Exploding the myths about ATP hygiene monitoring

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

There is little doubt that the application of ATP bioluminescence for 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 optimised cleaning with cost savings of 25-50% on cleaning chemicals, improvements in product quality and shelf life, the provisions of data for real time trend analysis and evidence of due diligence.

The technology and application has been in use for over 30 years and there are many publications on the subject.

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.

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), 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 that of a chemical assay.

Sample distribution and collection can have a significant 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 and body fluids is very large (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.

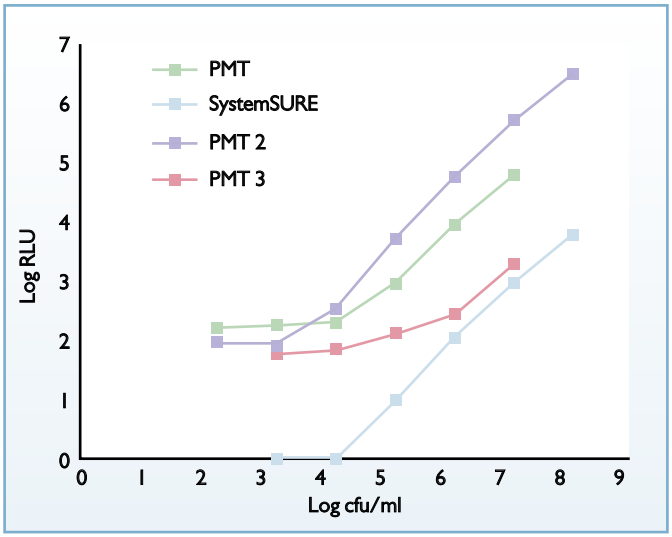

Fig. 1. ATP detection of bacteria.

The ATP hygiene test detects ATP from all sources and cannot differentiate ATP from different sources. Contamination (organic matter or microbes) are not evenly distributed on product 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 coincidental relationship. The primary purpose of cleaning is to remove product residue for product contact surfaces. Effective cleaning simultaneously removes the material capable of supporting microbial survival and growth, as well as many of microbes themselves.

Accordingly there will be a direct relationship between ATP hygiene monitoring and microbial enumeration as methods. This coincidental relationship cannot be expected to be 100% because both methods are measuring different analytes and both are variable biological tests.

Some published data 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.

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

Table 1. Comparison of ATP test and traditional microbiology tests.

Test method		No. 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**

Can the ATP test detect bacteria?

Yes, if they are present in large enough numbers (typically >10,000 cfu/ml) and there is no ATP from any other sources.

Fig. 1 shows microbial detection limits in different detection systems in the absence of ATP from other sources, and also that there is little practical difference between PMT-based systems and photodiode-based systems.

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 amount of ATP.

Similarly, the cleaning standard for product contact surfaces in the food industry is typically <100-800 cfu/100cm^2 which is below the detection limit of ATP test.

Accordingly, performance claims for ATP tests for hygiene applications based solely on the detection

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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 (or RLU). This is not a standardised unit of measurement such as length (inches or metres) or weight (kilograms).

The RLU value is dependent on the instrument construction and reagent/swab formulations. Each supplier has its own luciferase formulations so the RLU output scale will be different for each supplier but all systems are linear in response to ATP and have similar performances. RLU does not equate to cfu for the reasons given above.

Care should be taken when comparing the RLU scale from different instruments or suppliers.

For routine industrial applications there is little value in examining individual RLU values when comparing different ATP systems because of the effects of sample variation.

It is better to compare the overall performance in terms of the number of pass 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 pass (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 sample variation and is independent of the system used.

How does the instrument detect light?

There are two detector systems in use today. Photomultiplier tubes (PMT) are glass vacuum tubes that amplify electronic signals 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'.

One of the key features of any analytical method is the background noise of the system 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, and 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, then the poorer the sensitivity of the system).

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.

In summary, the application of ATP bioluminescence for rapid hygiene monitoring has been established for >25 years and 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 and there is coincidental direct correlation between the results of the two methods.

The ATP test is not suitable for the enumeration of microbes on product 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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References are available from the author on request.

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
Fail by BioTrace/Pass by Hygiena	20 (10.6%)
Pass by BioTrace/Fail by Hygiena	21 (11.2%)

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

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