Kikkoman Biochemifa Company

ATP Depletion - The Risk of False-Negatives in Hygiene Monitoring

A quick view

ATP tests for hygiene monitoring are widely used in all areas of the food, pharmaceutical and medical industries because they are fast, easy-to-use, and cost-effective. Increasingly, however, the capabilities of conventional ATP tests are being questioned and their limitations are becoming more recognized. These limitations can lead to the generation of false-negative assessments meaning that, although the surfaces in question are contaminated, the ATP test does not indicate the presence of soil. The implication of such false-negatives is that a manufacturing facility may proceed with processing even though the surfaces remain soiled. These false-negative assessments are based in fundamental chemistry and biology upon which ATP tests operate.

How ATP tests operate

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Adenosine triphosphate (ATP) is a molecule found in many materials, including foods, plant, and animal tissues, and within microorganisms such as yeasts, molds, and bacteria. In food processing, these same materials – foods, plant and animal tissues, and microorganisms – constitute the vast majority of contaminating soils. Because of this nearly universal presence of ATP within these soils, we assume that if ATP is found, then contaminating soils are present as might be the case after inadequate cleaning. Furthermore, the amount of ATP can correlate with the extent of soil contamination. In other words, if ATP concentrations are high, contaminating soils levels are high and conversely, if ATP concentrations are low, contaminating soil levels are low. In practice, when surfaces are cleaned to the point that assessments of ATP concentrations are below a defined threshold, the surface is declared sufficiently clean and the subsequent processing steps can proceed. However, ATP is a reactive molecule and is readily consumed by spontaneous and metabolic activities of contaminating soils. Therefore, ATP-based assays will correspondingly indicate lower readings which will be interpreted as the absence of soil when no change in soil mass has occurred.

The basis of test sensitivity losses

Research from the University of Wisconsin – Madison published in the *Journal of Food Protection*[1] demonstrates that under conditions common in food processing and service facilities, ATP within contaminating soils will degrade (hydrolyze) to the chemical homologues, adenosine diphosphate (ADP) and adenosine monophosphate (AMP). The shift from the target molecule for hygiene assessment, ATP, to ADP and/or AMP accounts for the loss of sensitivity when ATP-only assessments are deployed. This chemical shift from ATP to ADP and AMP occurs independent of the level of soil contamination, meaning that the same degree of soil contamination, including food debris and viable microorganisms, still remains. Other University of Wisconsin-Madison studies demonstrate that the shifts from ATP within food and microbial soils in typical environments can represent dramatic changes in concentrations from ATP to ADP and AMP. These shifts effect significant changes in assay sensitivity.[2] Clearly, an additional advancement was needed to remedy this loss of sensitivity with ATP-based assays.

ATP+ADP+AMP (A3) is discovered

In 2017, Kikkoman researchers developed a technology to remedy this shortcoming of current ATP test technologies. Termed "Kikkoman A3"[3], this development could cumulatively account for the loss of ATP by simultaneously quantifying the presence of all three homologues, ATP, ADP, and AMP. As a result, Kikkoman A3 has shown markedly improved sensitivity to ATP depletion. For example, in one applied study, University researchers compared the performance of a conventional ATP assay and the novel A3 assay in two licensed, inspected food processing facilities - one a Grade A dairy and the other a meat processing plant.[1] A total of 960 data points for each type of assay were collected in parallel over the course of several months and seasons. The study showed that the A3 assay detected contamination events as frequently as the existing technology, but, more importantly at significantly higher rates where ATP deletion was likely to occur (Fig. 1).

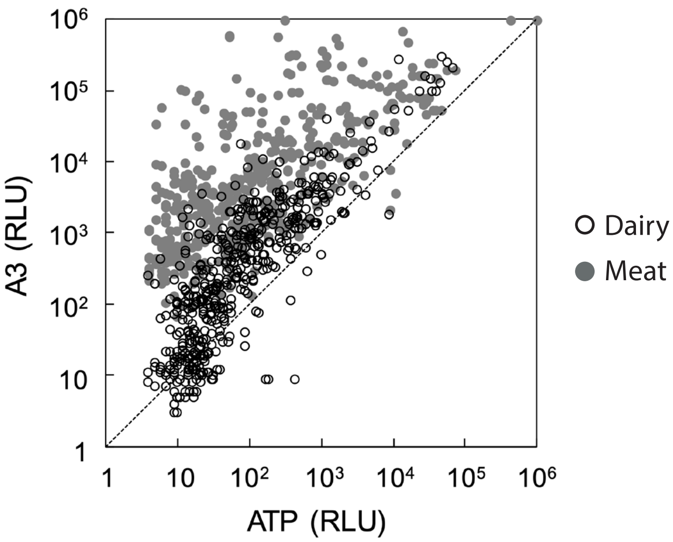

FIGURE 1. Plot of data comparing ATP and A3 at dairy (open circle) and meat (closed gray circle) processing facilities at the University of Wisconsin-Madison. These data demonstrate that the Kikkoman A3 advancement finds soil contamination at a higher rate than current technologies (Modified citation from reference Whyte C. et al. 2022.)

Another study[4] focused on the hydrolysis phenomenon of ATP in fish (flounder) processing. It showed that the ATP content continuously decreased during processing and completely diminished in sliced fish in as little as 24 hours. As evidenced by another study,[5] raw meat and fish start out containing mainly ADP. Raw whole eggs, shellfish, certain processed meats and seafood contain a large amount of AMP with only trace amounts of ATP suggesting that such contaminants are much less sensitive to detection using ATP-only assays. Other studies have confirmed this phenomenon showing that with many types of meat and fish-based soils, ATP is similarly depleted. Furthermore, they invite affiliated industries to carefully examine the influence of this technological advancement as a means of managing hygiene.

Conclusion

Cleaning and sanitation verification practices throughout the food manufacturing environment are critical and form the basis of every food safety plan. Rapid, sensitive verification assays are an important step in such programs and are why ATP tests were developed and continue to be used. However, these new studies demonstrate the loss of ATP and illustrate the implications of these losses in generating false-negatives from ATP-only assays. Therefore, processors working within adenylate-rich environments, such as those involving the processing of beef, pork, chicken, fish, dairy, cheese, and fermented foods should carefully consider these research findings especially in this era where food hygiene verification is such a critical requirement.

Swab and get *Better* results in 10 seconds.

- Tests are collected in the same way as conventional ATP tests
- The difference is in the chemistry
- Higher sensitivity creates a better level of detection

[1] Whyte, C., T. F. Ma, J. Sindelar, and S. A. Rankin 2021. Rapid hygiene assay sensitive to cumulative adenylate homologues exhibits equal or higher frequencies of soil contamination detection than assay limited to ATP detection. J. Food Prot. 84: 1937–1944. https://doi.org/10.4315/JFP-21-028

[2] Smith, N. W., J. J. Sindelar, S. A. Rankin 2019. Quantities of adenylate homologues (ATP+ADP+AMP) change over time in Prokaryotic and Eukaryotic cells. J. Food Prot. 82: 2088–2093. https://doi.org/10.4315/0362-028X.JFP-19-223

[3] Total adenylate is ATP+ADP+AMP, known as A3

[4] Shim, K. 2019. Estimating postmortem interval by bioluminescent determination of ATP content in the muscle of olive flounder (Paralichthys olivaceus). J. Food Prot. 82:703-709. https://doi.org/10.4315/0362-028X.JFP-18-440

[5] Bakke, M., and S. Suzuki. 2018. Development of a novel hygiene monitoring system based on the detection of total adenylate (ATP+ADP+AMP). J. Food Prot. 81:729-737. https://doi.org/10.4315/0362-028X.JFP-17-432org/10.4315/0362-028X.JFP-19-223

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