Comparing the Performance of ATP Hygiene Monitoring Systems

Hygiena® vs Kikkoman™

ATP Hygiene Monitoring

ATP hygiene monitoring is a simple, rapid and quantitative testing method to verify cleaning effectiveness. For a surface to be verifiably clean, all food debris and other organic matter must be removed. Food debris, organic matter, and microorganisms contain ATP. Microorganisms are very tiny and individually contain only small amount of ATP. Thus, large numbers of microbes (~10,000) are required to be detectable by ATP test systems, which measure ATP residue in Relative Light Units (RLU). Systems are highly sensitive and can detect extremely low levels of ATP molecules, which means they can detect extremely small amounts of organic matter or food debris on surfaces. Effective cleaning removes both microbes and food residues. This means the lower the ATP reading is, the higher the cleaning standards are, resulting in a lower risk of microbial contamination.

What Has Changed?

Over the past 10 years, some systems have been re-designed, and some have received 3rd party certification by AOAC-RI under the *Performance Tested Methods℠* Program.

Hygiena Changes

- Hygiena released the EnSURE® Touch to complement its SystemSURE *Plus*™ and EnSURE® luminometers.
- UltraSnap® Surface ATP Test pre-moistened swab device with liquid-stable luciferase reagent remains the same and is fully compatible with all three luminometers.
- UltraSnap is an AOAC-validated method when used with EnSURE and EnSURE Touch.

Kikkoman Changes

- Kikkoman's Lumitester™ PD 20 and LuciPac™ swabs detect both ATP and AMP using a combination of enzymes freeze-dried as single dosage test in a device containing a dry cotton swab.
- Kikkoman's Lumitester™ PD 30 (similar to PD 20) and Lumitester™ Smart, with the LuciPac™ A3 swab, detect ATP, ADP and AMP using a combination of enzymes freeze-dried as single dosage test in a device containing a dry cotton swab.
- Lumitester Smart, released in 2019, works with LuciPac A3 swabs to detect ATP, ADP and AMP.
- Kikkoman recommends wetting the swab before use with tap water.

Critical Performance Characteristics of ATP Hygiene Systems

Sensitivity
The smallest amount of ATP and food residues detectable

Consistency
The variation of result from repeated tests of the same sample

Accuracy
The measured ATP value compared to the true value

Precision
The repeatability of the test to produce the same result

These parameters are determined using samples containing several different concentrations of ATP, including a sample without ATP. Ten replicates at each concentration level are tested. The data generated is used to calculate the limit of sensitivity, consistency, accuracy and precision. All systems showed excellent linear response to all sample types ($R^2 > 0.98$).

Sensitivity

To ensure the highest level of cleanliness is achieved, a test is needed that can reliably detect the smallest amount of contamination. The table below shows the smallest amount of ATP detectable by all ATP hygiene monitoring systems. Both Hygiena and Kikkoman systems have shown a continual improvement over the past 10 years. Kikkoman has improved the sensitivity of its systems, but they are still 6 – 7 times less sensitive than Hygiena's ATP monitoring systems. This means that Hygiena systems can measure to higher cleaning standard than Kikkoman systems.

If greater sensitivity is required for high risk operations, then Hygiena's SuperSnap® High-Sensitivity Surface ATP Test provides an additional 5-fold increase in sensitivity (not shown in table).

	Hygiena UltraSnap			Kikkoman LuciPac		
Lowest amount of ATP (fmols) detected = greater sensitivity	EnSURE Touch	EnSURE	SystemSURE Plus	Lumitester PD 20	Lumitester PD 30 LuciPac A3	Lumitester Smart LuciPac A3
	<1.0	**1.0**	**1.0**	**10.0**	**3.1 - 5.2**	**7.6**

*Data provided by Hygiena AOAC certificate #101803 and Kikkoman AOAC certificate #051901.

Key Factors Affecting Sensitivity

Each detection system will generate a response when there is no ATP in the sample. This is called background noise and is caused by impurities in the chemistry. If not removed, these impurities significantly affect the performance of the system. For freeze-dried reagents like that found in Kikkoman's LuciPac and LuciPac A3, these impurities are locked in during the manufacturing process. In contrast, Hygiena's liquid stable chemistry remains active and impurities are removed, resulting in lower background noise. Low background noise means more reliable and sensitive measurement, particularly at low level detection required for cleaning verification.

The AOAC data* shows the background noise for ATP detection by Kikkoman systems is 10 –12 RLU, compared to Hygiena's superior performance of 1 – 2 RLU. In food residue and bacteria studies, Kikkoman's ranged from 7 – 42 RLU compared to 1 – 5 RLU for Hygiena systems.

This means that in food residue detection Kikkoman's result of < 40 RLU is unreliable.

Accuracy and Precision

For the detection of ATP and food residues, both Hygiena and Kikkoman systems show similar variation from which accuracy and precision were calculated.

Hygiena's EnSURE Touch and UltraSnap swab has an accuracy of 85.8%

Kikkoman's PD 30 and LuciPac A3 swab has an accuracy of 79.9%

Detection of Food Residues

Dilution of different food groups were applied to surfaces to assess the lowest amount detectable by swabbing. Both Kikkoman and Hygiena detected dilutions of foods at 1/1000 to 1/300,000, although there was some slight difference between food types. Overall there was little difference between the two systems (see table). Both Kikkoman and Hygiena systems are capable of detecting complex matrices from surfaces to verify cleaning. Accordingly, Kikkoman's claim that the A3 swab is more sensitive because it detects ATP, ADP and AMP is not substantiated by the data generated by AOAC third-party lab studies (Kikkoman certificate #051901).

The ATP surface cleaning verification test is not intended to be a surrogate bacteria test because it does not have the required sensitivity (typically 250/100cm^2 swab area).

Food residues on surface (AOAC study)	ATP System	
	Hygiena EnSURE Touch UltraSnap	Kikkoman Lumitester PD 30 LuciPac A3
Orange juice	> 1 in 100,000	> 1 in 100,000
Baked goods	> 1 in 10,000	> 1 in 5,000
Yogurt	> 1 in 1,000	> 1 in 30,000
Raw meat	> 1 in 10,000	> 1 in 30,000
Cooked meat	> 1 in 40,000	> 1 in 100,000

*Data provided by Hygiena AOAC certificate #101803 and Kikkoman AOAC certificate #051901.

Swabbing

The importance of correct swabbing technique was highlighted in the Kikkoman AOAC certificate #051901 which is probably a reflection of Kikkoman's short and dry swab (see quote below).

> *Method Developer Studies demonstrated that pure analyte solutions yielded <20% RSD_r but RSD_r values of each matrix solution for swabbing assays were <30%. Independent laboratory studies demonstrated RSD_r values of each matrix solution for swabbing were <26.7% (orange juice) and <42.5% (ham). The higher variations of matrixes were likely caused by additional factors i.e. swabbing technique. Additionally, regarding insoluble food samples, solid and liquid are separated soon even after careful homogenization. This unavoidable heterogeneity may cause variability in the amount of matrix applied onto plates. It should also be considered that all cotton swabs may not be able to pick up the dried particles completely. Consistent swabbing technique is important to minimize the variability. Swabbing an object thoroughly using the entire surface of the swab with rotation is ideal. Ideally the swab should be slightly bent when exerting appropriate pressing force.*

Effects of Chemical Sanitizers

Previous data on Kikkoman LuciPac showed hypochlorite and quaternary ammonium sanitizer, benzalkonium chloride, resulted in ~10% inhibition of the test. However, the AOAC studies* showed that LuciPac A3 swab showed a 25 – 30% inhibition by benzalkonium chloride. Conversely, peracetic acid amplified the RLU output to 160 - 187% of the control giving rise to a potential false positive reaction.

The AOAC report* says:

"The A3 test is recommended to be used after rinsing away sanitizing agents for accurate assessments"

Hygiena's UltraSnap is also affected by some sanitizer. However, over 20 years' experience has shown that this is not a problem in routine use which is acknowledged in the AOAC report*. If harsh samples are encountered, then Hygiena's SuperSnap can be used because its formulation is more robust and more sensitive.

Detection Microbes

The AOAC studies* showed that both Hygiena and Kikkoman systems were equally able to detect Gram-positive and Gram-negative bacteria as well as yeast.

The smallest number of microbes detected by Hygiena systems was 50,000 – 75,000 bacteria and 1,000 yeasts.

The smallest number of microbes detected by Kikkoman systems was 20,000 – 100,000 bacteria and 100 yeasts.

Summary

- Hygiena's UltraSnap swab used with EnSURE and EnSURE Touch is more sensitive than Kikkoman PD30 and Lumitester Smart with either LuciPac or A3 swabs.
- Hygiena and Kikkoman systems both have similar accuracy and precision for detecting ATP and food residues.
- Kikkoman systems have high background noise that limits sensitivity, particularly when detecting food residues on surfaces, which is partly a function of its dry swab and swabbing technique.
- Kikkoman's A3 swab that detects ATP, ADP and AMP offers little benefit over systems that detect ATP only.

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*Data provided by Hygiena AOAC certificate #101803 and Kikkoman AOAC certificate #051901.