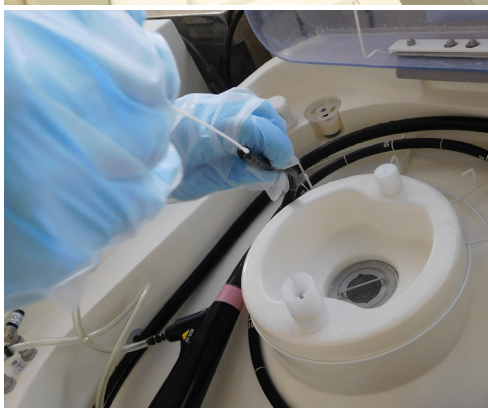
New Technology for Healthcare Surface and Equipment Sanitation Applications

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 many clinical and hospital applications because they are easy to use and provide immediate feedback and verification of sanitation processes. In hospitals, they have been successfully used as a method of monitoring environmental contamination and verifying sanitation processes in hospital rooms, operating rooms and in instrument reprocessing operations.

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 adenylate 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.

Now infection control professionals have an alternative tool available to provide a clearer picture. Offering higher sensitivity and better detection of residue than conventional ATP tests, Kikkoman A3 technology uses this advanced chemistry to detect ATP and its degradation products - ADP+AMP - to guide and assure better sanitation outcomes and support a more effective environmental hygiene program.

1.0 Introduction

1.1 Background

Whether the contamination comes from blood, bacteria or body fluids, ineffective environmental hygiene can leave behind biofilms or residue that can harbor opportunistic pathogens in patient rooms or on medical devices.

Although many hospitals and healthcare institutions are currently using ATP-based swab tests to assess and verify cleaning procedures, research shows that testing for ATP alone can be ineffective. ATP has been shown to quickly degrade on surfaces, reducing the amount of ATP available for detection causing conventional ATP tests to miss contamination and produce false negative results.

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 1). If the ATP left behind on hospital surfaces or on medical devices has degraded, a conventional ATP test will have limited signal to detect, showing a false negative, and can fail to be a reliable indicator of the effectiveness of sanitation.

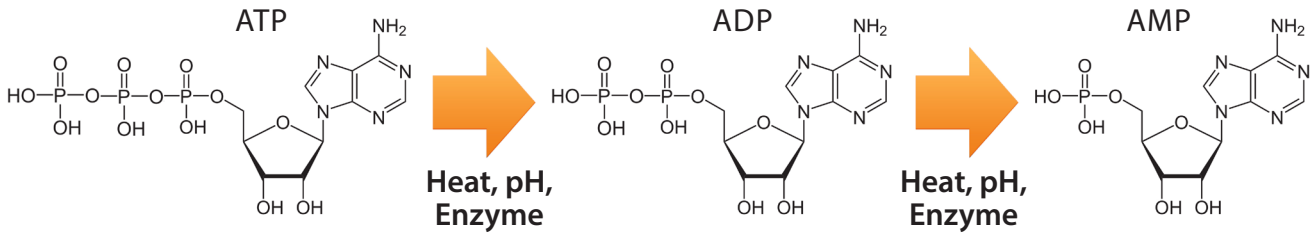


Figure 1: Degradation of ATP through ADP to AMP.

It has been shown, however that even as ATP degrades, the concentration of total adenylates (ATP+ADP+AMP) remains relatively stable (Figure 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.

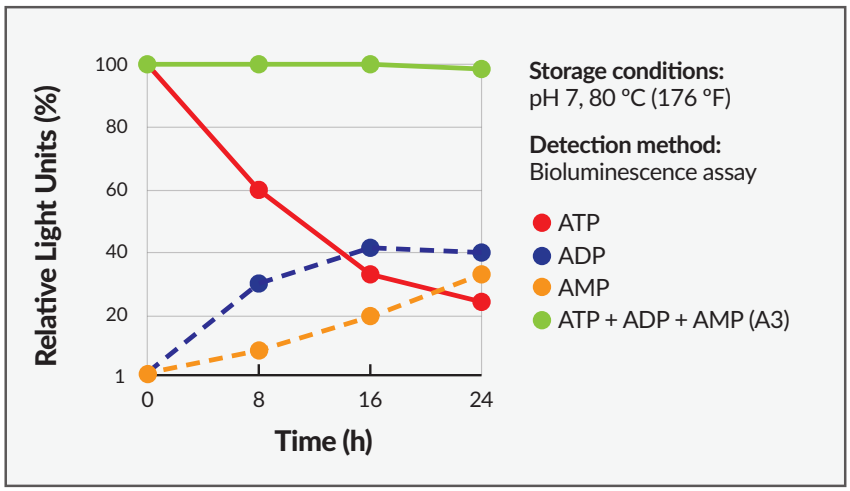


Figure 2: Stability of total adenylate concentration.

2.0 The Technical Solution: Kikkoman A3 Technology

2.1 Principle of Testing Method

Firefly luciferase can produce light using luciferin 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 and conventional ATP tests cannot detect these degradation products. 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 (Figure 3). This allows the test to detect and quantify total adenylate and dramatically increases the signal available to the test.

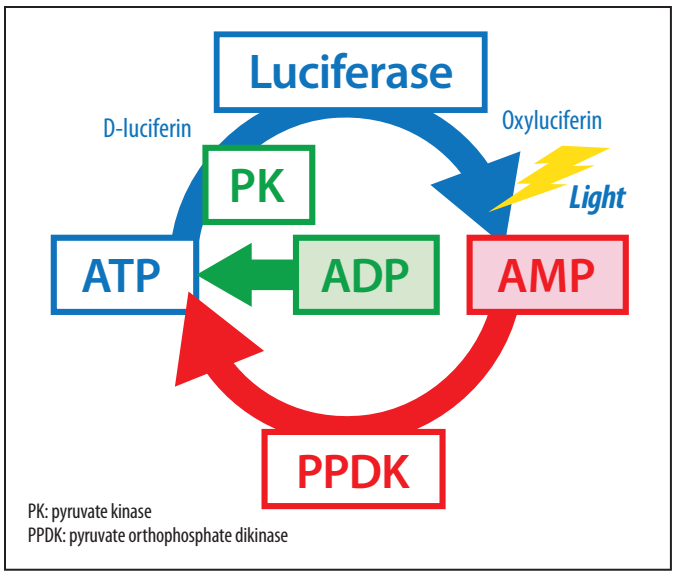


Figure 3: Innovate recycling using advanced enzyme chemistry.

2.2 ATP, 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 ADP and AMP, compared to three of the leading competitive ATP products.

As part of the study, separate solutions of ATP, ADP and AMP were prepared in Nuclease Free Water at 10^{-4} M to 10^{-10} M solutions. Ten microliter aliquots were pipetted onto ten separate swabs, and RLUs were measured immediately on each of the devices.

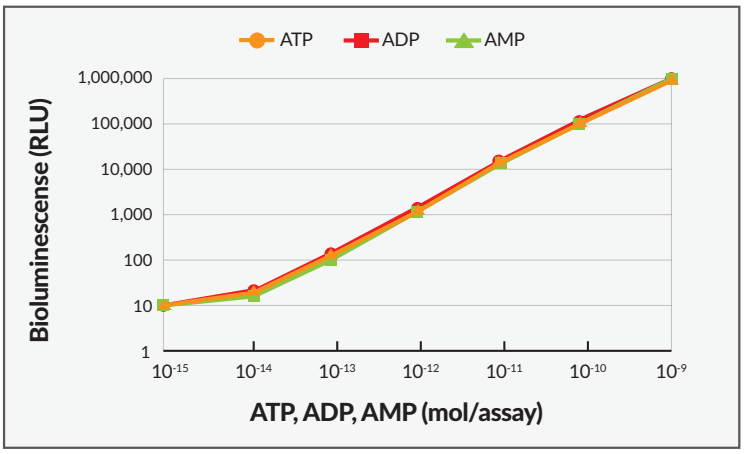


Figure 4: Detection of dynamic range of ATP, ADP and AMP for LuciPac A3.

As can be seen in Figure 4, the Kikkoman A3 technology showed superior detection of not only ATP but also ADP and AMP, with a detectable concentration at 10^{-15} mol. 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 (data not shown).

3.0 Healthcare Sanitation Applications

3.1 Detection of adenosine nucleotides from Blood

Blood was diluted by water (x10) and stored at 35 °C (95 °F). Measurement by three conventional ATP methods and the Kikkoman A3 (LuciPac A3 Surface/Lumitester PD-30) method were carried out. Figure 5 shows that the ATP was degraded dramatically after hemolysis. Yet, the concentration of total adenylates (or "A3") was stable and detected by the Kikkoman A3 technology. This shows that A3 is a more reliable marker for the detection of blood contamination.

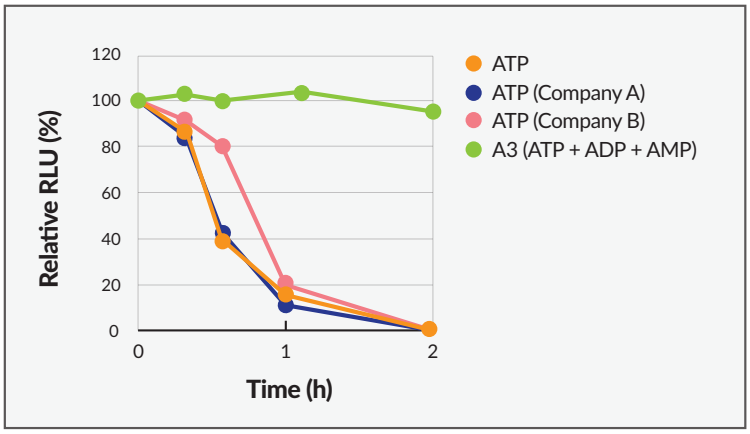


Figure 5: Deletion of blood over time by Kikkoman A3 with comparison to conventional ATP tests.

3.2 Detection of adenosine nucleotides from Sweat

Sweat secreted on the skin surface of hands were recovered by glove juice sampling. Conventional ATP and Kikkoman A3 (LuciPac A3 Surface/Lumitester PD-30) assays were carried out. The detection shown by the Kikkoman A3 method was approximately 20 times higher than that of a conventional ATP method (Figure 6).

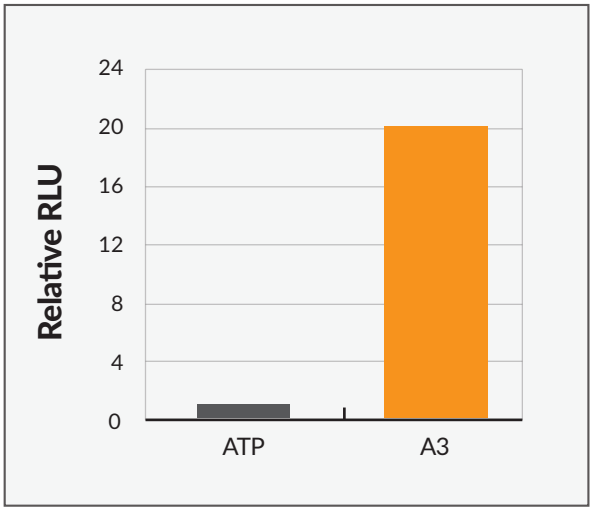


Figure 6: High sensitivity of sweat by Kikkoman A3 technology.

3.3 Detection of adenosine nucleotides from Gastroscopes and Colonoscopes immediately after patient use

In gastroscopes and colonoscopes, Kikkoman LuciSwab – a long stem swab with a stem length of 400 mm and a 2.8 mm or 3.2 mm swab designed for endoscopes and colonoscopes, respectively - was used to sample the inside of the instruments. The biospecimen attached to a cotton bud was recovered in 5% glucose solution, then Kikkoman A3 and conventional ATP assays were carried out.

The detection sensitivity of the A3 method on residues derived from gastroscopes and colonoscopes (Figure 7 and 8) were between 3 and 8 times higher than those of ATP method. Thus, A3 method is more sensitive for monitoring gastrointestinal endoscope hygiene.

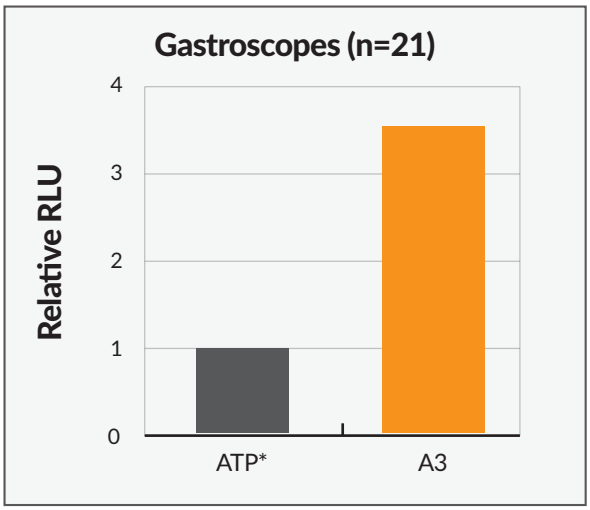


Figure 7: Kikkoman A3 sensitivity in Gastroscope samples.

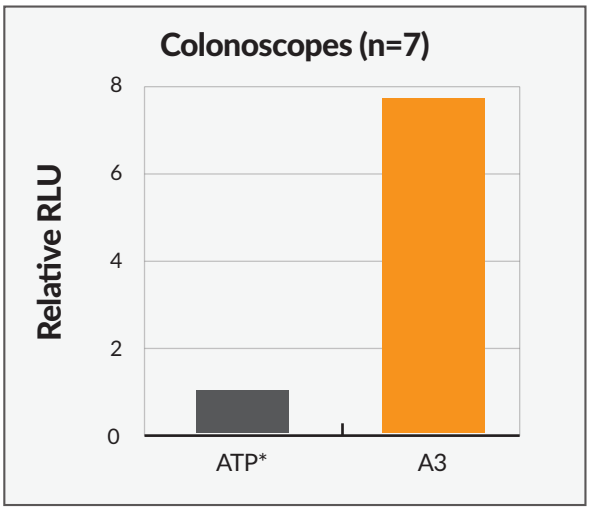
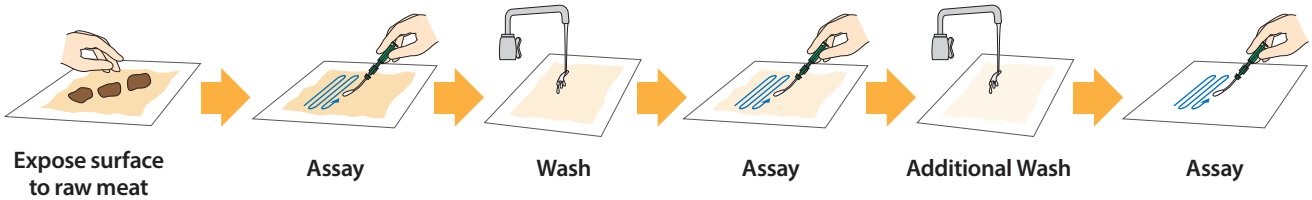


Figure 8: Kikkoman A3 sensitivity in Colonoscope samples.

3.4 Surface Sanitation

Six stainless steel coupons were exposed to 3 kinds of raw meats (as a surrogate for blood and body residue). 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. After each wash, ATP and Kikkoman A3 assays were carried out.



Test results using a conventional ATP method are shown by the red lines in Figure 9. The first and second rinse steps with simple cold and hot water produced a result of less than 200 RLU. This is significant as many hospitals 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.

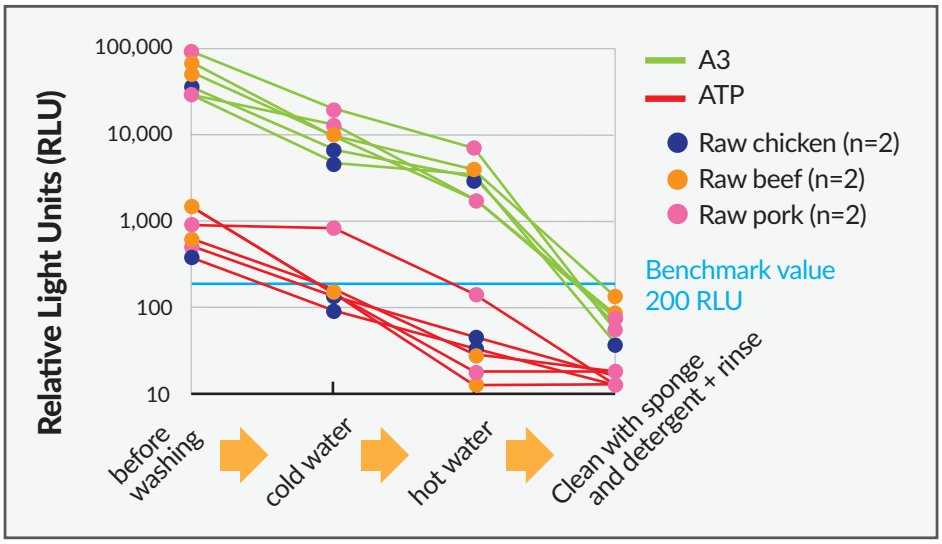


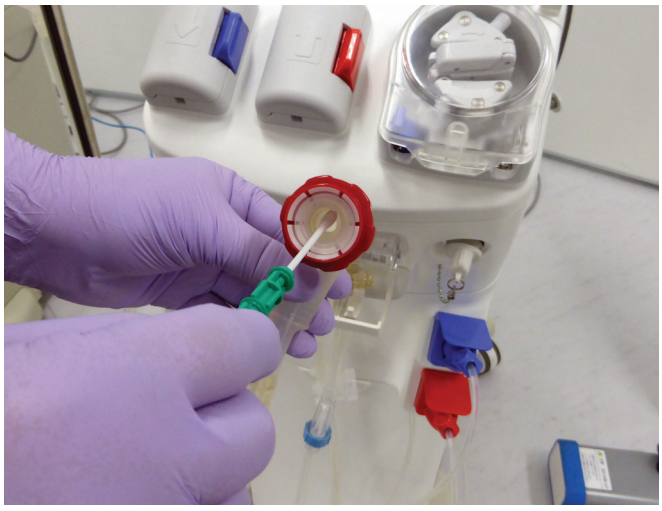
Figure 9: Comparison of Kikkoman A3 technology in surface sanitaion verification, compared to conventional ATP tests.

Using the Kikkoman 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 to verify effective sanitation processes of surfaces.

Conclusion

In summary, we have shown that a new innovative hygiene monitoring assay based on the detection of total adenylate could be developed based on the existing ATP luciferase assay with the combination of two additional enzymatic reactions.

In this method, we could detect all three adenylate molecules and the Kikkoman A3 method is far more sensitive for detections of residue on hospital surfaces and devices than conventional ATP assays.



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