATP monitoring systems of Set 1

ATP Unit	**Swab Device**	ATP Solution	Microorganism						Food			
			Escherichia coli	*Lactobacillus plantarum*	*Pseudomonas aeruginosa*	*Saccharomyces cerevisiea*	*Salmonella* Typhimurium	*Staphylococcus aureus*	Ground beef	Milk	Orange juice	Salad
Biocontrol Lightning MVP	Hygiena Snapshot SBC 1575	0.987	0.986	0.955	0.995	0.984	0.947	0.868	0.951	0.972	0.981	0.998
Biocontrol Lightning MVP	Lightning	0.982	0.931	0.928	0.986	0.989	0.921	0.810	0.945	0.960	0.996	0.992
Clean Trace NG Luminometer	Clean Trace	0.988	0.993	0.975	0.989	0.988	0.974	0.920	0.890	0.974	0.997	0.995
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333	0.988	0.987	0.985	0.992	0.997	0.990	0.984	0.855	0.986	0.984	0.996
Charm Science	Hygiena Snapshot CH 1616	0.982	0.984	0.998	0.995	0.984	0.945	0.997	0.954	0.909	0.971	0.937
Charm Science	Pocketswab Plus	0.949	0.972	0.997	0.984	0.983	0.983	0.998	0.990	0.982	0.986	0.986
Hygiena SystemSURE	Hygiena Ultrasnap	0.988	0.962	0.991	0.974	0.979	0.980	ND [a]	0.855	0.986	0.984	0.996
Neogen Accupoint	Neogen Accupoint	0.976	0.643	0.970	0.972	0.928	0.967	0.961	0.995	0.920	0.938	0.985

[a] Not determined; only 0 dilution had RLU readings.

Table 8. Correlation coefficient of ATP monitoring systems of Set 2

ATP Unit	**Swab Device**	ATP Solution	Microorganism						Food			
			Escherichia coli	*Lactobacillus plantarum*	*Pseudomonas aeruginosa*	*Saccharomyces cerevisiea*	*Salmonella* Typhimurium	*Staphylococcus aureus*	Ground beef	Milk	Orange juice	Salad
Biocontrol Lightning MVP	Hygiena Snapshot SBC 1575	0.990	0.999	0.986	0.989	0.995	0.997	0.986	0.964	0.976	0.991	0.933
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333	0.992	0.998	0.978	0.991	0.982	0.997	0.952	0.978	0.988	0.993	0.997
Hygiena SystemSURE	Hygiena Supersnap	0.987	0.987	0.990	0.991	0.980	0.996	0.992	0.984	0.986	0.950	0.986
Hygiena SystemSURE	Hygiena Ultrasnap	0.989	0.974	0.989	0.991	0.991	0.989	0.968	0.966	0.985	0.978	0.985

Table 9. Coefficient of variation (CV%) of ATP solution, microorganisms and food samples when tested by different ATP monitoring systems-Set 1

ATP Unit	**Swab Device**	**APT Solution**	*Escherichia coli*	***Lactobacillus plantarum***	***Pseudomonas aeruginosa***	***Saccharomyces cerevisiea***	***Salmonella* Typhimurium**	***Staphylococcus aureus***	**Ground beef**	**Milk**	**Orange juice**	**Salad**	**Ground beef soiled surface**	**Milk soiled surface**	**Average (Range)[2]**
Biocontrol Lightning MVP	Hygiena Snapshot SBC 1575	19	20	18	24	53	20	20	19	21	14	12	35	15	22 (12-53)
Biocontrol Lightning MVP	Lightning	39	62	22	51	31	49	30	15	52	20	20	24	32	34 (15-62)
Clean Trace NG Luminometer	Clean Trace	26	24	20	24	27	27	23	15	18	15	10	31	23	22 (10-31)
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333	27	22	16	31	25	20	24	22	26	17	10	30	29	23 (10-31)
Charm Science	Hygiena Snapshot CH 1616	68	7	8	16	16	18	14	15	22	9	19	26	15	19 (7-68)
Charm Science	Pocketswab Plus	86	45	14	31	30	15	15	22	31	17	17	19	22	28 (14-86)
Hygiena SystemSURE	Hygiena Ultrasnap	59	28	15	51	31	20	11	31	40	17	17	58	50	33 (11-59)
Neogen Accupoint	Neogen Accupoint	123	186	30	36	63	142	113	78	19	19	17	33	27	68 (17-186)

[2] CV% Average does not include 0 and NA readings

Table 10. Coefficient of variation of ATP solution when tested by different ATP monitoring systems-Set 2

ATP Unit	**Swab Device**	**APT Solution**	***Escherichia coli***	***Lactobacillus plantarum***	***Pseudomonas aeruginosa***	***Saccharomyces cerevisiea***	***Salmonella* Typhimurium**	***Staphylococcus aureus***	**Ground beef**	**Milk**	**Orange juice**	**Salad**	**Ground beef soiled surface**	**Milk soiled surface**	**Average (Range)[3]**
Biocontrol Lightning MVP	Hygiena Snapshot SBC 1575	10	11	10	44	28	19	6	17	48	16	19	21	25	21 (6-48)
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333	15	18	14	35	17	20	16	27	19	13	18	36	44	23 (13-44)
Hygiena SystemSURE	Hygiena Supersnap	9	18	17	41	14	17	18	34	32	18	18	28	34	23 (9-41)
Hygiena SystemSURE	Hygiena Ultrasnap	28	15	13	30	18	12	14	13	158	13	17	23	32	30 (12-158)

[3] CV% Average does not include 0 and NA readings

Figure 1. Relative frequency distribution for CV% values

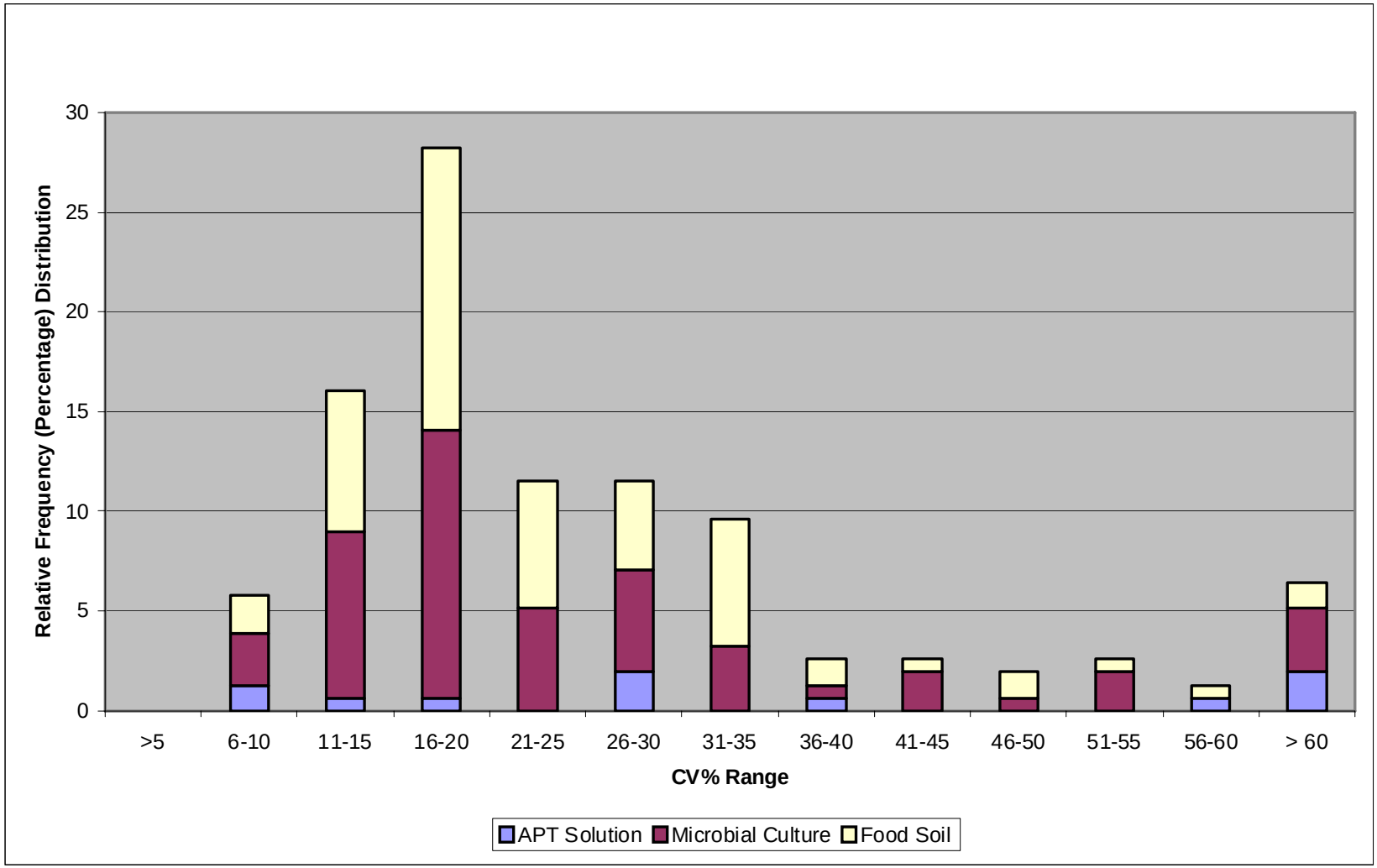

Table 11. Relative Light Unit (RLU) per femtomole (fmole) and limit of detection (LOD) values of Set 1

ATP Unit	Swab Device	RLU/fmole	LOD (fmole)
Biocontrol Lightning MVP	Hygiena Snapshot SBC 1575	552	0.60
Biocontrol Lightning MVP	Lightning	698	1.10
Clean Trace NG Luminometer	Clean Trace	5.4	1.30
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333	6.8	0.42
Charm Science	Hygiena Snapshot CH 1616	582	5.0
Charm Science	Pocketswab Plus	218	10.0
Hygiena SystemSURE	Hygiena Ultrasnap	1.0	1.0
Neogen Accupoint	Neogen Accupoint	12	10.0

Table 12. Relative Light Unit (RLU) per femtomole (fmole) and limit of detection (LOD) values of Set 2

ATP Unit	Swab Device	RLU/fmole	LOD (fmole)
Biocontrol Lightning MVP	Hygiena Snapshot SBC 1575	825	0.40
Clean Trace NG Luminometer	Hygiena Snapshot SPXL 1333	9.0	0.39
Hygiena SystemSURE	Hygiena Supersnap	6	0.17
Hygiena SystemSURE	Hygiena Ultrasnap	1.0	1.0

Table 13: Summary of ATP performance characteristics of 5 commercial detection systems

System	Linearity	Output (RLU)		Variability	Sensitivity
	(r)	Blank (Background at zero ATP)	Maximum (at 1000 fmols ATP)	(CV%)	Limit of detection (fmols ATP)
BioControl MVP with Lightning swab	0.982	283	975,941	39	1.1
3M Clean Trace NG Luminometer with CleanTrace swab	0.988	4	7382	26	1.3
Charm Science 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

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

Figure 2: Recovery of ATP by different ATP detection systems

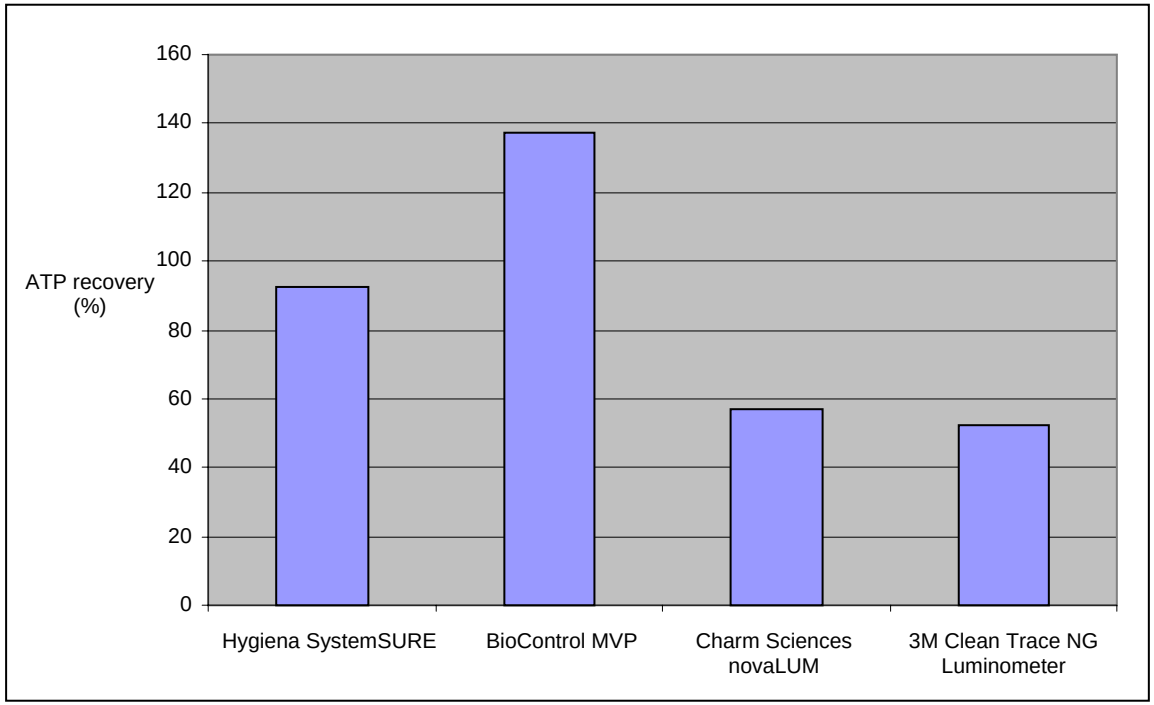

Table 14: SnapShot performance in different luminometer compared to manufacturers own swabs

System	Linearity	Output (RLU)		Variability	Sensitivity
	(r)	Blank (Backgrounda t zero ATP)	Maximum (at 1000 fmols ATP)	(CV%)	Limit of detection (fmols ATP)
BioControl MVP with Lightning swab	0.982	283	975,941	39	1.1
BioControl MVP with Snapshot swab	0.990	199	927,161	10	0.4
3M Clean Trace NG Luminometer with CleanTrace swab	0.988	4	7382	26	1.3
3M Clean Trace NG Luminometer with Snapshot swab	0.992	2	12620	15	0.42
Charm Science novaLUM With Pocketswab Plus	0.949	0	418,517	86	10.0
Charm Science novaLUM with Snapshot swab	0.982	0	783,031	68	5.0

Figure 3: Snapshot sample recovery from dry surfaces compared with other swabs

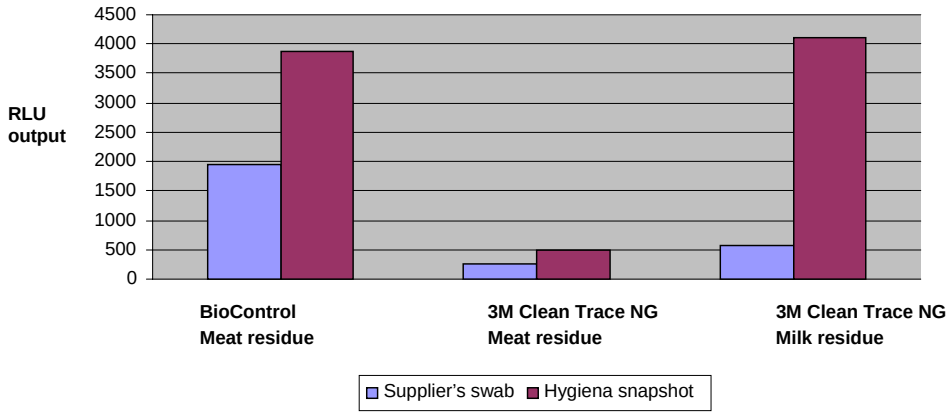

Table 15. Summary of lowest dilution levels for RLU output due to ATP derived from microbiological culture dilutions tested by different ATP monitoring systems

ATP Unit	Swab Device	Order of best extraction (mean of lowest dilutions detected)
Hygiena SystemSURE (Set 2)	Hygiena Supersnap	-3.00
Biocontrol Lightening MVP (Set 2)	Hygiena Snapshot SBC 1575	-3.00
Clean Trace NG Luminometer (Set 2)	Hygiena Snapshot SPXL 1333	-3.00
Clean Trace NG Luminometer (Set 1)	Clean Trace	-2.50
Clean Trace NG Luminometer (Set 1)	Hygiena Snapshot SPXL 1333	-2.33
Hygiena SystemSURE (Set 2)	Hygiena Ultrasnap	-2.17
Biocontrol Lightening MVP (Set 1)	Lightening	-2.17
Biocontrol Lightening MVP (Set 1)	Hygiena Snapshot SBC 1575	-2.00
Charm Science (Set 1)	Hygiena Snapshot CH 1616	-2.00
Charm Science (Set 1)	Pocketswab Plus	-2.00
Hygiena SystemSURE (Set 1)	Hygiena Ultrasnap	-1.83
Neogen Accupoint (Set 1)	Neogen Accupoint	-1.50

Table 16. Summary of lowest dilution levels for RLU output due to ATP derived from food samples suspensions tested by different ATP monitoring systems

ATP Unit	Swab Device	Order of best extraction (mean of lowest dilutions detected)
Hygiena SystemSURE (Set 2)	Hygiena Ultrasnap	-3.75
Biocontrol Lightening MVP (Set 2)	Hygiena Snapshot SBC 1575	-3.75
Clean Trace NG Luminometer (Set 1)	Hygiena Snapshot SPXL 1333	-3.50
Hygiena SystemSURE (Set 2)	Hygiena Supersnap	-3.25
Hygiena SystemSURE (Set 1)	Hygiena Ultrasnap	-3.00
Biocontrol Lightening MVP (Set 1)	Hygiena Snapshot SBC 1575	-3.00
Clean Trace NG Luminometer (Set 2)	Hygiena Snapshot SPXL 1333	-2.75
Clean Trace NG Luminometer (Set 1)	Clean Trace	-2.75
Biocontrol Lightening MVP (Set 1)	Lightening	-2.75
Charm Science (Set 1)	Pocketswab Plus	-2.50
Charm Science (Set 1)	Hygiena Snapshot CH 1616	-2.50
Neogen Accupoint (Set 1)	Neogen Accupoint	-2.50

Table 17 Compendium Ranks of ATP Hygiene Monitoring Swabs for ATP Detection, ATP Recovery from swabs, Microbial Extraction and Food Extraction Levels

ATP Limit of Detection	**ATP Recovery from Swab**	**ATP Extraction from Microbial Cultures**	**ATP Extraction from Foodstuffs**
Hygiena Supersnap	BioControl Lightning (100%)	Hygiena Supersnap	Hygiena Ultrasnap
Hygiena Snapshot 1333	Hygiena Ultrasnap (93%)	Hygiena Snapshot 1575	Hygiena Snapshot 1575
Hygiena Snapshot 1575	Charm Pocketswab (57%)	Hygiena Snapshot 1333	Hygiena Snapshot 1333
Hygiena Ultrasnap	3M Cleantrace (52%)	3M Cleantrace	Hygiena Supersnap
BioControl Lightning		Hygiena Ultrasnap	3M Cleantrace
3M Cleantrace		BioControl Lightning	BioControl Lightning
Hygiena Snapshot 1616		Hygiena Snapshot 1616N	Hygiena Snapshot 1616N
Charm Pocketswab		Charm Pocketswab	Charm Pocketswab
Neogen Accupoint		Neogen Accupoint	Neogen Accupoint