Kikkoman Biochemifa Company

Comparison and interpretation of the sensitivity between ATP Test (Kikkoman A3) and conventional ATP tests.

What are Relative Light Units (RLU)?

The principle of the ATP test is based on the firefly luciferase reaction, which can produce light using luciferin and ATP. The amount of light produced is proportional to the amount of ATP in a sample and can be quantified by measuring the light. The results are displayed in relative light units (RLU). RLU are not a standardized unit of measurement like inches or centimeters, and each manufacturer sets their own value for 1 RLU based on their reagent formulations and light detection systems. Therefore, a system that can indicate larger RLU values for a certain amount of ATP is not necessarily more sensitive.
Pass/fail limits are set to determine regular cleanliness in a facility. They are generally set from tens to thousands of RLU depending on sites.

Comparison of detection sensitivities for between the ATP and Kikkoman A3 when detecting pure ATP

The precision of ATP detection may be one of the key criteria for the selection of commercially available ATP tests. However, considering the ATP tests that have received approval from the Association of Official Analytical Collaboration Research Institute's (AOAC-RI) *Performance Tested Method*SM (PTM) program, they seem to show equivalent performance for detection of pure ATP (1-6). According to data from studies of detection of pure ATP, the tests showed good linearity between ATP and RLU (Table 1). The Kikkoman A3 measured 174 RLU for 100 fmol[1] ATP, which is equivalent to or lower than measurement values from conventional ATP tests (211-659 RLU, Table 1). The Kikkoman A3 also demonstrated less variation over repeated measurements (RSD_r = 9.6%). As shown by the results, limits of detection
(LOD) for these ATP/Kikkoman A3 were equivalent, by an order of magnitude of femtomoles ATP detected (Table 1). It seems to be difficult to find significant differences for the efficiency of detection of pure ATP detection among these ATP tests.

[1] Femtomole = 10^{-15} mol

Table 1. Pure analyte results of adenosine triphosphate (ATP) tests by method developers for the approval of the Association of Official Analytical Collaboration Research Institute (AOAC-RI) Performance Tested Method℠ (As of November 2021).

Manufacturer	Kikkoman Biochemifa	Neogen	3M	Hygiena
Object	ATP+ADP+AMP	ATP	ATP	ATP
Luminometer	Lumitester™ Smart	Accupoint® Advanced	Clean-Trace™ LM1	EnSURE™ Touch
Device	LuciPac™ A3 Surface	Accupoint® ATP Surface Sampler	Clean-Trace™ Surface ATP	UltraSnap™
Line Equation x: ATP (fmol) y: RLU	y = 1.65x+9.37 (0-100 fmol) (for ATP)	y = 5.91x+7.77 (0-1000 fmol)	y = 4.05x+17.94 (0-100 fmol)	y = 2.13x-0.68 (0-100 fmol)
RLU for 100 fmol ATP	174	659	422	211
RSD_r, %[b]	9.6	14.0	35.3	8.1
Limit of detection for ATP	1.5 fmol (11.9 RLU)[a]	1.9 fmol (6.5 RLU)[a]	3.0 fmol (30.2 RLU)[a]	1.3 fmol (2.1 RLU)[a]

These data are based on the method developer pure analyte (ATP) study results. Each data set can be downloaded through AOAC RI-PTM website. [Search "ATP" on analyte at https://members.aoac.org/AOAC/PTM_Validated_Methods.aspx.]

[a] *Values were calculated using each line equation.*

[b] *Relative standard deviation of repeatability.*

Superiority of the Kikkoman A3 for detection of organic debris

Residual food debris is recognized as an indicator of insufficient cleaning. It is also a direct hazard that may cause promotion of bacterial growth caused by 1) food debris interfering with the antimicrobial activity of disinfectants, 2) raw ingredients that can contain and introduce bacteria, and 3) food residues that can be a source of nutrients for microorganisms. Moreover, allergen cross-contact can be caused by allergenic food residues left behind after insufficient cleaning. Therefore, the monitoring of food residue on equipment and surfaces should be a great concern for hygiene management.

Using ATP for this monitoring is a common practice. This use of conventional ATP tests has a weakness in that ATP in food residue on surfaces can rapidly degrade to ADP and AMP, leaving little or no signal for a conventional ATP test to detect. In fact, ADP and AMP are overwhelmingly abundant in many foods due to the degradation of ATP potentially leaving little or no ATP in the residue to detect. Therefore, conventional ATP tests may fail to detect food residues after insufficient cleaning even if they show excellent sensitivity for pure ATP detection. When the abilities to detect the adenylates present in different types of food matrices of the Kikkoman A3 and ATP tests were compared, the Kikkoman A3 showed much higher signal intensity than any of the other three ATP test kits (Fig. 1) (7). It is also shown that a variety of foods, which are widely known allergens, also contain a large amount of ADP and/or AMP (8).[2]

[2] Caution: The ATP/A3 tests are not feasible to assay the allergen protein directly or identify the allergen.

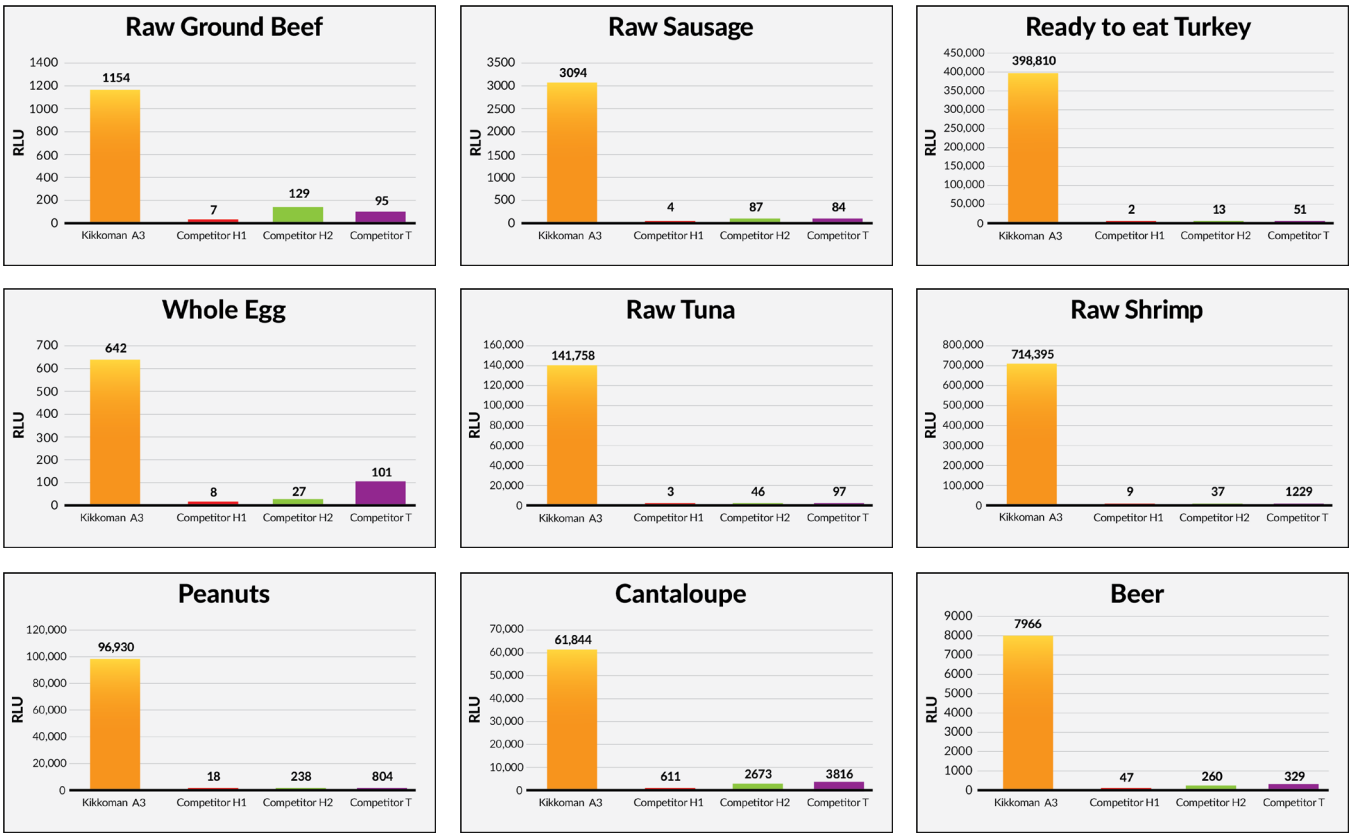

Fig 1. Detection of foods and beverages using the Kikkoman A3 and three commercially available ATP tests. ATP-1 and ATP-1H were from the same manufacturer, and ATP-1H was that manufacturer's "high sensitivity offering" that combines a high-sensitivity device and a luminometer.

Another good indicator of insufficient cleaning is adenylates from people's hands because hands are the main source of contamination found on high-touch surfaces. A previous study demonstrated that the mean ratio of ATP:ADP:AMP in debris from gloved-hands was 5%:59%:36%, with the amount of Kikkoman A3 being 20 times higher than that of ATP (9). In a study on high-touch surfaces, the repeat cleanings of plastic coupons contaminated with a mixture of debris recovered from gloved-hands plus feline calicivirus, (a typical surrogate for norovirus) was also evaluated using conventional ATP tests, the Kikkoman A3, and the standard cell culture plaque assay for virus (10). The viruses and Kikkoman A3 from organic debris were easily detectable before cleaning (21000 plaque-forming units and 3988 RLU). The two conventional ATP tests, however, could detect only substantially lower levels of organic debris (146 and 112 RLU). Following a single cleaning of the plastic coupon with microfiber cloth, the level of virus decreased by 96%, while the results from the Kikkoman A3 indicated inadequate cleaning (803 RLU). The conventional ATP tests indicated that the surface was clean (61 and 14 RLU). After cleaning the surface four times, the measured level of the virus on the surface declined by 99.5% with the Kikkoman A3 measuring 135 RLU which is below the manufacturer's recommendation for effective cleaning. These results suggest that the Kikkoman A3 is a superior method for the assessment of the cleaning of various high-touch surfaces while conventional ATP tests produced questionable correlations and results.[3]

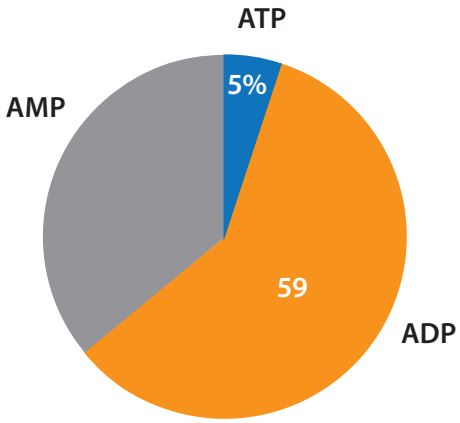

Fig. 2 Ratio of ATP:ADP:AMP in gloved hand samples.

[3] Caution: The ATP/A3 tests cannot measure viruses directly because viruses do not generate or store adenylates.

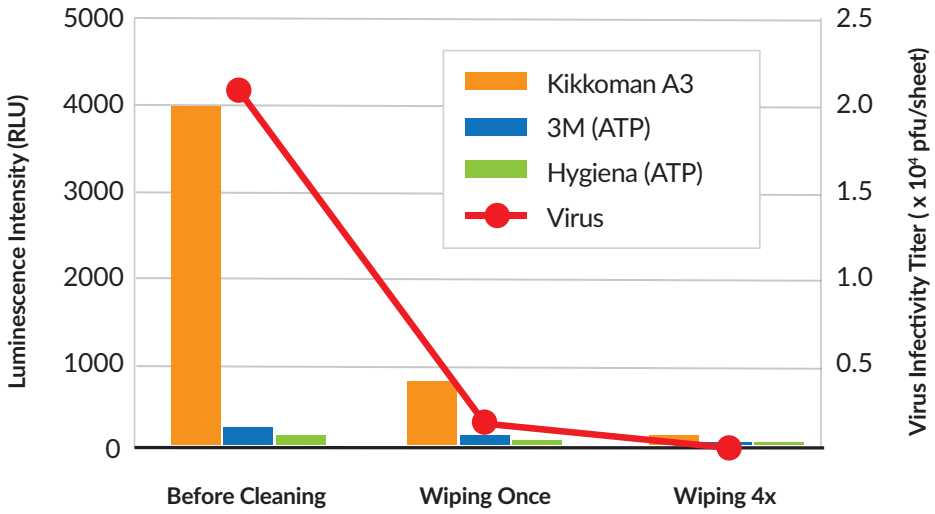

Fig. 3 Virus, A3 and ATP levels on plastic test coupons.

Misunderstanding about detection of bacteria using the ATP/Kikkoman A3

Many users have inquired as to whether better precision of the detection of pure ATP makes for a test effective for bacterial detection. This is not the case and, unfortunately, ATP/Kikkoman A3 should not be considered as a replacement for microorganism testing (11). First, it is generally known that an ATP/Kikkoman A3 will not detect bacterial cultures unless their number exceeds approximately 10^2-10^4 (12). Second, another main reason that ATP tests cannot accurately predict the presence of microorganisms or quantify the populations of microorganisms is they detect ATP/Kikkoman A3 from all organic debris (e.g. food residues and microorganisms simultaneously) and cannot differentiate the source of the ATP. Moreover, the ATP content of microorganisms differs depending on their species, cell size, and metabolic state (13,14). High detection sensitivity of detection of pure ATP is not indicative of the specific detection and determination of bacteria.

Reviewing the principle of hygiene monitoring using the ATP/Kikkoman A3, they are acceptable methods for assessing cleaning effectiveness through the detection of ATP/Kikkoman A3 from food residues, debris from hands, body fluid, and bacteria in total. If cleaning is effectively performed (using proper techniques and cleaning chemistry) to the point that ATP/Kikkoman A3 is reduced to below levels validated to represent clean surfaces, definitive tests will show that bacteria have been reduced to lower levels. For example, a field study in a hospital demonstrated that cleaning with microfiber cloths surely decreased both Kikkoman A3 and aerobic plate counts on high touch surfaces (9). Furthermore, in 15 food processing plants and commercial kitchens, the Kikkoman A3 was correlated with the aerobic plate counts indicative of clean surfaces in food processing environments associated with raw food ingredients that would otherwise have a high microbial load (15).

A University of Wisconsin-Madison study also demonstrated that the concentrations of the different adenylates in bacteria varied in that ATP was predominant at initial stages of bacterial incubation and growth stages, but AMP became predominant at later time points (13). Another study also suggested that Kikkoman A3 in microorganisms was more stable than ATP content, which was affected by the nutritional conditions present (16). It is also known that ATP levels in spores are very low, but the levels of ADP and AMP in spores are much higher (17). Bacteria that may be present, may or may not be growing or may exist in various states of nutrient deficiency or injury that change their growth phase. Therefore, the detection of Kikkoman A3 is more effective as an indicator of the hazards present, including bacteria, from insufficient cleaning.

Conclusion

Regarding detection of pure ATP, the ATP/Kikkoman A3 that are approved by AOAC-PTM show good and similar detection performance. These tests are acceptable methods for assessment of cleaning effectiveness through the detection of ATP/Kikkoman A3 from food residues, debris from hands, body fluid, and bacteria in total, using pass/fail limits for ATP/Kikkoman A3 set from tens to thousands. Therefore, the precision of the tests for detection of pure ATP detection at several RLU scale and LOD may not have a strong impact on the effectiveness of the tests for hygiene monitoring.

On the other hand, since organic debris onsite contains remarkably large amount of ADP and AMP, the Kikkoman A3 can detect organic debris more effectively than conventional ATP tests. Accordingly, one can see that the primary consideration for selection of an ATP tests to manage the effectiveness of cleaning is the ability of the test to detect ADP and AMP as well as ATP rather than the precision of the test's detection of pure ATP.

References

1. Bakke, M. et al. J. AOAC Int. 2020, 103, 1081-1089.
2. Bakke, M. et al. Available at: https://members.aoac.org/AOAC_Docs/RI/21PTM/21C_051901_kikkoman%20LuciPacA3.pdf.
3. Viator, R. et al. J. AOAC Int. 2017, 100, 537-547.
4. Viator, R. et al. Available at: https://members.aoac.org/AOAC_Docs/RI/21PTM/21C_091601_NeoATP.pdf.
5. Bakken, H. et al. Available at: https://members.aoac.org/AOAC_Docs/RI/20PTM/20C_041901_3MCleanTrace.pdf.
6. Todd, R. et al. Available at: https://members.aoac.org/AOAC_Docs/RI/20PTM/20C_101803_HygATP.v2mod.pdf.
7. Bakke, M., and S. Suzuki. J. Food Prot. 2018, 81, 729-737.
8. Saito, W. et al. J. Food Prot. 2020, 83, 1155-1162.
9. Bakke, M. et al. J. Prev. Med. Hyg. 2019, 60, E140-146.
10. Kikkoman Biochemifa Company. 2021. Available at: https://biochemifa.kikkoman.co.jp/download/?id=197&k=2.
11. Mildenhall, K. B. and Rankin, S. A. J. Food Prot. 2020, 83, 1619-1631.
12. Gracias, K.S., and J. L. McKillip. Can. J. Microbiol. 2004, 50, 883-890.
13. Smith, N. W. et al. J. Food Prot. 2019, 82, 2088-2093.
14. Venkateswaran K. et al. J. Microbiol. Methods 2003, 52, 367-377.
15. Chai, L. C. et al. J. Food Prot. 2021, 84, 973-983.
16. Sakakibara, T. et al. Anal. Biochem. 2003, 312, 48-56.
17. Hausenbauer, J. M. et al. J. Bacteriol. 1977, 129, 1148-1150.

kikkoman®

Kikkoman Biochemifa Company

2-1-1 Nishi-Shinbashi, Minato-ku, Tokyo
105-0003 JAPAN
biochemifa@mail.kikkoman.co.jp
https://biochemifa.kikkoman.com/e/kit/