What is really important in ATP measurement for hygiene monitoring?

Rapid surface hygiene monitoring using ATP bioluminescence has been established for 30 years and is a recognised contribution to global 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 organic product residues.

ATP hygiene monitoring provides cost savings to the food business operator as well as improvements in product quality, food safety and brand protection.

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Biological tests such as ATP bioluminescence have much greater inherent variability compared to tests for chemical analytes and the hygiene monitoring application is not intended to be a precise determination of ATP content because the sample itself (a surface swab) is variable and operator dependent.

Apart from technical performance the other important considerations are consistency, reliability, robustness, maintenance, and service and support.

Sources of variation

ATP measurement systems consist of two main parts; the reagent and instrumentation, both of which need to be precisely engineered and optimised to give minimum background noise and variation that confers repeatability and sensitivity for best performance.

ATP hygiene monitoring systems use one of two detector systems. Photomultiplier tubes (PMTs) are glass vacuum tubes with embedded electrodes. The disadvantage of PMTs is that they are expensive, require high voltages, have 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 and EnSURE are simpler, smaller, lighter, more robust, self-calibrating, virtually maintenance free and lower cost.

Instrument construction and electronics can impact significantly on performance irrespective of the detector itself. The unit of measurement of the ATP test is called a Relative Light Unit (or RLU). This is not a standardised unit of measurement and it 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 will be different for each supplier. Although all ATP systems are linear in response to ATP, they do not all have the same performance in terms of sensitivity and repeatability.

It can be difficult to compare the performance of different ATP system when using only the results from routine factory surface testing. 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.

Sample error is one of the largest sources of variation and is entirely user dependent.

Sample distribution is dependent on the surface type and material under test as well as the efficacy of cleaning procedures.

Sample collection is dependent on the type and material of the sampling device as well as the operator technique. For these reasons, ATP test results are given as bands of Pass, Caution and Fail.

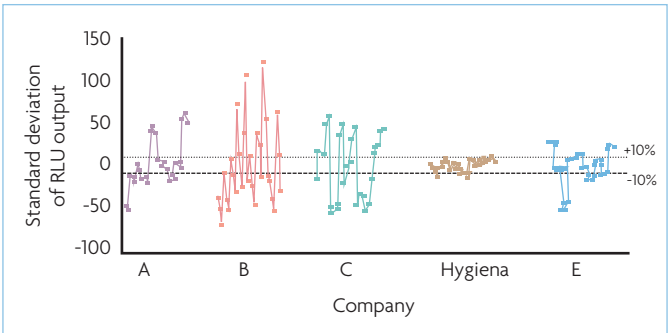

Fig. 1. Variation of ATP detection systems.

Table 1. Comparison of Hygiena UltraSnap with a competitor product.

ATP (fmols)			Results (RLU)			
Hygiena (EnSURE UltraSnap)	**Avg (10 reps)**	**Std Dev**	**CV (%)**	**Min**	**Max**	**Detected (%)**
20	54	4.6	8	48	62	100
200	486	26.2	5	453	535	100
2000	4839	568.3	12	3944	5673	100
RLU/fmol	2.4					
Combined variation	8%					
Competitor's product	**Avg.**	**Std Dev**	**CV (%)**	**Min**	**Max**	**Detected (%)**
20	24	26.7	112	0	68	0
200	255	184.0	72	71	615	70
2000	2427	1677	69	651	6069	100
RLU/fmol	1.21					
Combined variation	84%					

Precision and accuracy

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. A test cleaning verification requires the ability to detect at low levels.

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 liquid stable reagent formulation. This combination delivers remarkable performance. The variation of any method determines its accuracy and precision. In an extensive study conducted by an independent international laboratory, the accuracy and precision of different ATP systems were studied under ideal conditions. Fig. 1 shows the results from 30 replicate samples of ATP (at relatively high levels of ATP of 100 fmols) tested directly in five test systems by the same analyst.

Hygiena SystemSURE Plus was shown to be the most accurate and

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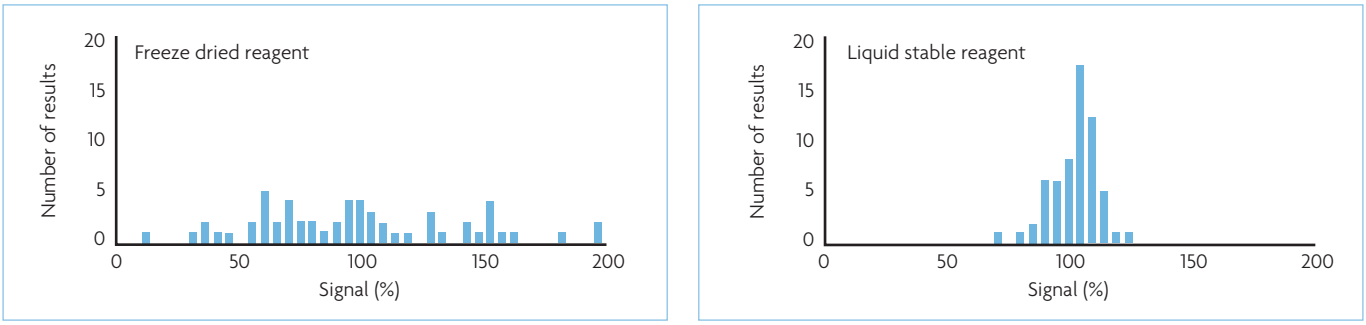

Fig. 2. Variation within an individual freeze dried test device and Hygiena's liquid stable devices.

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precise ATP test system because results have a good standard error limits of ±10%.

Other systems can vary up to 130%, which is highly imprecise and very inaccurate. At lower ATP levels (10fmols) which would be a target for most industrial food processors in high care facilities, variation was greater for most ATP systems except those of Hygiena.

The study also showed that the Hygiena system recovered >90% ATP from the swab and with the least variation, thus enabling reliable, repeatable and accurate sample collection and detection. Other systems detected only 50% of the available ATP from the swab.

Swab size is claimed by some studies to offer greater coverage, and faster/better sample collection. However the quality of the sample collected is dependent on the diligence and training of the operator as well as the construction of the swab device.

The dimensions of the swab itself need to be practical and function for all surface types. The formulation of the swab wetting agent needs to be balanced with the test reagent to give optimum performance with repeatable output and consistency from batch to batch.

Table 1 shows that a competitor's advanced system with a large swab has poorer repeatability that could generate false negative results.

Robustness and reliability

Luciferase reagent preparations and their delivery devices for ATP detection vary between suppliers and are optimised for each system. Each reagent system is a balanced cocktail of enzyme, co-factors, buffer, and extractant.

Some comparative studies have over-analysed the impact of time and temperature on RLU output of ATP systems to imply greater scientific performance.

However, this surface swab is intended to be an instant semi-quantitative verification of cleanliness. It is not conducted under controlled laboratory conditions but rather at the point of use in working factory facilities where robustness is a more important attribute.

Reliability and consistency of output from batch to batch is a critical key parameter that is often taken for granted.

Different reports have shown that some suppliers' devices vary between 5 and 10 RLU per fmol ATP, whereas studies involving another system varied between 1 and 10 RLU per fmol ATP. Similarly, claims of shelf stability are often made without revealing the assessment criteria and providing substantiating data.

Hygiena was the first to make a liquid-stable luciferase reagent more than 15 years ago and continues to make improvements.

The benefits include greater consistency, repeatability, robustness, sensitivity and shelf stability.

For example UltraSnap has a shelf life of 15 months at +2 to +8°C. It can be stored for four weeks at ambient storage (21°C) or one week at 37°C without significant loss of activity. UltraSnap can be treated as an ambient stable short shelf life product.

Freeze dried reagent formulations generally require refrigerated storage and single test devices have much greater variation (see Fig. 2).

Most ATP hygiene monitoring systems detect only residual ATP in a sample, however Hygiena has made a family of products (MicroSnap and ZymoSnap) that, for the first time, make the ATP test reaction specific for certain analytes.

MicroSnap can detect low numbers of bacteria (1-5) in seven hours from a wide variety of sample types, including surface swabs, raw materials and finished products. It can also be used with filtration to detect low numbers in water and filterable beverages of 100ml or more.

MicroSnap is available for total aerobic bacteria, enterobacteriaceae, coliforms and E. coli, and a listeria test will be available soon. All tests can be run on the same instrument.

A quality product needs to be supported by excellent service and support that only the manufacturer can supply – from packaging and delivery to training and high calibre technical support.

These are some of the many reasons why Hygiena is the system of choice for many users. ■

References are available from the author on request