Rapid hygiene tests in support of food safety

– exploding the myths about ATP hygiene monitoring.

Cleaning and hygiene is a primary preventative measure for all food business operators both large and small, and is a key component of many food safety initiatives such as HACCP, Safe Food Better Business and Scores on the Doors.

Food Hygiene is one of the three main risk-rating scores of the Code of Practice of the new Local Authority Enforcement Monitoring System (LAEMS) in the UK. The intention of LAEMS is to implement 'effective inspection and enforcement to maintain and improve the compliance of food establishments with food law'.

ATP bioluminescence provides a simple rapid test method for monitoring cleanliness, hygiene and risk. The technology and application has been in use for >25 years and there are many publications on the subject (Griffith et al 1997; Dillon & Griffith, 1999; Illsey et al., 2000).

There is little doubt that the application of ATP bioluminescence for rapid hygiene monitoring applications is widely recognised as an effective test for hygiene and the verification of cleaning procedures. The broader benefits of this rapid alternative test include the provision of:

- real time information for early warning of risk and immediate corrective action
- data for key performance indicators and trend analysis
- data as evidence of due diligence
- cost savings of 25–50% from optimised cleaning procedures and chemical usage
- improvements in product quality and shelf life.

Senior technical professionals from leading independent organisations around the world concur that the ATP test is a direct, objective method that detects product residues on surfaces, and that the test is not intended to be a direct replacement for the traditional cultural microbiological test. Put simply, ATP hygiene monitoring is a product residue test, not a bacteria test.

There are several misconceptions regarding the application of ATP bioluminescence for hygiene monitoring, and this article will address the issues.

What is ATP hygiene monitoring?

The method uses the enzyme luciferase to convert a chemical compound (Adenosine Triphosphate, ATP) into a light signal which is measured by the instrument that gives results in Relative Light Units (RLU). The enzyme is very specific for ATP only and does not detect ADP or AMP. The test is very sensitive (limit of detection is typically 10–15 mols ATP), and gives results in seconds that are linear, repeatable and reproducible. However, the test is a biological assay and is therefore inherently more variable (lower precision and accuracy) than a chemical assay. Sample distribution and collection can also have an impact on the results. It is important to understand the sources of error to obtain reasonable expectations of the test results, particularly when comparing different systems.

ATP is the universal energy carrier and is found in all living organisms from the food we eat, our own body fluids and micro-organisms. The ATP content of foodstuff

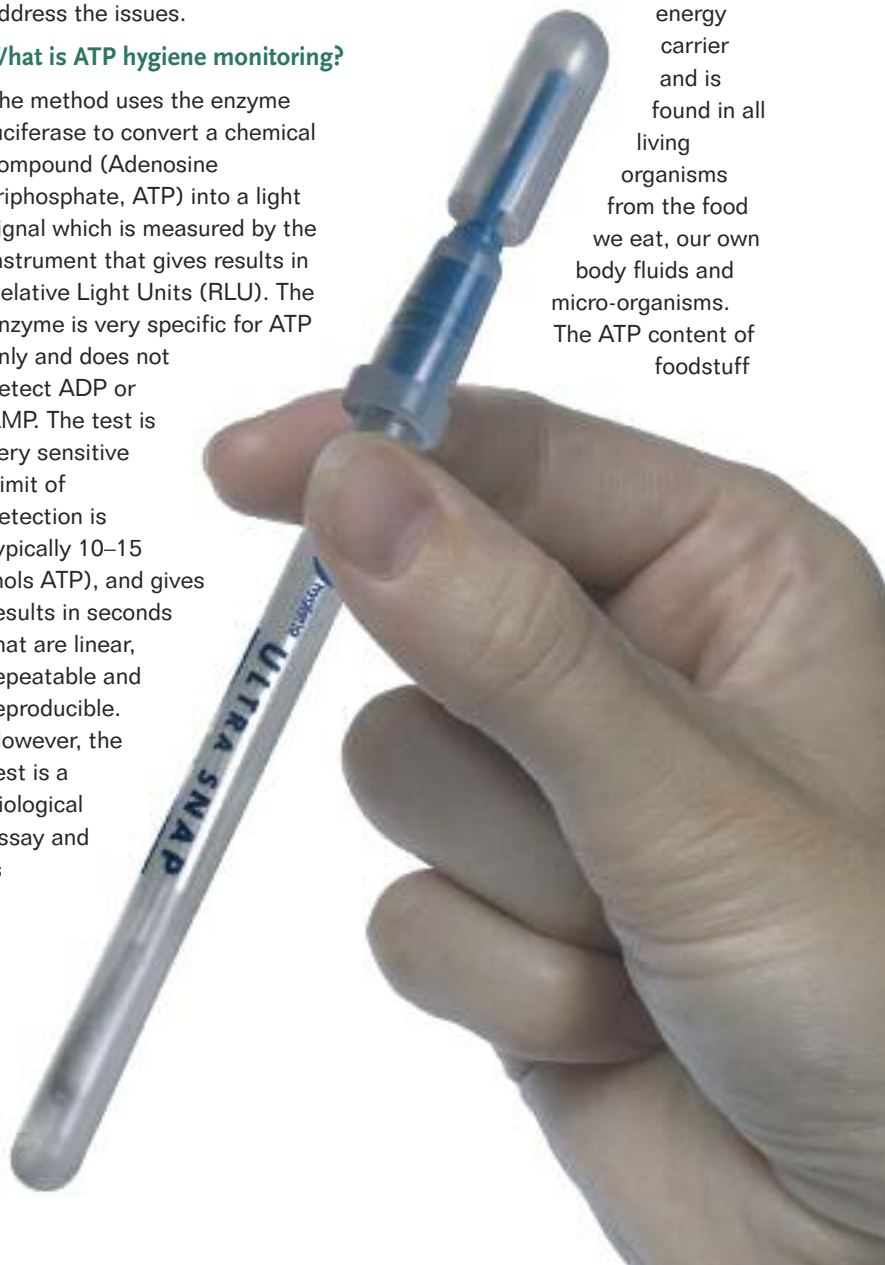
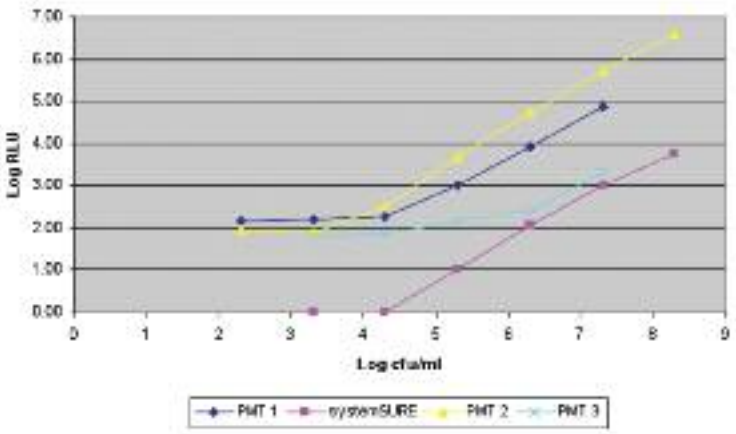


Table 1: Comparison of ATP test and traditional microbiology tests

TEST METHOD			NOS OF SAMPLES	PERCENTAGE
ATP Hygiene monitoring (RLU)	Microbial plate counts (CFU)			
>500	>300		59	36.4
<500	<300		49	30.2
		Sub-total	108	66.6
>500	<300		37	22.8
<500	>300		17	10.5
		Sub-total	54	33.3
		Totals	162	99.9

Figure 1: ATP detection of bacteria



and body fluids is very large and is usually millions of times greater than that of micro-organisms. This is largely due to the size differences but is also a function of metabolic condition.

The ATP hygiene test detects ATP from all sources and cannot differentiate ATP from different sources. Contamination (organic matter or microbes) is not evenly distributed on a product's contact surface. Accordingly, the ATP hygiene test should not be considered as an absolute, precise measurement of surface contamination. It is a sophisticated sensitive indicator test of hygienic status and potential risk.

Is there a relationship between the ATP test result and microbial numbers on food production equipment?

Yes, but it is a concurrent relationship. The primary purpose of cleaning is to remove product residue from product contact surfaces. Effective cleaning simultaneously removes the material capable of supporting microbial survival and growth, as well as many of the microbes themselves. Accordingly, there will be a direct relationship between ATP hygiene monitoring and microbial enumeration as methods. This concurrent relationship cannot be expected to be 100% because both methods are measuring different analytes and both are variable biological tests. Some published data (Ilsey, 2000) shows 80–90% agreement, whereas other data shows 67% agreement (see Table 1), due to the presence of product residues that are not detected by the microbial test.

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Accordingly, the ideal test to measure cleaning efficiency is a product residue test that gives rapid results so that corrective action (e.g. re-cleaning) can be implemented immediately in support of GMP and HACCP. This is what the ATP hygiene test delivers.

Can the ATP test detect bacteria?

Yes, if bacteria are present in large enough numbers (typically >10,000 CFU/ml), and there is no ATP from any other sources (Stanley, 1989; Kyriakides et al., 1991, 1994). Figure 1 shows microbial detection limits in different detection systems in the absence of ATP from other sources, and also demonstrates that there is little practical difference between PMT-based systems and the photodiode-based systemSURE.

In most manufacturing facilities it is unlikely that there will be a high number of microbes in the absence of organic matter, particularly as foodstuffs contain large amounts of ATP. Similarly, the cleaning standard for product contact surfaces in the food industry is typically <100 – 800 CFU /100cm^2 (Dillion and Griffiths, 1999), which is below the detection limit of the ATP test. Accordingly, performance claims for ATP tests for hygiene applications based solely on the detection of numbers of micro-organisms are irrelevant.

What does the RLU mean?

The unit of measurement of the ATP test is called a Relative Light Unit (RLU). This is not a standardised unit of measurement such as length (inches or metres), or weight (kilograms or pounds). RLU does not equate to CFU (microbial numbers), for the reasons given above.

The RLU value is dependent on the instrument construction and reagent/swab formulations. Each supplier has its own luciferase formulations and instrument design so the RLU output scale

Table 2: Comparison of ATP hygiene monitoring systems by an international soft drinks manufacturer

PARAMETER		ALL SAMPLE LOCATIONS
Total samples		189
Total Passes;	– BioTrace	160
	– systemSURE II	159
Total Fails;	– BioTrace	29
	– systemSURE II	30
Passes by both systems		139
Fails by both systems		9
Fails by Bio Trace / Pass by Hygiena		20 (10.6%)
Pass by Bio Trace / Fail by Hygiena		21 (11.2%)

Table 3: Effect of background on the determination of sensitivity of an ATP system

PARAMETER	systemSURE (II AND PLUS)	PMT SYSTEM SUPPLIER A	SUPPLIER B	SUPPLIER C
Average Blank RLU (10 replicates)	0.1	21	63	23
Std Dev of Blank	0.3	6	40	11
Slope (RLU/fmol)	1.1375	27.5	7.1	5.2
Sensitivity (ATP) (Limit of detection) {Average x 3(sd) / slope}	0.8	0.6	16.9	6.2

will be different for each supplier, but all systems are linear in response to ATP and have similar performances in terms of sensitivity and repeatability.

It is a common mistake for users to expect a close agreement between RLU measurements when comparing different systems. This is due to the differences in RLU scales and outputs, the inherent variation of this biological assay and variations due to sample distribution and sample collection. Therefore the ATP hygiene test should not be considered as a precision assay. The ATP hygiene test application is a sensitive, direct, objective test of cleaning efficiency and risk.

Care should be taken when comparing the system performance of different instruments or suppliers. For routine industrial applications there is little value in examining individual RLU values for the reasons given above. It is better to compare the overall performance in terms of the number of passes and fails by both systems at equivalent settings. Table 2 shows almost 100% agreement on the correct classification of results when two different systems were compared in routine test applications i.e. number of passes (159/160) and fails (29/30). Both systems show an equal number of samples (~10%) were passed by one system and failed by the other system. This is a function variation from sample distribution and collection and is independent of the system used.

How does the instrument detect light?

There are two detector systems in use today. Photomultiplier tubes (PMT) that are glass vacuum tubes that electronically amplify the light signal and require high voltages to function. The disadvantage of PMTs is that they are expensive, fragile (made of glass), have a high background noise, drift with time and require regular service and calibration.

By contrast, the photodiode detectors are solid-state, semi-conductor devices that are robust, have low background noise, require low voltage and do not drift with time. Accordingly instruments using photodiode detectors such as systemSURE are simpler, smaller, lighter, more robust, self-calibrating, virtually maintenance-free and significantly cheaper.

The relative merits of light detectors are described by Godfrey, and although, in theory, a PMT is potentially a more sensitive detector, the complexity of their design and operation, and high background noise can limit the working performance of the system.

Instruments offering large RLU numbers do not necessarily mean that there is a greater sensitivity. The RLU scale is a function of the instrument design and construction that can be made to show any number scale which is all 'relative'. Similarly a large RLU number scale may suggest a finer discrimination between results, but this only applies if the test results show a high degree of precision, which is not possible with the ATP hygiene test. Accordingly care should be exercised in assessing supplier claims.

One of the key features of any analytical method is the background noise of the systems

because this directly affects the reliability of the measurements at low levels and hence the limit of detection (or sensitivity) of the test. For ATP bioluminescence there are several sources of noise which can come from both the instrument detection system and reagent formulation. SystemSURE Plus is a unique system that has low background from both its photodiode instrument and reagent formulation. This combination delivers remarkable performance. Table 3 shows the impact of high background noise of PMT instruments on the system's performance: the larger the background noise and variation from blank samples, the poorer the sensitivity of the system. Similarly, as variation increases so precision decreases.

Systems offering low background give better performance by showing less variation and more reliable detection at low RLU values which in turn delivers better sensitivity and reliable early warning from trend analysis.

SystemSURE Plus is a unique system that has low background from both its photodiode instrument and reagent formulation

In summary, the application of ATP bioluminescence for rapid hygiene monitoring has been established for >25 years and it now makes a well recognised contribution to food quality and safety systems. These systems deliver a rapid, direct, objective measurement of cleaning efficiency, hygienic status and risk, primarily by the measurement of product residues. ATP hygiene monitoring provides cost savings to the business as well as improvements in product quality. The results from ATP 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 but there is concurrent direct correlation between the results of the two methods. The ATP test is not suitable for the enumeration of microbes on products' contact surfaces because it does not have the desired sensitivity. ATP detection systems with low background noise deliver the better performance. ■

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