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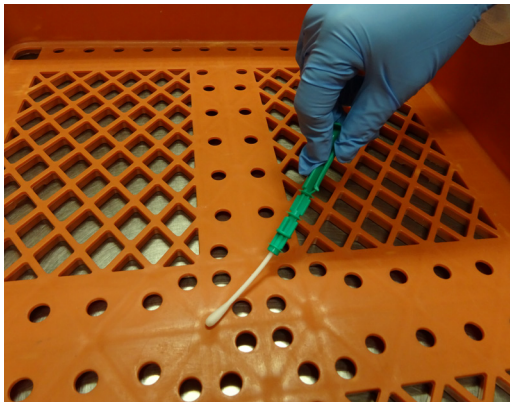
New Technology for Food Safety Surface Sanitation Applications

Kikkoman Biochemifa Company



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Abstract

Adenosine triphosphate (ATP) is the universal energy molecule found in all living things. ATP hygiene monitoring tests are widely used in all types of food processing plants because they are easy to use and provide immediate feedback and verification of sanitation processes. In food processing, they have been successfully used as a method of monitoring environmental contamination including food residues and bacteria, including detection of biofilms that can harbor pathogens and allergens.

Yet the conventional ATP test has a weakness in that the method can detect only ATP. ATP has been shown to quickly degrade on surfaces to adenosine diphosphate (ADP) and adenosine monophosphate (AMP). But as the ATP in a sample degrades, the total andenylate concentration - ATP+ADP+AMP - can be shown to be maintained. A test that could detect total adenylate would provide a more sensitive and reliable indicator of sanitation. Kikkoman has been able to incorporate advanced enzyme chemistry into the ATP test to detect total adenylate and develop a test that is significantly more sensitive than conventional ATP tests.

1.0 Introduction

1.1 Background

Sanitation is critical to food safety and ineffective cleaning can affect the appearance and taste of food, harbor microorganisms and promote the production of biofilms. The rapid results and immediate feedback for sanitation verification provided by ATP measurements have become a crucial tool for monitoring food plant sanitation processes. Most food processing facilities in every category use ATP test for sanitation verification and experts estimate that more than 40 million tests are conducted every year worldwide.

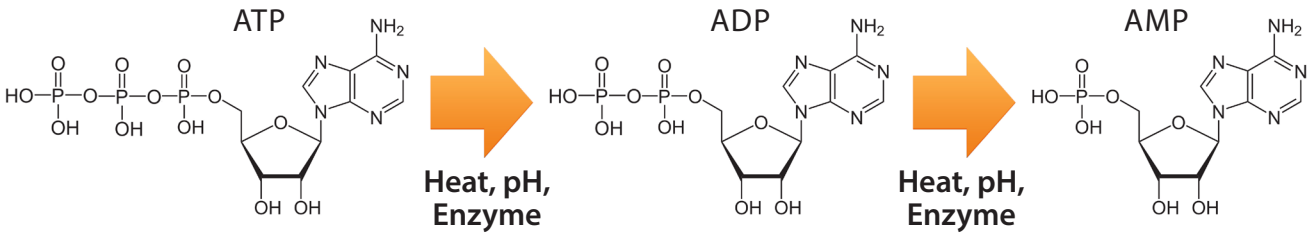


Fig. 1 Dephosphorization reaction of ATP through ADP to AMP.

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) (Fig. 1). If the ATP in food residues 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.

It has been shown, however that even as ATP degrades, the concentration of total adenylate (ATP+ADP+AMP) remains relatively stable (Fig. 2). A test that can detect total adenylate (or "A3") would provide higher sensitivity due to an increase in signal to be detected, would be less likely to produce false negative results, and would provide for an overall more accurate verification of sanitation.

The Kikkoman A3 test uses advanced enzyme chemistry to allow for the detection of A3 and greatly increases the sensitivity of sanitation swab testing.

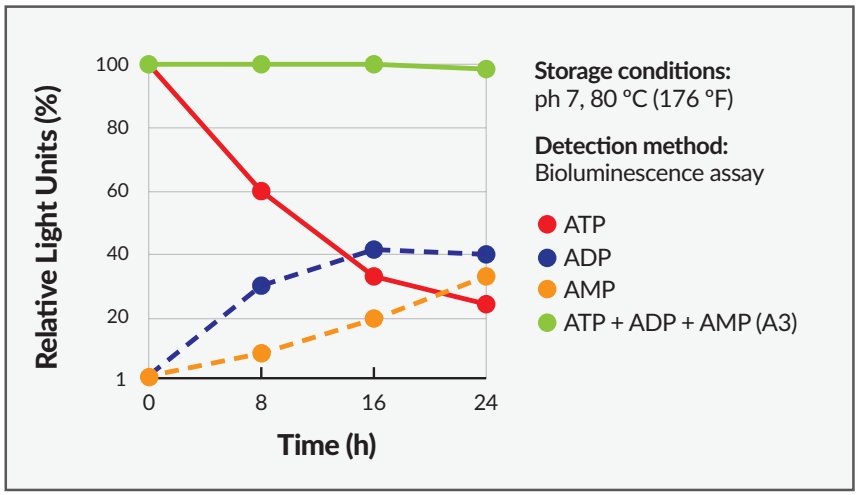


Fig. 2 Degradation of ATP through ADP to AMP.

2.0 The Technical Solution: Kikkoman A3 Technology

2.1 Principle of Testing Method

Firefly luciferin can produce light using luciferase and ATP. The amount of light produced is proportional to the amount of ATP in a sample and therefore ATP can be quantified by measuring the light produced through this reaction using a luminometer, producing a reading of Relative Light Units (RLUs). We have shown, however that ATP can quickly degrade into ADP and AMP. A conventional ATP test cannot detect these adenylate species. But with the use of two additional enzymatic reactions. AMP can be recycled to ATP using pyruvate orthophosphate dikinase and ADP is converted to ATP by pyruvate kinase (Fig. 3). This allows the test to detect and quantify total adenylate and dramatically increases the signal available to the test.

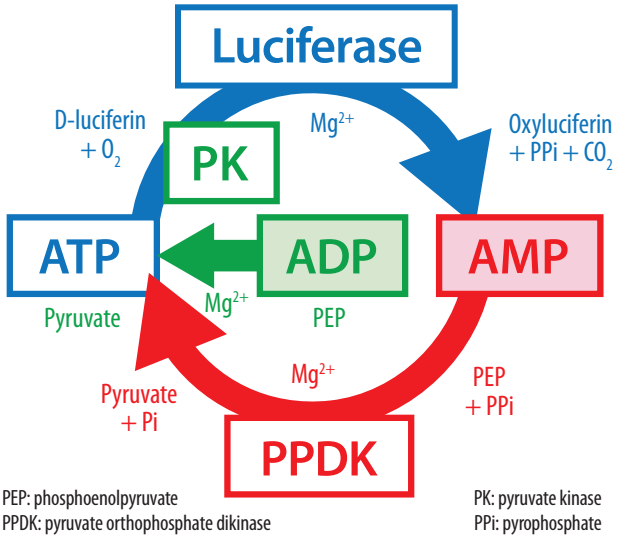


Fig. 3 The principle of A3 detection.

2.2 ADP and AMP Detection Dynamic Range and Repeatability

During an independent evaluation study conducted by Food Safety Net Services, San Antonio, TX, the Kikkoman A3 technology was shown to have superior detection of ATP, ADP and AMP, and compared to three of the leading competitive ATP products.

Separate solutions of ATP, ADP and AMP were prepared and 10-fold serial dilutions were carried out in Nuclease Free Water. Ten micro litter aliquots were pipetted onto ten separate swabs, and RLUs were measured immediately on each of the devices.

As can be seen in Fig. 4, the Kikkoman A3 technology showed superior detection of not only ATP but also ADP and AMP. On the other hand, the conventional three ATP products showed below of limit of detections of either ADP or AMP even at 10-12 mol. These data show that the innovative A3 technology can detect surface contamination that conventional ATP tests cannot.

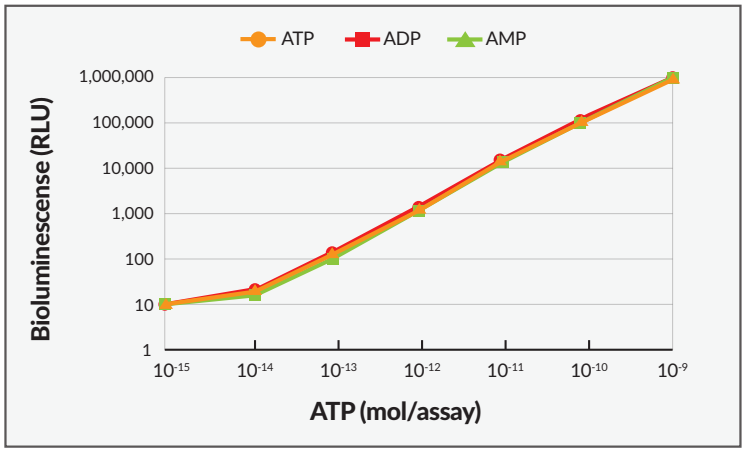


Fig. 4 Detection of dynamic range of ATP, ADP and AMP for LuciPac A3. The conventional three ATP products showed no detection of either ADP or AMP even at 10-11 mole/assay. These data were taken by independent laboratory.

3.0 Food Sanitation Applications

Sanitation is critical to food safety and ineffective cleaning can affect the appearance and taste of food, harbor microorganisms and promote the production of biofilms. This is one of the reasons that so many food processors use ATP as part of their sanitation programs to verify the effectiveness of their processes. But, as we have shown, ATP can be unstable and decompose into ADP and AMP, and conventional systems that detect ATP alone can fail to detect the food residues and may not be a reliable indicator of sanitation. As part of the technology verification conducted by FSNS, Kikkoman A3 technology and the three leading ATP systems were compared for their ability to detect various types of foods.

3.1 Food Samples

The Kikkoman A3 technology was assessed along with three of the leading ATP tests for their ability to detect adenosine nucleotides from different types of food matrices. The target foods for this study were beef, sausage, RTE turkey, seafoods, egg, beer, nuts, fruits and vegetables.

Portions (10 g) of each food matrix were mixed with 90 ml of sterile distilled water and homogenized for 2 min in a masticator to generate the 10-1 dilution. A 1 ml aliquot of homogenate was diluted in 9 ml sterile distilled water. Liquid food samples were not diluted. Aliquots of 10 µl of each dilution were pipetted to appropriate swabs or sponges for each type of detection device and food matrix. Measurements were performed at 23 ± 1°C. Solid foods were homogenized with water, and all samples were diluted appropriately. The measurements were repeated 3 times and average values were obtained.

The samples were applied to swabs of LuciPac A3 surface and three conventional ATP detection devices. RLUs were measured (n = 5) using the Kikkoman Lumitester PD-30 and other corresponding luminometers.

The results of these tests are shown in Fig. 5. Surprisingly, very little ATP was shown to be present in the meat and egg products. The level of total adenylate (A3) in the samples was significantly higher, and was detected by the Kikkoman technology at concentrations two orders of magnitude lower than the conventional ATP tests.

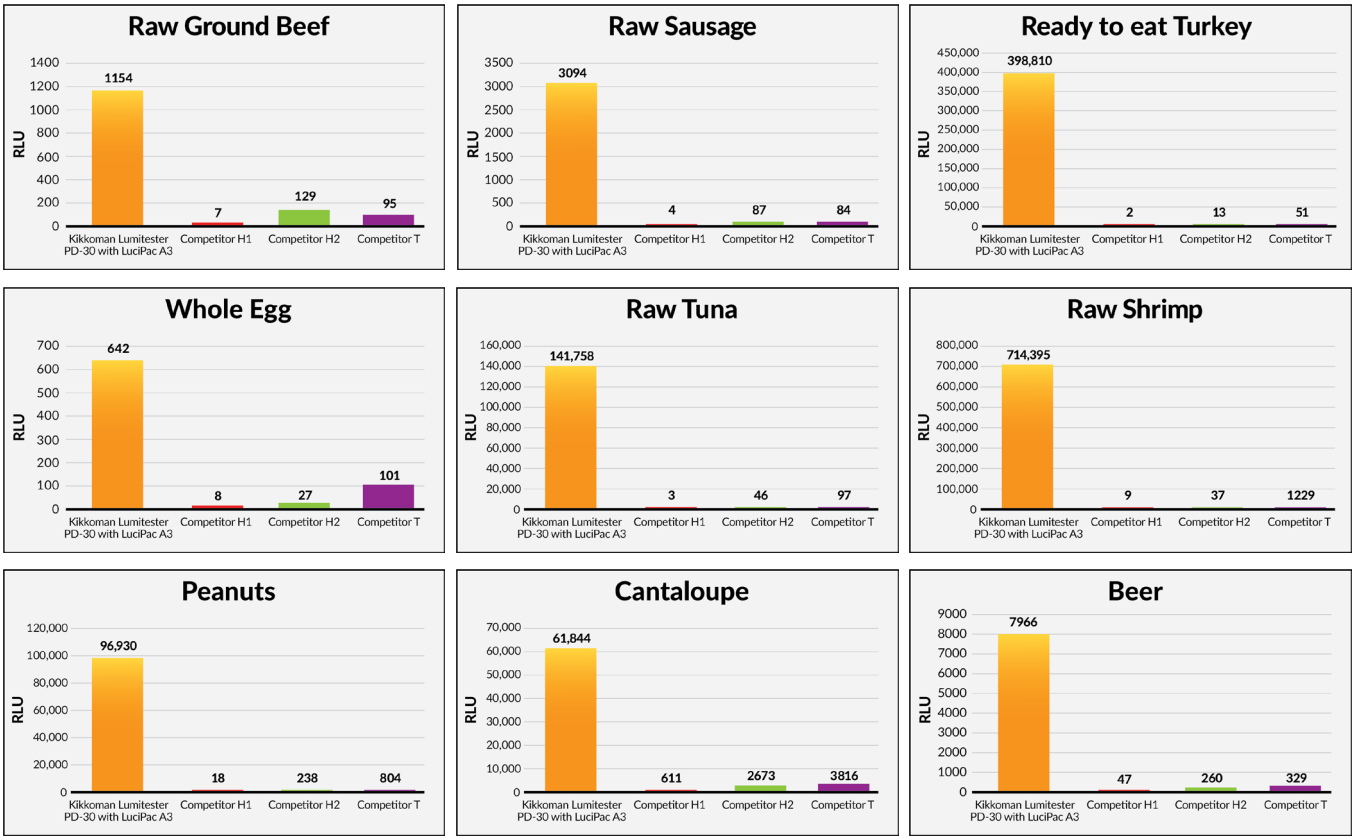


Fig. 5 Detection of foods and beverages using the A3 monitoring test kit and three commercially available ATP monitoring tests. The producer of ATP-1 and ATP-1H were same and ATP-1H was the combination of high sensitive device and luminometer.

Similar results were seen in Seafood. ATP method also shows a very low response in seafood while the A3 technology shows more than two orders of magnitude greater sensitivity.

In another important food type – nuts – a low response was seen from the conventional ATP tests yet the A3 technology showed more than two orders of magnitude higher response than the most sensitive conventional test. Many processors will recognize the RLU responses from the conventional tests as being comparable to a typical pass/fail criterion used in sanitation programs. Yet the A3 technology shows that there is still a considerable amount of product residue remaining in the samples. This is an important finding in that these products are common allergens and any residue left behind can contaminate a processor's product. The A3 assay is shown to be a more sensitive method to detect trace amounts of surface contamination from nuts.

Fruits and vegetables were also tested and the A3 method was also shown to be superior. Beer was also tested with similar results.

As part of this study, the Kikkoman A3 technology and ATP method were compared with a protein swab test. Portions (10 g) of peanut were mixed with 90 ml of sterile distilled water and homogenized with blender to generate the 10-fold dilution, and 10-fold serial dilutions were carried out. These samples were evaluated using ATP assay, A3 assay, protein swabbing assay and lateral flow detection method. Regarding lateral flow detection, the sample was additionally diluted 10-fold by phosphate buffer according to the instruction and could detect 105-fold diluted samples. A3 method also showed a response of more than 200 RLU at 105-fold dilutions although ATP method showed around 30 RLU even at 102-fold dilutions. Supposing that 100 RLU is seen as positive, the calculated concentrations that was equivalent to 100 RLU for ATP assay and A3 assay is shown in Fig. 6. Protein swab test could detect just 102-fold diluted samples at most. The concentration of target protein or total protein were evaluated by ELISA and Bradford assay, respectively, then the theoretical dilution factors to afford 10 ppm solutions were also shown in Fig. 6. These results indicated that LFD and A3 assay can detect 10 ppm levels of peanuts protein. Thus, A3 method is enough sensitive to detect peanuts allergen and the sensitivity of A3 method were superior to that of protein swab assay. Some kinds of immunological detection tests for allergens, such as seafood and specific tree nuts are not easily available. Moreover, fermentation is known to reduce the sensitivity of immunological allergen detection due to proteolysis of allergen structures. The A3 assay also likely assist to check remaining such allergens that is difficult to be detected by immunological method.

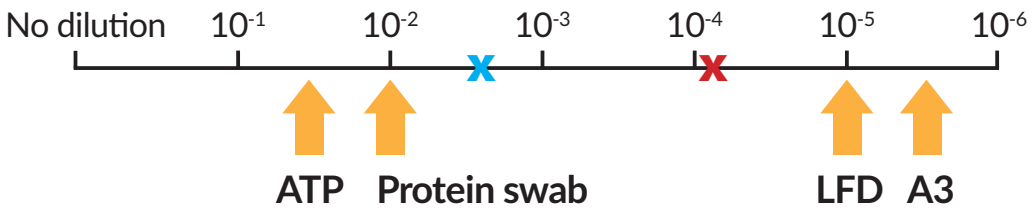


Fig. 6 Comparisons of detection limits of the ATP and A3 monitoring test kit, protein swabbing assay and lateral flow detection using homogenized and diluted peanuts.

Sample volume of A3, ATP assay and protein swab: 100 µl

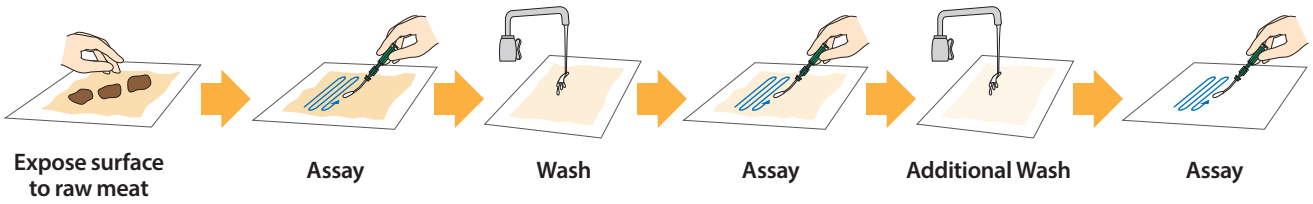
A3, ATP assay: Calculated concentration equivalent to 100 RLU

X: *Calculated 10 ppm Target Protein (ELISA)*

X: *Calculated 10 ppm Protein (Bradford Protein Assay)*

3.2 Surface Sanitation

Six stainless steel coupons were exposed to three kinds of raw meats. These coupons were washed three times – the first a rinse with cold water, the second with hot water, and the third using sponge with detergent and rinse (Fig. 7). After each wash, ATP and A3 (LuciPac A3 Surface/Lumitester PD-30) assays were carried out.



Test results using a conventional ATP method are shown by the red lines in Fig. 7. The first and second steps rinse with cold and hot water produced a result of less than 200 RLU. This is significant as many food processing facilities will use

200 RLU as a typical pass/fail criterion. It is clear that simply washing a stainless-steel surface with cold or hot water will not be sufficient to achieve effective sanitation yet the conventional ATP tests would have indicated that the procedure would have been verified to do exactly that. Using the A3 method, as indicated by the green lines, the 200 RLU level was not achieved until after complete washing with detergent and rinse. These results showed that A3 is better indicator for sanitation processes of stainless steel.

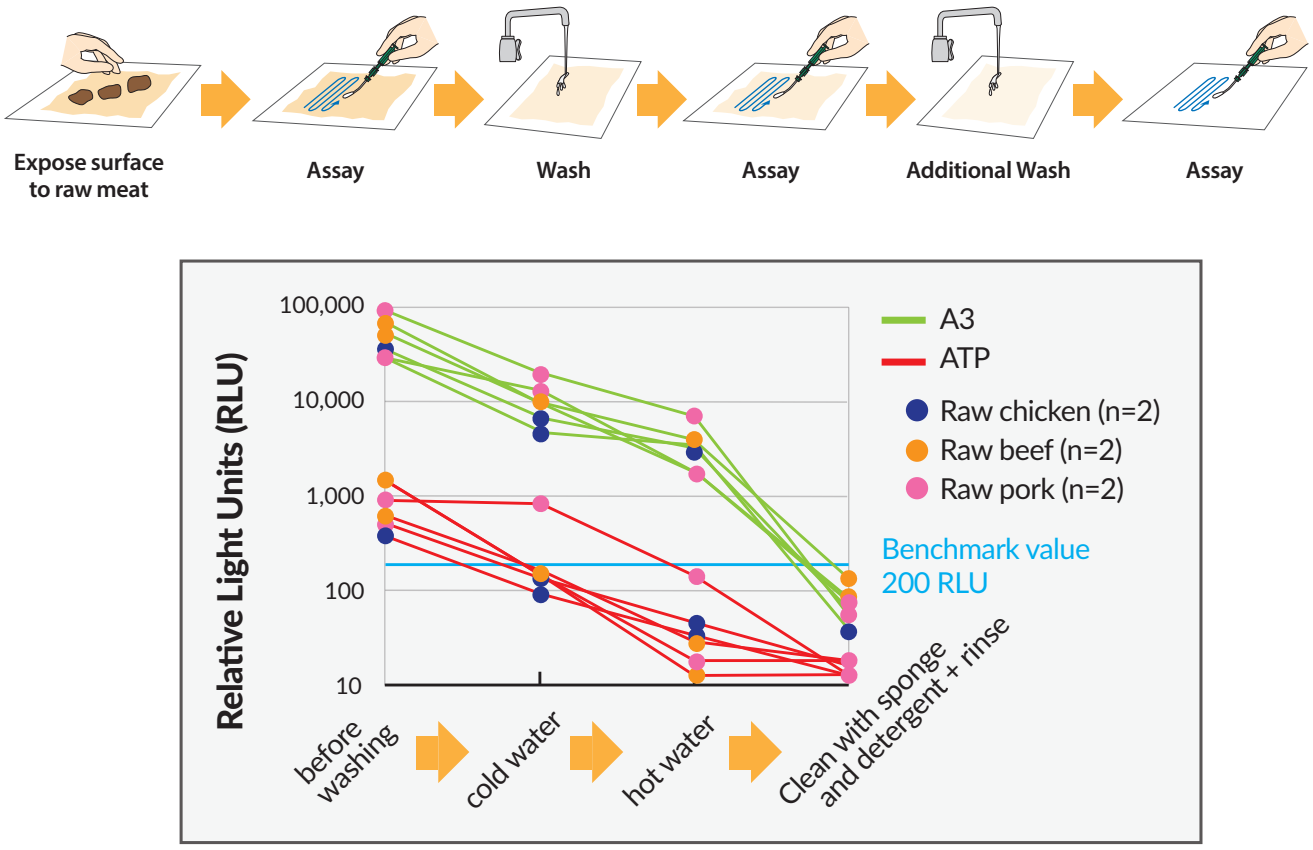


Fig. 7 The method and results of sanitation monitoring of stainless steel exposed to raw meat using ATP and A3 methods.

4.0 Conclusion

In summary, we showed that the new hygiene monitoring assay could be developed based on luciferase assay with the combination of two additional enzymatic reactions. In this method, we could detect all three adenylate molecules (A3). The Kikkoman A3 technology that detects total adenylates was shown to be a far more sensitive method for detections of food residue and allergens than conventional ATP assays.