Looking at further developments in ATP bioluminescence

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Since 2003 there has been a rapid and continued development in ATP bioluminescence in both simple, portable, sensitive instruments and novel reagent formulations that have extended the performance capabilities, ease of use and, importantly, made the technology more affordable and therefore more accessible to a wider user base and range of applications.

Here we report on the evaluation of a new, improved system for hygiene monitoring and describe a new bioluminogenic test that, for the first time, confers specificity to the technology permitting microbial detection in seven hours.

Key to these developments has been the use of solid-state detectors in instruments with reduced size and power consumption, as well as liquid-stable reagents both providing low background noise, which is essential for maximum performance and sensitivity. It is the background noise in any analytical system that governs performance and determines the limit of detection and sensitivity of the results.

Instrument	No. tested*	Performance output (RLU) at 15 fmols ATP Min.	Max.	Av.	%CV
Supplier A Photomultiplier	7	36	319	143	64
SystemSURE Photodiode	6	23	29	28	10

*Instruments in routine use for several years

Table 1. Variation in instrument performance in the field.

Verification of cleaning

ATP hygiene monitoring is intended to be used for the verification of cleaning where it measures low level residual organic contamination, so reliable detection at low ATP values is very important.

The unit of measurement is RLU (Relative Light Units) which is 'relative' to the system i.e. the instrument and reagents and ATP present. A system with a higher RLU and large dynamic range is not necessarily more sensitive than another system with a smaller RLU scale.

For example, a single length of material can be measure as 12 inches or 30cm but is the same length. The only difference is measurement scale. Similarly, speed can be measured on different scales, for example, 50 miles per hour = 80 kilometres per hour. Bigger is not always better.

It is often incorrectly assumed that instruments with photo-multiplier tube detectors should be more sensitive than instruments with solid-state photodiode detectors.

The sensitivity and performance of all ATP systems is determined by the background noise within 'the system as a whole'.

Background noise comes from instrument construction, electronics, and detector as well as the reagents. Sensitivity and performance are not governed by detector technology alone, which is a small part of the overall system.

Unlike PMTs that drift and are fragile, the photodiode is stable and robust which means that they do not drift and require little service and calibration giving greater reliability and lower capital and maintenance costs.

Table 1 shows the variation of PMT instrument performance compared to photodiode instruments after several years' usage in the field. ATP systems with high RLU output may imply a greater dynamic range and sensitivity, however high background can mask the underlying poor performance of the systems.

Fig. 1. shows the effect of high background

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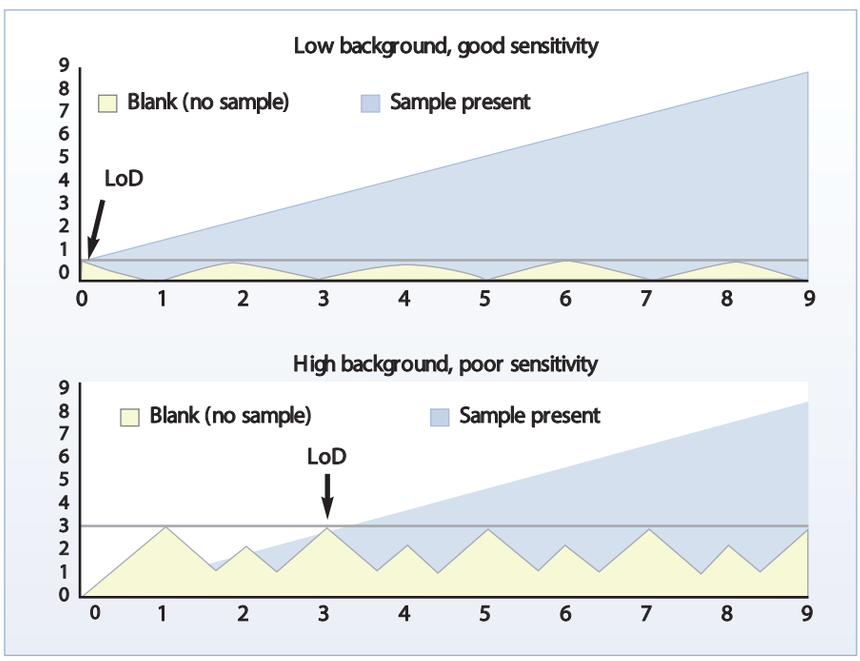
Fig. 1. The influence of background noise on test performance.



Performance parameter	SystemSURE Ultrasnap*	EnSURE SuperSnap*	Supplier 1 system A**	Supplier 2 system B**	Supplier 3 system C**	Supplier 4 system D*
Background Noise (RLU)	0 - 1	0 - 1	2 - 11	100 - 570	0 - 511	0 - 48
Limit of detection (fmols)	1.0 – 1.4	0.1 - 0.2	1.3 - 2.7	1.1	10.0	10.0
%CV at 10 fmols ATP	6.2 – 10.4	6.9	17.1	52.6	213.8	114.4
*Photodiode detector	**PMT detector					

Table 2. Summary of results from three different independent evaluation studies.

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noise on the sensitivity of a system irrespective of the detector system within the instrument. Liquid-stable reagent gives consistent batch performance with low background noise that provides:

- A high degree consistency/ repeatability that cannot be matched by single dosage forms of freeze dried reagents.
- High sensitivity.
- Reliable detection and trending at low ATP and low RLU values.

Independent validation studies have demonstrated the performance benefits of solid-state ATP systems over other detection systems based on photomultiplier tube technology (see Table 2)

Recent evaluations

The most recent ATP system development – EnSURE with Supersnap and AquaSnap – was evaluated by Campden BRI and compared with another leading ATP system.

The Hygiena EnSURE luminometer was very simple and easy to use requiring very little instruction. Its hand-held format was very portable and sturdy which allows it to be used in a brewery situation where hygiene assessment may be required.

The calibration of the equipment did not show any drift over a one month period and the provided standards were stable over this time. Measurements demonstrated good repeatability.

In comparison with a competitor luminometer the Hygiena unit appeared about 10 times more sensitive at the low ATP levels of 1fmol and lower.

This was also demonstrated by the difference in limit-of-detection which was 0.21 fmol ATP for the EnSURE luminometer and 2.72 fmol ATP for the competitor system.

In comparison with the competitor device the Hygiena luminometer appeared more sensitive at low ATP levels and showed slightly better repeatability.

However, at the higher bioluminescence levels it showed signal saturation (EnSURE + AquaSnap) which was not seen with the competitor device. This is not a cause for concern as, at these ATP levels of residue, the hygiene test would be considered a fail anyway.

Generally, linearity between ATP bioluminescence and product concentration was achieved on both systems with linear correlation coefficients mostly lying between 0.92 and 0.97.

The readings would be expected to decrease by one log order for each dilution and the bioluminescence measurements showed this for most of the drinks (down to dilution 1:1000 for the beverages with the highest readings and down to 1:100 dilution for beverages with intermediate readings).

However, the drinks showing the lowest values did not follow this pattern. There was no indication of signal saturation at the higher concentrations.

However, a levelling off at lower concentrations was noticeable and this was more pronounced for the competitor's system for which ATP bioluminescence levelled off at about 15 RLU for both of the lowest product concentrations tested.

The EnSURE system, on the other hand, did show better linearity at these low concentrations indicating that the sensitivity of the EnSURE system at these low ATP concentrations is better than for the competitor system.

There was a linear correlation between yeast cell concentration (up to $1x10^4$ cells/ ml) and bioluminescence output and yeast cells were reliably detected at 100 cells/ ml and above. This equates to 10 yeast cells per sample presented to the test device.

Table 3. Supersnap and resistance to hypochlorite.

Hypochlorite (ppm)	Inhibition of ATP test reaction (%) SuperSnap	UltraSnap	Supplier A
0	0	0	0
62	0	0	17
125	0	0	41
250	0	30	70
500	0	20	86
1000	0	72	100

The system can therefore be used for detection of yeast cell residue, for example in CIP rinse waters.

SuperSnap not only gives improved sensitivity but it also has improved tolerance to harsh samples and chemicals.

It is five times more resistant to acid and 10 times more resistant to alkali than Ultrasnap, and is totally resistant to 1000ppm hypochlorite (Table 3).

The enhanced sensitivity of SuperSnap makes it suitable for high care cleaning verification and in support of allergen cleaning programs where it has been shown to detect food residues below those detectable by specific allergen test.

Hence a super ATP test provides better evidence of cleaning verification than specific allergen tests and is more cost effective.

New bioluminogenic tests

MicroSnap and Zymosnap are a family of products that for the first time make the ATP test reaction specific for certain analytes.

The speed and sensitivity of the ATP test reaction is linked to specific substrate such that the light generation reaction can only occur when the substrate is utilised by a specific enzyme and a specific bacteria.

The systems 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 number in water and filterable beverages of 100ml or more. MicroSnap tests specific for coliforms, enteros, E. coli are available and tests for listeria, Staphyl-ococcus aureus and total counts are under development. Zymosnap can detect enzymes such as alkaline phosphatase for dairy processors, and protease for a number of different industrial applications.

The latest product Cross-Check specifically detects raw meat residues and provides a rapid test to monitor cross contamination hazards in food processing and quick service restaurants.

The EnSURE system provides a simple, affordable platform on which multiple tests can be performed that are suitable for a wide range of applications. ■

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