Millipore®

Preparation, Separation,
Filtration & Monitoring Products

Application Note

Do Disinfectants and Sanitizers Interfere with ATP Testing in the Food Industry?

The effects of eight frequently used compounds on commercial ATP-based hygiene monitoring systems

Introduction

To avoid microbial contamination of food and beverage products during the manufacturing process, it is common practice in the industry to disinfect the work surfaces that come into contact with organic materials. Different disinfectants and sanitizers are used, containing either a single active compound or a mixture. To monitor whether a cleaning process has been adequate, rapid tests are used that deliver results almost instantly. The most frequently applied one in the industry is based on the detection of ATP (adenosine triphosphate), the primary energy carrier of living organisms. As all living cells including bacteria, yeasts and molds contain ATP, its presence can serve to indicate biological contamination.

Rapid ATP testing systems are based on the enzymatic reaction of luciferin to oxyluciferin, catalyzed by luciferase in the presence of Mg^{2+} and oxygen. This reaction leads to light being emitted (bioluminescence), which can be measured to quantify ATP. Commercially available systems typically comprise different pen-like swabbing devices for sampling, their own reagents, proprietary luminometers to measure the light, and dedicated instrument software.

Some substances are known to inhibit ATP detection, particularly when they occur in high concentrations. These include the active compounds of disinfectants and sanitizers. The purpose of this study was to investigate the impact of eight different active compounds of disinfectants, at both low and high working concentrations, on the results of five commercially available ATP detection systems for hygiene monitoring in the food and beverage industry.

Method

Five commercial ATP detection systems were used in this study, two of which are Millipore® systems: the HY-LiTE® 2 system and the MVP ICON® system. The three other systems are designated as System H, System K and System M in this study.

The impact of disinfectants and sanitizers on the ATP signal was investigated using a panel of commercially available, industrial products selected to cover the following broad range of active compounds, commonly applied in the food, beverage and other regulated industries:

- Chlorine
- Peroxyacetic acid (PAA)
- Di-amine
- Benzalkonium chloride (BAC)
- Dimethyl didecyl ammonium chloride (DDAC)
- Tri-amine
- Guanidine
- Glutaraldehyde

Each sanitizer or disinfectant was tested at two concentrations: the minimum and the maximum working strength as recommended by the manufacturer. Freshly prepared Milli-Q® water was used as the controls as well as for dilutions. Its signal was considered 100%, i.e. no inhibition at all.

The ATP detection systems were used according to the recommendations for water testing given in their user manuals. Bioluminescence was measured by the luminometers of each system in relative light units (RLUs). The test procedure and RLU calculations were performed as follows:

1. Dip the device's sampler tip into the disinfectant solution (or water).
2. Activate the device to start the ATP reaction.
3. Measure the signal RLU value in the respective instrument and note the displayed value, which is for the background light.
4. Open the device and add 20 µL of 2.5×10^{-8} M ATP solution.
5. Close the device, mix, and measure once more. Note the displayed value.
6. Subtract the RLU value measured in step 2 from the value measured in step 5 to determine the net RLU result.
7. Repeat steps 1 to 6 four times (for five values in all) and calculate the average net RLU result.

Because RLU values are not directly comparable between systems, each system's average net RLU results for a disinfectant was set against its average measurement result for Milli-Q® water, and expressed as percent inhibition:

$100\%*(1- (\text{Mean RLU}_{(\text{disinfectant+ATP})}/\text{Mean}\ _{\text{RLU(water+ATP)}}))$

The calculated inhibition results were subsequently categorized as described in the results section.

Results

Inhibition percentages must be seen in the context of the typical measurement precision of ATP based hygiene monitoring where, for example, a 2-fold difference is usually barely significant in practical terms. This is why, for a clearer and more graphic presentation of the study's results, the inhibition values were categorized into five groups of inhibition levels:

Negligible: < 20% signal reduction

Low: 20-50% signal reduction

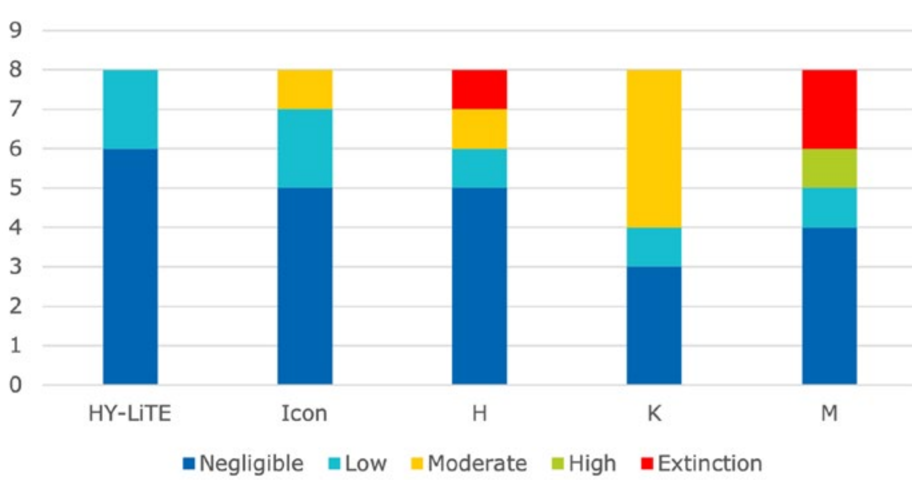
Moderate: 50-80% signal reduction

High: 80-95% signal reduction

Extinction: ≥ 95% signal reduction

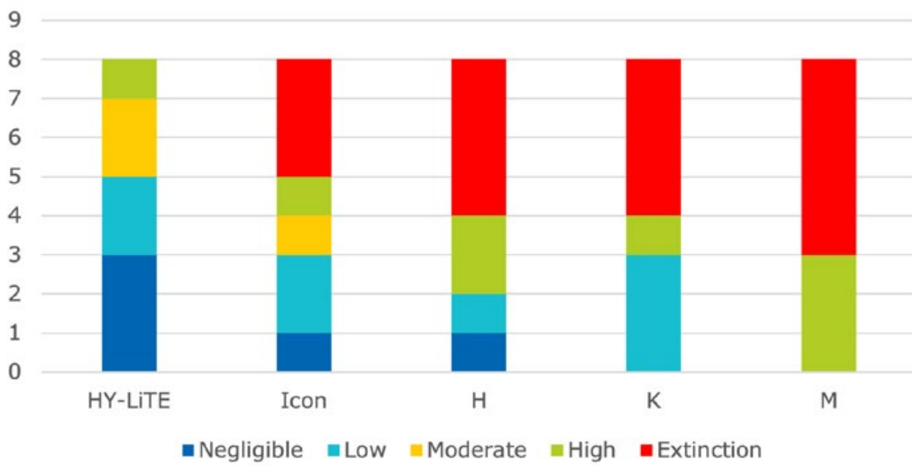
The graphs below show, separately for each ATP detection system, how many of the eight tested disinfectants fell under each of the five inhibition categories. **Graph 1** shows the results for the lowest recommended concentration, **graph 2** for the highest recommended concentration.

Frequency of inhibition lowest recommended working strength



Graph 1: Distribution of the 8 tested disinfectants at their lowest recommended working concentration into the 5 inhibition levels, shown separately for each ATP detection system.

Frequency of inhibition Highest recommended working strength



Graph 2: Distribution of the 8 tested disinfectants at their highest recommended working concentration into the 5 inhibition levels, shown separately for each ATP detection system

Most of the ATP detection systems show some resistance to most of the compounds at the recommended minimum concentrations of the disinfectants.

The pictures changes dramatically at the recommended maximum concentrations. Three of the five systems showed almost no signal at all (<5% of RLU value for water) for at least half of the tested disinfectants. The two Millipore® systems clearly outperformed the other three.

Discussion

When using ATP measurements to monitor cleaning results, the reliability of the test result is key. Residues of disinfectant or sanitizers can severely impact the results and lead to wrong conclusions about the effectiveness of the cleaning program and the readiness of the manufacturing location for resumed production.

The results of this study suggest that inhibition of ATP detection by eight commonly used disinfectants at their lowest working concentrations, as recommended by their manufacturers, is of practical relevance only for a few combinations of ATP detection systems and disinfectants.

However, at the highest recommended disinfectant concentrations, inhibition of ATP detection is a significant issue for three of the five systems, whereas the two Millipore systems and particularly the HY-LiTE® 2 system performs well at such concentrations, with no disinfectant causing extinction level inhibition and only one a high inhibition level. The HY-LiTE® 2 system is thus the ATP test of choice where inhibition is of concern. Where convenience and a hand-held solution is priority, the MVP Icon system is recommended.

It is worth noting that disinfectants and sanitizers are not the only potential inhibitors of ATP detection. Soluble salts and organic acids, often contained in food samples, do so, too, at varying concentrations. It is a reasonable assumption that systems sensitive to disinfectants and sanitizers may also be prone to inhibition from other substances.

Apart from the concentration of inhibitor in the sample, differences in assay chemistry and sample-to-assay volume ratio are likely to be causing the observed differences in performance of the five tested ATP detection systems.

In summary, this study demonstrates the superior performance of both the HY-LiTE 2® and MVP ICON® systems in the presence of varying levels of disinfectants typically used in food and beverage manufacturing facilities.

Article Code	Product Description	Units
78300	MVP ICON® (ATP Monitoring)	1
64003-100	MVP ICON® Surface Sampling Device	100
64004-100	MVP ICON® Liquid Sampling Device	100
1301000001	HY-LiTE® 2 Hygiene Monitoring System	1
1301010021	HY-LiTE® Surface ATP Test Kit	100
1301020021	HY-LiTE® Liquid Test Pens for Total ATP	50
1301940021	HY-LiTE® Liquid Test Pens for Free ATP	50

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