Novel developments in ATP bioluminescence

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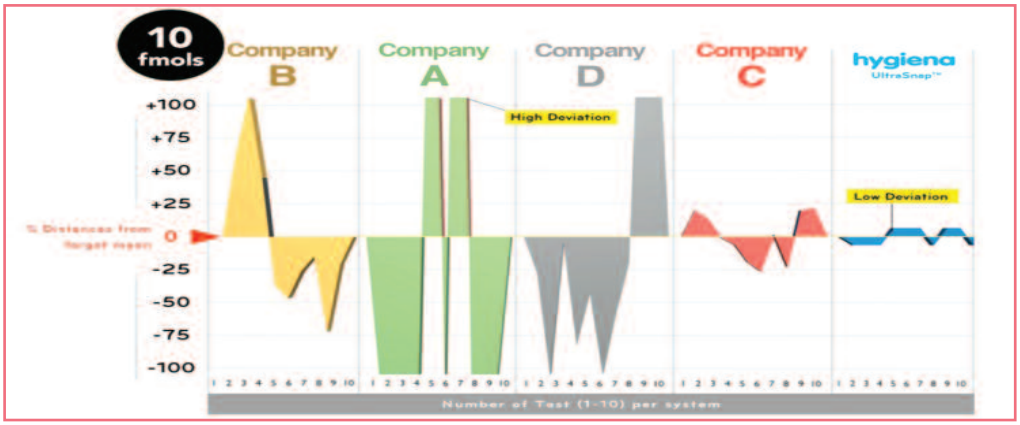


Fig. 1. Variation of ATP hygiene monitoring results.

Several industrial applications of ATP bioluminescence have been developed since the 1970s. These have largely been non-specific applications primarily for the direct objective assessment of cleaning verification and also as a gross monitor of microbial biomass.

Significant developments have been made, particularly over the past 10 years.

This article describes the most recent developments in reagents and instrumentation leading to the first specific test application for the detection of low numbers of pathogenic and indicator organisms within a working day.

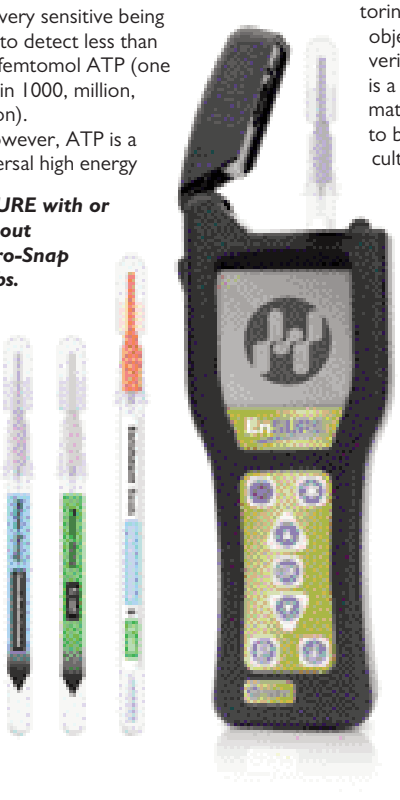
Traditional ATP bioluminescence relies on the use of the enzyme luciferase. This test is very rapid giving results in seconds and is also very sensitive being able to detect less than one femtomol ATP (one part in 1000, million, million).

However, ATP is a universal high energy molecule that is abundant in most organic materials but the test cannot differentiate between ATP from different sources.

The most widely used and accepted application of ATP bioluminescence is surface hygiene monitoring that provides a direct objective test for cleaning verification. This application is a test for residual organic matter and it is not intended to be a replacement for the cultural bacteria test.

Extensive sample preparation procedures and/or pre-enrichment procedures are required to enable the test to detect microbes above the high levels of endogenous ATP present in most products.

These sample preparation procedures therefore limit the sensitivity, duration and complexity of the test. The limit of detection for bacteria using a direct instant ATP test and in the absence of organic matter is typically 10,000cfu/ml, whereas samples with low biomass 10-100cfu/g require 24 hours enrichment to produce a qualitative result (present or absent).



EnSURE with or without Micro-Snap swabs.

Some ATP tests are formulated from native luciferase extracted from the fire fly. However, genetically engineered luciferase preparations have been developed that give enhanced performance and stability.

This has enabled liquid-stable formulations to be prepared that offer a number of advantages including low background noise, improved repeatability, increased sensitivity and lower cost. Packaging these improved reagents into all-in-one, ready-to-use, sample collection and testing devices enables products to be made that offer simplicity, convenience and affordability.

Other tests have used luciferase in combination with Adenylate Kinase to enhance their sensitivity or speed of result (for example Celsis AKuScreen) but overall the time to detection of low levels of biomass is still 18-24 hours and the sample effects still need to be ameliorated or negated.

Instrumentation

The novel reagent technology described below uses a modification of the standard luciferase reaction to make it both specific and sensitive, while reducing the time to detection to <8 hours.

Most instruments use photomultiplier tube (PMT) and this has remained unchanged for 30 years. Advances in CCD cameras make it possible to detect micro-colonies treated with luciferase but this is very expensive and the test requires >14 hours to produce results (Millipore MilliFlex Rapid).

Most luminometers detect the light generated by the bioluminescence signal using PMT detectors which are fragile, expensive, require high voltage and drift with time.

Accordingly, these instruments are complex, high cost and require regular calibration and service. In addition, these instruments can suffer from high background noise which limits their performance and sensitivity irrespective of the reagent formulation and performance.

By contrast, modern solid-state detectors such as photodiodes have a similar sensitivity but they require low voltage, have low background noise, do not drift with time and are lower cost.

In the largest independent study of leading ATP hygiene monitoring systems, the Hygiena SystemSURE Plus was shown to give the best performance in all criteria, particularly repeatability, precision and accuracy (see Fig. 1). The new EnSURE instrument and new SuperSnap reagent swab (Hygiena) both provide additional sensitivity with the same low background noise and low variation for precise accurate

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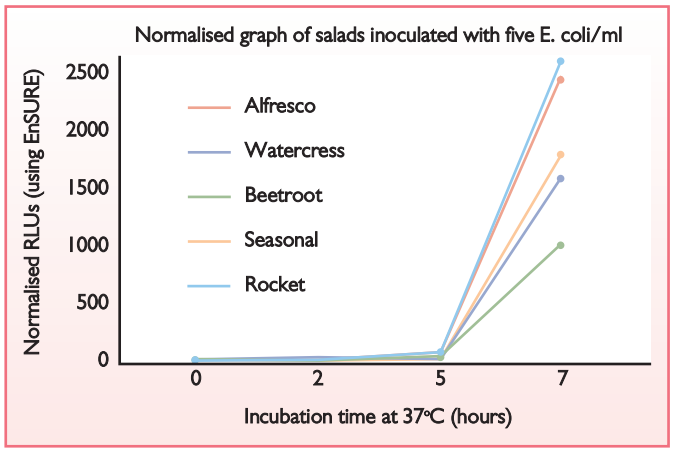

Fig. 2. Detection of E. coli in salad.

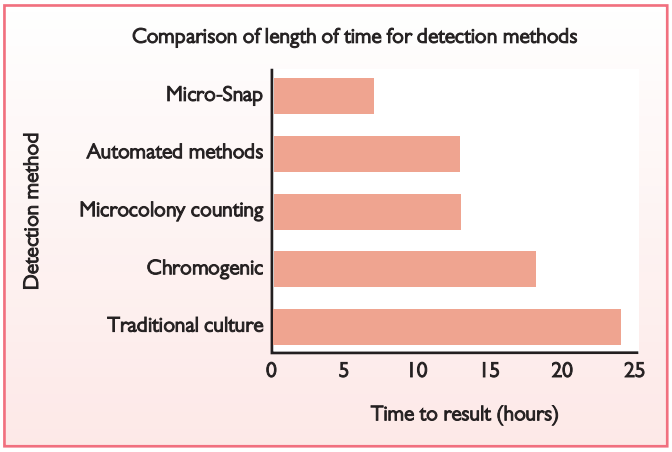

Fig. 3. Comparison of enterobacteriacae methods.

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and consistent results.

This means that this system is 10 times more sensitive than Hygiena SystemSURE Plus and products from Company B and C, and is 100 times more sensitive than products from Company A and D.

Hence EnSURE and SuperSnap are the most suitable for high care food and beverage production operations and in support of allergen control programs.

The EnSURE luminometer also provides a simple, sensitive and convenient test platform for the new bioluminogenic tests that includes specific tests for microbes called Micro-Snap and specific enzyme tests called Zymo-Snap.

New bioluminogenic assay

Micro-Snap uses a novel reagent formulation where some of the essential co-factors of the standard luciferase reaction are immobilised on to a specific substrate that can only be released in the presence of specific enzymes. Hence the light generation will only occur if the substrate is utilised and is diagnostic of certain microbes, for example beta-galactosidase for coliform and beta-glucuronidase for E. coli.

Enzyme activity and copy number are greater than microbial numbers thus providing an early amplified signal such that one organism can be detected in seven hours (see Table 1).

Table 1. Detection limit of Micro-Snap EC.

Incubation time (37°C/hours)	Lowest inoculum detected
1	500,000
2	100,000
3	10,000
4	1,000
5	100
6	10
7	1-5

The test is dependent only on the specific enzyme-substrate reaction, hence there is little interference from the sample which has historically limited the applications of ATP bioluminescence. Consequently, a wide range of products can be tested.

The bioluminogenic assay can also be engineered to detect enzymes of industrial significance such as proteases and phosphatases.

Zymo-Snap is a test for alkaline phosphatase that is used to measure pasteurisation efficiency.

Hence the EnSURE with Super-Snap, Micro-Snap and Zymo-Snap provides a package of tests that are particularly well suited for the dairy industry.

Easy to use components

The Micro-Snap system consists of two easy to use components:

- Sample collection and enrichment device.
- The end detection device (Micro-Snap).

Different substrate mixes permit the detection of different bacteria – Micro-Snap EN detects enterobacteriacae and Micro-Snap EC detects E. coli.

The sample collection and enrichment device is fitted with a swab for testing solid surfaces such as processing equipment or carcases.

Alternatively, a liquid sample (1ml) can be added directly to the swab tube. The liquid can be a suspension of solid sample prepared in the same way and with the same materials as traditional microbiological methods.

After adding the sample, the device is activated by breaking the patented snap valve and squeezing the bulb to mix the sample and enrichment broth. The whole device is then incubated for up to eight hours at 37°C.

At the desired time points, an aliquot (0.1ml) of enrichment culture is transferred to the Micro-Snap device; this can be repeated on 3-4 separate occasions from the same enrichment broth during the enrichment period.

The Micro-Snap device is activated by breaking the snap valve and is incubated for 10 minutes at 37°C and then placed in the luminometer and the result is generated in 15 seconds.

A result of >10 RLU is considered a positive and the greater the RLU output the higher the contamination in the original sample. The total recommended test time is seven hours and 10 minutes. The sensitivity of the test can be further increased by extending the period of the Micro-Snap detection device to 20 or 60 minutes if required.

Micro-Snap has been independently verified by a leading food safety and quality laboratory and shown to have a sensitivity of 89% and a specificity of 99%, which is better than alternative cultural methods (see Table 2).

Micro-Snap EN for enterobacteriacae has 94% sensitivity and 99% specificity. For low numbers of organisms (1-5) the detection time was confirmed as seven hours (as in Table 1) both in pure culture and in inoculated foodstuffs even where the target organism was outnumbered >10,000:1 by competing natural microflora (see Fig. 2).

The traditional method detected 95% of samples, whereas Micro-Snap detected 99% of samples.

The specification for many industrial applications for coliforms and E. coli is <10 per gram which is effectively a presence/absence test for a 1 in 10 dilution of a solid sample (<1 colony per plate.)

Hence Micro-Snap gives equivalent results to the traditional method but in a fraction of the time (see Fig. 3).

If two or more sequential tests are made throughout the enrichment period, the resultant kinetic rate can be used to provide a quantitative measurement of contamination present in the sample.

Large volume liquid samples (~100ml) such as water and beverages can also be tested using Micro-Snap.

The microbes are concentrated from solution by filtration, and the enrichment broth is added to the filter and incubated.

This delivers a sensitivity of one organism in 100ml that can be detected in seven hours. Other applications for Micro-Snap include instant colony characterisation for coliforms and E. coli from semi-selective agars. The use of other specific substrates enables the detection of other organisms such as Listeria monocytogenes.

Summary

Modern technology has delivered improvements in reagent performance and instrumentation to produce small, low cost ATP detection systems with improved sensitivity and repeatability.

Further developments of this very rapid and sensitive technology have now made it possible to detect specific bacteria and to deliver a same shift seven hour test result on a low cost portable instrument.

The same technology can also provide an instant test for enzymes of industrial importance such as alkaline phosphatase. ■

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Table 2. Specificity and sensitivity of Micro-Snap.

	Testing methods for E. coli detection		
	Petrifilm EC	DryCult Coli	Micro-Snap EC
Sensitivity (%)	40	33	89
Specificity (%)	91	79	100