ATP hygiene monitoring
Do sanitisers really affect measurements?

The use of adenosine triphosphate (ATP) as a marker of cleaning and hygiene has been well established in industrial food processing since the early 1980s. The test method is called ATP bioluminescence which uses an enzyme called luciferase and its properties were determined by McElroy and Strehler in 1949.

The natural enzyme was extracted from fireflies and as with all enzyme reactions, the test has a pH optimum (7.75) and a temperature optimum (20–22°C). The test requires magnesium ions and so it can be adversely affected by other divalent cations or chelating agents. Similarly the turbidity of the sample or reaction mix can also affect the analysis.

The influence of sanitisers

Several authors in the 1990s reported that the ATP bioluminescence test was influenced by cleaning chemicals and sanitisers which could result in enhanced or decreased light output depending on the concentration (Velazquez & Feirtag, 1997; Green et al, 1999). The inhibition of bioluminescence activity by chemicals is generally referred to as quenching. An internal standard or 'caged ATP' has been suggested as a means to assess the amount of quenching and provide a more precise determination of the ATP present (Calvert et al, 2001).

The application of ATP bioluminescence for hygiene monitoring is intended to provide a simple, rapid, direct, objective test for cleaning verification, primarily for the removal of organic matter. The test results are expressed as Relative Light Units (RLU) and are interpreted in broad categories of Pass/Caution/Fail for many reasons. The method and application are not intended to be a precision quantitative determination for ATP. Experienced industrial practitioners have reported that "the interpretation of the results (i.e. as clean or dirty) was not affected by the phenomenon" of quenching (Kyriakides, et al, 1991; Kyriakides, 1994)

Evaluating modern cleaning agents

The firefly as natural source of luciferase is still used by some suppliers today, however, many manufacturers and suppliers use modern recombinant forms of the enzyme with significantly improved characteristics of stability and robustness. There have been many recent developments in instrumentation and reagent formulation, as well as changes to the formulation of cleaning chemicals and sanitisers. Accordingly a series of laboratory tests were conducted to evaluate a selection of modern cleaning agents on the ATP hygiene monitoring test application.

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Methodology

Nine cleaning agents were tested representing different categories of chemical agents supplied by the leading international chemical supply company, JohnsonDiversey. The chemicals were tested at seven concentration levels (above and below the manufacturer's recommended working

Table 1: Effect of chemical sanitizers on ATP response from SystemSURE Plus and Ultrasnap

Detergent Concentration	TEGO 2001	Enduro	DellaDet	Acifoam	MultiClean	UltraClean	ShureClean	HD 141	CleanGel
Basis	Amphoteric	Alkaline	Alkaline	Acidic	Alkaline	Alkaline	Neutral	Alkaline	Alkaline
Solvent	Water	Water	Water	Water	Water	Water	Water	Water	Water
MRWC (v/v %)	1 - 2%	4 - 10%	1 - 2%	3 - 10%	1 -10%	0.5 - 5%	0.1 - 1%	2-10%	1-5%
Percentage response compared to ATP positive control									
0.10%	97	98	102	108	107	98	102	100	102
1%	95	94	91	92	108	100	52	100	100
10%	94	45	106	51	70	85	1	100	100

MRWC = manufacturer's recommended working concentration

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Figure 1: Effect of Tego 2001 at 10% v/v on ATP detection by five different systems

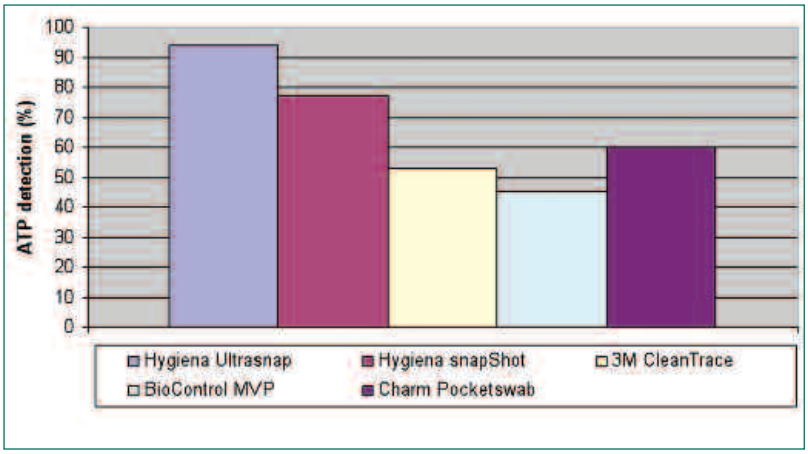

Table 2: Effect of cleaning chemicals at maximum recommended working concentration on 5 ATP detection systems

Chemical	Ultrasnap	snapshot	CleanTrace	BioControl	Charm
TEGO 2001	94	77	53	45	60
Enduro HD	45	53	60	98	75
DellaDet	106	105	119	52	54
Acifoam	51	65	77	4?	89
MultiClean	70	68	99	90	100
UltraClean	85	100	93	97	100
ShureClean	1	5	8	6	25
HD 141	100	100	99	99	102
CleanGel	100	100	100	100	100

concentration (MRWC)) in the presence and absence of a known amount of ATP (1000 fmols). The effect of the chemicals on the RLU output was compared using five different ATP detection systems including Hygiena SystemSURE Plus with Ultrasnap swabs, and the universal ATP swabs snapshot.

Results

The results are described as the percentage recovery of ATP. Table 1 shows that the Hygiena SystemSURE Plus and Ultrasnap were tolerant to most chemicals at MRWC (typically 0.1–10%). Six chemicals showed no or slight reduction in test performance even at the highest MRWC. Enduro and Acifoam caused a reduction in RLU output but only at the maximum MRWC, however, there were sufficient activities' remains to enable the detections of product residues. ShureClean exhibited most quenching particularly at its maximum recommended strength.

Quenching only really appears to happen at high concentrations for some chemicals in some ATP detection systems

At the lowest MRWC there was no significant quenching on the ATP detection observed in any of the ATP detection systems tested. However quenching and some minor enhancement of light output were observed at the highest MRWC for some chemicals in some systems; but not all.

A comparison of ATP test systems

In comparison with other ATP test systems, the Hygiena Ultrasnap and snapshot devices were shown to be more tolerant to the effects of Tego than 3M CleanTrace, BioControl MVP Lightning and Charm Pocketswab in their respective instruments (see Figure 1), however this was not consistent across the range of chemicals tested.

Only at the highest MRWC were ATP detection systems affected by some cleaning chemicals (Table 2). Different detection systems exhibited different responses to the chemical agents. ShureClean appeared to be the most inhibitory chemical tested and affected all systems similarly. Accordingly, care should be exercised when using ATP detection systems in the presence of this chemical at its maximum MRWC. None of the ATP systems were resistant to all the chemical tests.

Quenching

The extent of the quenching is dependent on the formulation of the reagents from the different suppliers. Optimum bioluminescence performance is a balance between gaining the maximum light output at the point of maximum robustness. A compromise needs to be reached between the robustness of a bioluminescent reagent, its real time stability and the detection parameters of the instrument.

So what does this mean in practice?

Quenching only really appears to happen at high concentrations for some chemicals in some ATP detection systems. ATP bioluminescence reagents are unlikely to be exposed to chemicals at or above maximum working concentrations since many would be removed or diluted by rinsing procedures, and test surfaces are usually allowed to drain and dry before surface swab

samples are collected for testing. In addition, any potential adverse effects should be identified during the introduction of alternative chemicals and the validation of modified cleaning procedures.

In the majority of cases, chemical quenching has relatively few consequences for ATP hygiene monitoring. Even in the worst case, a reduction of 50% luciferase activity will still leave sufficient activity to differentiate clean from dirty surfaces. The test results are categorised as Pass/Caution/ Fail based on the RLU unit which is by definition 'Relative'. The ATP hygiene test application is a qualitative determination of cleanliness and it is not intended to be an absolute determination of ATP content.

In summary

- Most cleaning chemicals at MRWC do not significantly affect the practical performance of ATP hygiene monitoring systems.
- The effects of quenching are only apparent at very high concentrations that are unlikely to occur in practice. The extent of quenching varies between ATP detection systems and is a function of the reagent formulation.
- There is little between ATP detection systems and their response to cleaning chemicals.
- No single ATP detection system is resistant to all chemical types. ■

References
McElroy, WD and Streghler, BL (1949) Arch. Biochem. Biophys., 22, 420 – 433.
Velazquez, M and Feirtag, JM (997) J. Food Protection, 60, (7), 799 – 803
Green, TA: Russell, SM and Fletcher, DL (1999) J. Food Protection, 62, (1), 86 – 90.
Calvert, RM; Hopkins, HC; Reilly, MJ and Forsythe, SJ (2001) Letters in Applied Microbiology, 30, (3) , 223 – 227
Kyriakides, A. L and Patel, P. D (1994) Luminescent techniques for microbiological analysis of foods. In; Rapid Analysis Techniques in Food Microbiology (ed P. D. Patel) 196 – 231. Blackie Academic and Professional, Glasgow
Kyriakides et al (1991) Rapid hygiene monitoring using ATP bioluminescence, In: Bioluminescence and Chemiluminescence: current status; 519 – 522. John Wiley and Sons, Chichester

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