What is really important in ATP measurement for hygiene monitoring?

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Rapid surface hygiene monitoring using ATP bioluminescence has been established for 30 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 organic product residues.

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

The results from ATP surface hygiene monitoring are different to those of microbial enumeration methods and give additional information that the microbial test can not provide.

ATP tests are not intended to replace microbial tests but there is concurrent direct correlation between the results of the two methods because cleaning simultaneously removes both organic residues and microbes.

Most compositional tests used in the food industry are based on chemical methods but the bioluminescence test for ATP (adenosine triphosphate) is an enzymic biological method that is more complex and has several variables.

Sources of variation

ATP hygiene monitoring systems use one of two possible detector systems. Photomultiplier tubes (PMT), that are glass vacuum tubes, 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. The complexity of their design and operation and high background noise can limit the working performance of the system.

Parameter	SystemSURE (Hygiena)	PMT system Supplier A	Supplier B	Supplier C
Average blank RLU (10 replicates)	0.1	21	63	23
Std deviation 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

Table 1. Effect of background noise on the sensitivity of an ATP detection system.

By contrast, 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 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 similar performances 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 largely outside the control of the supplier and entirely user dependent.

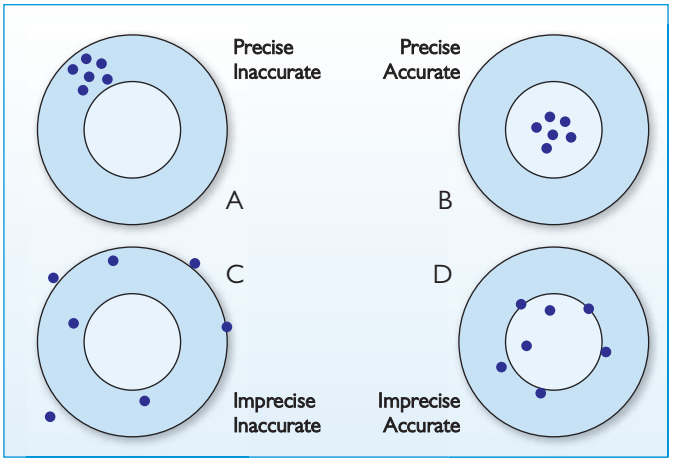
Sample distribution is dependent on the surface type and material under test as well as the efficacy of cleaning procedures.

Sample collection is dependent on the type and material of the sampling device as well as the operator technique. The ATP hygiene test should not be considered as a precision assay in the same way as chemical methods of compositional analysis. Hence, most ATP test results are described in bands of pass, caution and fail.

The ATP surface hygiene test application is a sensitive, direct, objective biological test of cleaning efficiency and risk.

Accordingly, great care should be taken when comparing the performance of different systems.

Fig. 1. Schematic description of accuracy and precision.



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'.

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 usually not possible with the biological tests.

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 1 shows the impact of high background noise of PMT instru-

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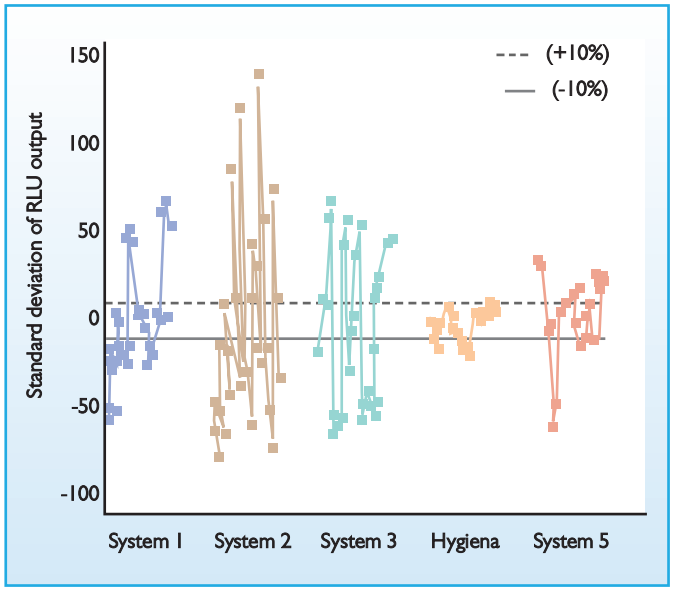

Fig. 2. Variation of ATP detection systems.

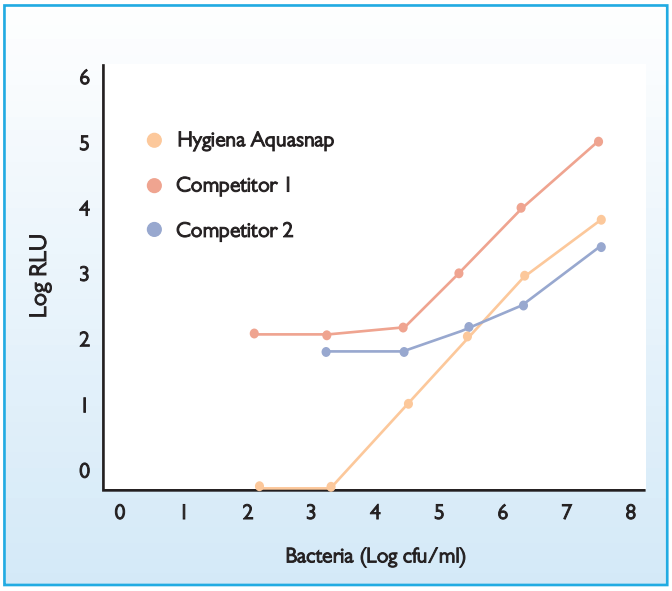

Fig. 4. Microbial detection in water using ATP bioluminescence.

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ments on the system's performance – the larger the background noise and variation from blank samples, then the poorer the sensitivity of the system. Similarly, as variation increases, then precision decreases.

The variation of any method determines its accuracy and precision (as shown in Fig. 1).

In an extensive study conducted by independent international laboratory, accuracy and precision of different ATP systems were studied.

Fig. 2 shows the results from 30 replicate samples of ATP tested directly in five test systems by the same analyst.

Hygiena SystemSURE Plus was shown to be the most accurate and 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.

In addition, the Hygiena SystemSURE recovered most ATP from the swab and with the least variation, thus enabling reliable, precise and accurate sample collection and detection.

Systems 3 and 5 detected only 50% of the available ATP sample (see Fig. 3).

These results were determined under controlled laboratory conditions where sampling error had been eliminated, hence the results under routine factory conditions can be expected to be more variable.

Continuous improvement

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.

The robustness and sensitivity of the ATP reagent preparations can be improved to meet the more stringent test requirements from harsh samples for a variety of different industrial applications.

Hygiena has developed a new formula with more robustness and sensitivity that is incorporated into both the Aquasnap and Supersnap devices.

Both devices have more resilience against samples at extremes of pH and chemical interference, more light output per unit of ATP, and better extraction of ATP.

These characteristics, combined with the low background to minimise the signal to noise ratio, deliver enhanced detection of ATP (0.05fmols) in SystemSURE Plus which is 10-100 fold better than comparable systems.

Supersnap has a swab format for collecting surface samples for hygiene monitoring and uses the patented double-snap valve device.

Aquasnap is a simple convenience device designed to collect and test liquid samples (0.1ml).

For the food and beverage industry the application of Aquasnap is mainly the detection of product residues in rinse water samples, however in certain situations it can be used to estimate microbial populations.

Control of biofilm

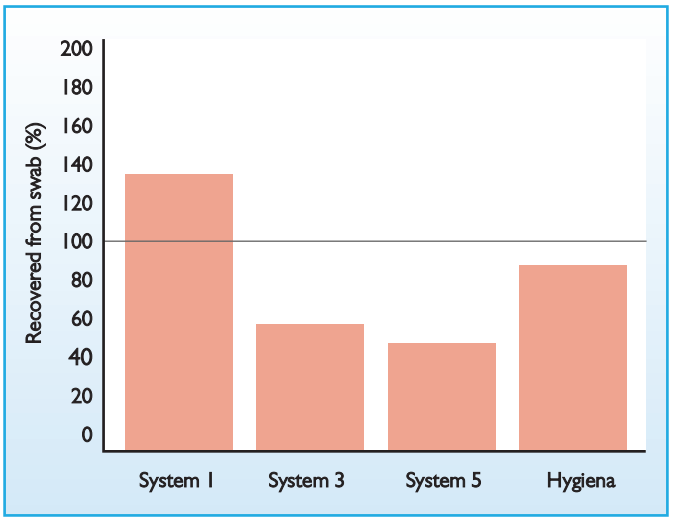
For industrial water samples, for example cooling towers, the monitoring and control of biofilm and biomass is important for process efficiency, biocide dosage and the control of waterborne risks such as legionella.

In these water treatment systems, ATP from organic sources is typically very low such that the main source of ATP is from microbial contamination and biofilm.

Historically the limit of detection for microbes in water using a direct ATP test on water was 10,000-100,000cfu/ml but Aquasnap and Supersnap can now detect 1000 cfu/ml (see Fig. 4).

The results from ATP surface hygiene monitoring are different to those of microbial enumeration methods and give additional information that the microbial test cannot provide.

Fig. 3. Detection of ATP by different swabbing systems.



Swabbing machinery.

Water testing.

Conclusion

ATP tests are not intended to replace microbial tests but there is concurrent direct correlation between the results of the two methods because cleaning simultaneously removes both organic residues and microbes.

Accordingly, SystemSURE Plus with its low background noise, consistent and reproducible reagent performance with excellent sample collection and recovery delivers the best performance of repeatable, accurate and precise results. ■

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