# New Technology Improves ATP Testing for Food Safety Applications

Kikkoman Biochemifa Company

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# Introduction

Sanitation is critical to food safety. Ineffective cleaning can affect the appearance and taste of food, harbor microorganisms and promote the production of biofilms. ATP[i] hygiene monitoring tests are one of the most commonly used methods to verify cleaning effectiveness as they are simple, easy-to-use and provide immediate results. ATP is a critical metabolite and energy source for living organisms and ATP-based hygiene assessments rely on the principle that surface contaminants contain ATP in concentrations sufficient for detection. The principle also takes advantage of the assumption that changes in ATP are proportional to the degree of residual contamination remaining on food processing surfaces or equipment. If cleaning is ineffective, organic debris from food may remain on food processing surfaces or equipment and remaining food residuals may promote microbial growth, shield bacteria from the action of sanitizing agents or allow allergen-containing residuals to remain. ATP testing is designed to verify the effectiveness of such cleaning and most food processing facilities in every processing category use ATP tests for sanitation verification and experts estimate that more than 50 million tests are conducted every year worldwide.

# Principle of ATP Testing

The method of action of conventional ATP tests involves the reaction of ATP with firefly luciferin (Figure 1) - the biochemical that allows a firefly to produce light. The amount of light produced is proportional to the amount of ATP in a sample and measured in Relative Light Units (RLU) using a luminometer.

The assumption underlying the principle in the use of ATP tests is that the concentration of ATP residuals remains consistent over time as long as the residuals remain in place. This may frequently not be the case.

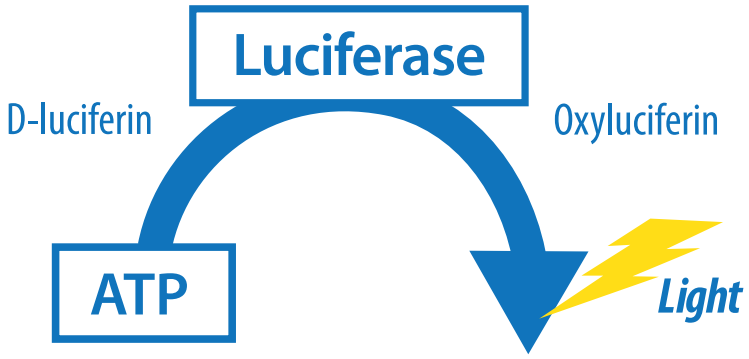



*Figure 1. Principle of Conventional ATP tests*

The problem with using ATP measurements as an indicator of sanitation is that the ATP molecule can be unstable and can rapidly decompose into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) (Figure 2). If the ATP in food residue or biofilm has degraded, conventional ATP tests that indicate ATP levels alone can fail to be a true sanitation indicator and can show false negatives. This problem has been shown to be a particular problem in the production of meats, including beef, pork and chicken. As ATP degrades, the concentration of total adenylate (ATP+ADP+AMP) remains relatively stable and a test that could detect the total concentration of adenylate (or "A3") would provide higher sensitivity due to an increase in the signal to be detected, would be less likely to produce false negative results, and would provide for an overall more accurate verification of sanitation.

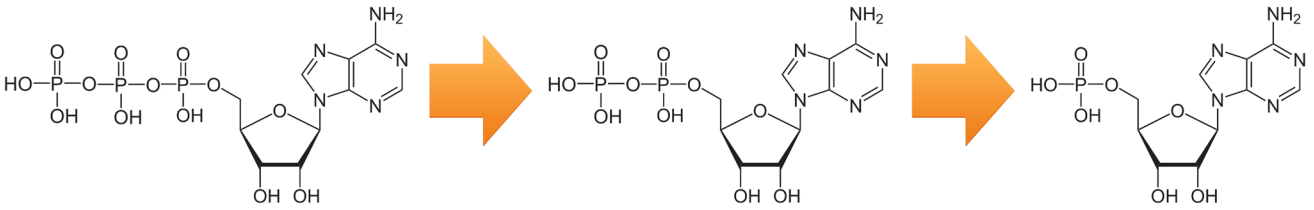



*Figure 2. Degradation of ATP*

# University of Wisconsin-Madison Study

Research recently published in the Journal of Food Protection[ii] has uncovered very interesting data about changes in ATP concentrations in foods and bacteria that confirm this question about the effectiveness of ATP tests. The results of this study revealed that ATP concentrations can vary by several orders of magnitude as the cells present in residual food or bacteria continue to metabolize ATP and produce higher concentrations of its metabolic products – ADP and AMP.

In this study, the researchers from the University of Wisconsin-Madison first examined the contribution of residual ATP from different types of raw meat (beef, pork and chicken). As with other foods, reliable cleaning and sanitation after the processing of raw meat is important to hygiene and ATP tests are frequently used by meat processors to assess the effectiveness of their cleaning processes. What the data from the Wisconsin-Madison study showed was that the concentration of ATP degraded in the raw meat samples over time, reducing the concentration of ATP present in the meat residuals and thus reducing the signal available to a test that detects only ATP (Figure 3). This can reduce the effectiveness of a traditional ATP test.

The study also showed that one of the ATP degradation products - ADP - was shown to remain stable in concentration in the raw beef, pork and chicken samples. The Wisconsin-Madison study provides quantitative data of the shift in concentration from predominately ATP to a state where ADP and AMP become the predominant adenylate species (Figure 3).

Food products are not the only source of residual ATP in food processing; microorganisms will also contribute to ATP on food processing surfaces. Bacteria that may be present may be growing or may exist in various states of nutrient deficiency or injury changing the growth phase at which they exist. In this same study, it was also shown that the concentrations of the different adenylates varied in that ATP was predominant at initial stages, but AMP became predominant at later time points (Figure 4). These results indicate that detection of ATP + ADP + AMP will be more effective than measuring ATP alone as an indicator for the potential presence of bacteria.[iii]

Under these conditions, a test that detects only ATP will fail to detect the concentration of either the ADP or AMP present and consequently fail to serve as an effective indicator of the presence of residuals. This agrees with another previously published study[iv] that suggested that a test that detects ATP plus the degradation products of ATP will be able to detect raw meat residue much more effectively than a test that detects ATP alone.

*Figure 3. The change of ratios of adenylate homologs in raw poultry.*

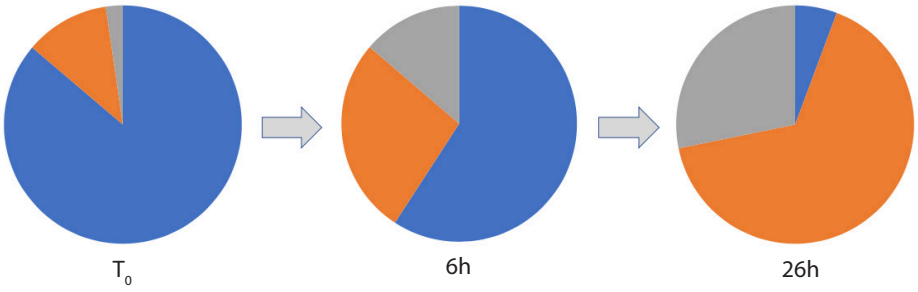
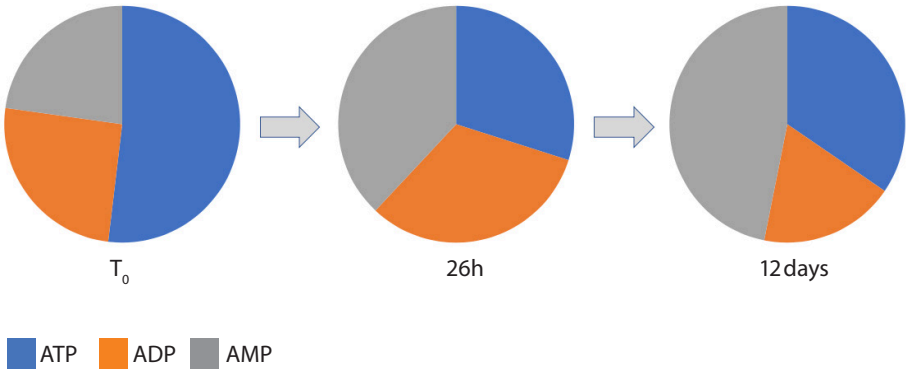



*Figure 4. The change of ratios of adenylate homologs after incubation of Escherichia coli*



# Why A3 Chemistry is Different

A test that can detect all three adenylates will be more effective than one that detects ATP alone.

Kikkoman's new LuciPac A3 Sanitation System detects ATP+ADP+AMP with one swab to give you the whole picture. With the use of two additional enzymatic reactions (Figure 5), AMP and ADP can be recycled back to ATP. This allows the test to detect the total adenylate concentration and dramatically increases the signal available to the test.

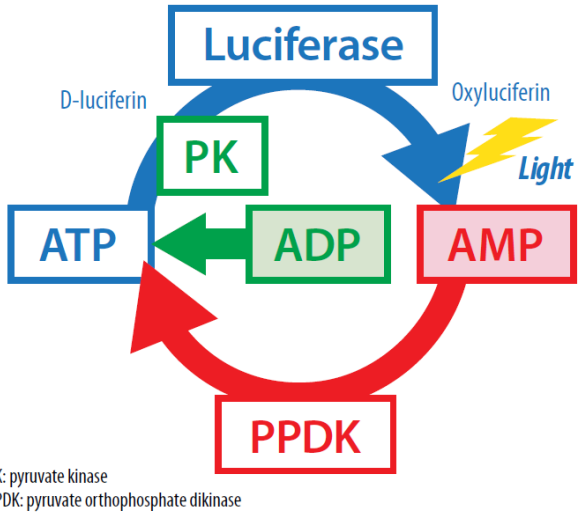



*Figure 5. Principle of A3 Technology*


*From left to right: Lumitester Smart, LuciPac A3 Surface, LuciPac A3 Water*

# Conclusion

When considering the use of ATP-based hygiene tests, food processors and hygiene specialists should consider that variables such as time prior to cleaning, source of the residual contamination, and physical conditions such as temperature may alter the quantity of ATP available for an ATP test potentially making them less effective for the intended purpose. The patented[v] Kikkoman A3 technology has been proven to detect residues that others miss. And it is just as easy to use as a conventional ATP test. Just swab the way you always have, but you'll detect what you have been missing.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 051901

Kikkoman LuciPac A3 Surface is AOAC-PTM certified.

[i] Adenosine triphosphate

[ii] Quantities of adenylate homologues (ATP+ADP+AMP) change over time in Prokaryotic and Eukaryotic cells. Smith N. W. et al., J. Food Prot. 2019, 82, 2088.

[iii] The presence of ATP of other adenylates is not a confirmatory test for the presence of bacteria but is used as an indicator of the effectiveness of cleaning processes.

[iv] Bakke M. et al., J. Food Prot. 2018, 81, 729.

[v] Patent pending

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