Measuring More, Meaning Less: Kikkoman A3 System Doesn't Pass

Introduction

In 2019, Kikkoman launched its Lumitester™ Smart, to replace the PD-30/PD-20 hygiene monitoring systems. They claim that its ability to measure ADP levels as well as ATP and AMP makes it a more sensitive and accurate detection method than systems that measure ATP alone. Adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) are chemicals within a cell that differ only by the number of phosphate groups (1, 2, or 3, respectively). Of the three, ATP stores the most energy and is measured with the luciferase enzyme to give simple, sensitive and instant test results that have been the gold standard for rapid monitoring of cleaning and sanitation effectiveness since 1980.

Kikkoman's PD-10 and PD-20 ATP+AMP hygiene monitoring systems have been in the market for over 10 years; the Lumitester SMART, for only 2 years. In 2019, Kikkoman updated the instrument to make it more user friendly and allow for storage in the cloud. Kikkoman claims that because its instrument is measuring more molecules (ADP and AMP), it is more sensitive than luminometers that only measure ATP. In addition, the same relative differences exist between clean and unclean surfaces, regardless of whether ATP alone is measured or whether ATP/ADP/AMP are measured.

In the study detailed below, the Kikkoman hygiene monitoring system was compared against the Hygiena™ EnSURE™ Touch monitoring system for linearity, sensitivity, repeatability, accuracy, and reproducibility when tested with food samples. Chemistry in both systems was also compared.

Equipment, Supplies

- Kikkoman Lumitester™ SMART luminometer with LuciPac® A3 Pen
- Hygiena™ EnSURE™ Touch with UltraSnap™ ATP test devices

Methodology

The study evaluated three Hygiena and three Kikkoman instruments:

- To compare **sensitivity** and **linearity** of results, relative light units (RLUs) were measured at ATP concentrations of: 0.1, 0.5, 1, 5, 10, 100, 1,000 and 5,000 femtomoles. RLUs were measured and recorded from 1 to 5,000.
- To measure **repeatability** of the system over many tests, concentrations of 100 femtomoles of ATP was added to both swab devices for 10 replicates each. The percentage variability of the 10 RLU results were determined and plotted. The same test was performed using 10 femtomoles of ATP with 5 replicates each.
- To compare **accuracy**, the study measured percent recovery from a single swab application from each instrument. The higher the recovery of ATP in RLUs, the more accurate the reading.
- For detection of instrument **reproducibility**, three EnSURE Touch systems were compared to three Lumitester SMART systems. The study tested instrument to instrument variability in detection of 100 femtomoles of ATP on either UltraSnap or LuciPac A3 test devices, respectively. Ten replicates were analyzed for each instrument.
- For detection of **specific food** soils, EnSURE Touch with UltraSnap was compared to Kikkoman Lumitester with with LuciPac A3. The study tested the ability of each system to detect ATP from the following food residues: natural yogurt, orange juice, baked goods, raw meat and cooked meat. Each food was added to surfaces in a series of dilutions from 1/10 to 1/300,000. Data is pulled from the AOAC study for the EnSURE Touch system.

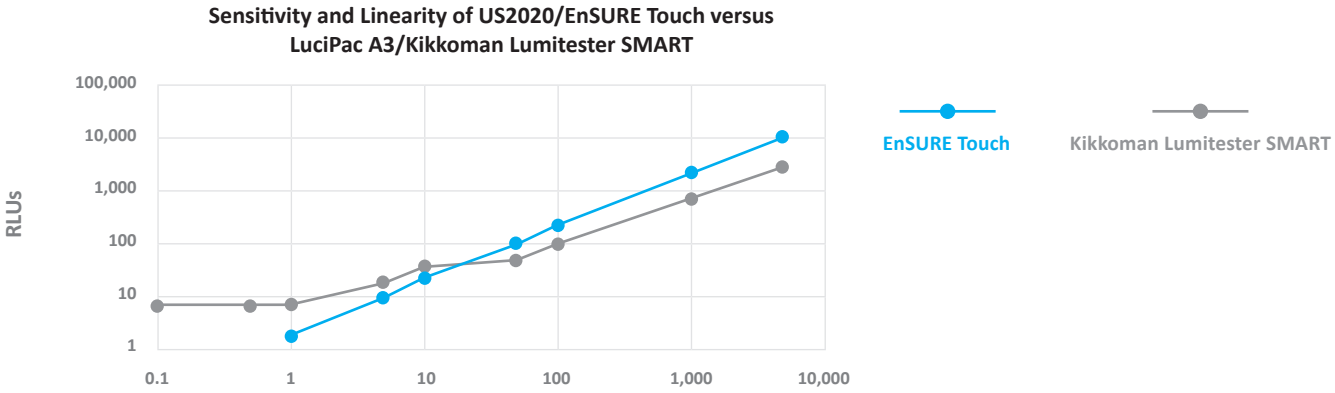
Results and Discussion

Linearity/Sensitivity

The Kikkoman Lumitester SMART and LuciPac A3 Pen showed background signal of 7.3 RLUs and limit of detection of ~10 fmoles.

The Hygiena system had a background signal of 0 RLU, and limits of detection of 1 femtomole when using UltraSnap test devices.

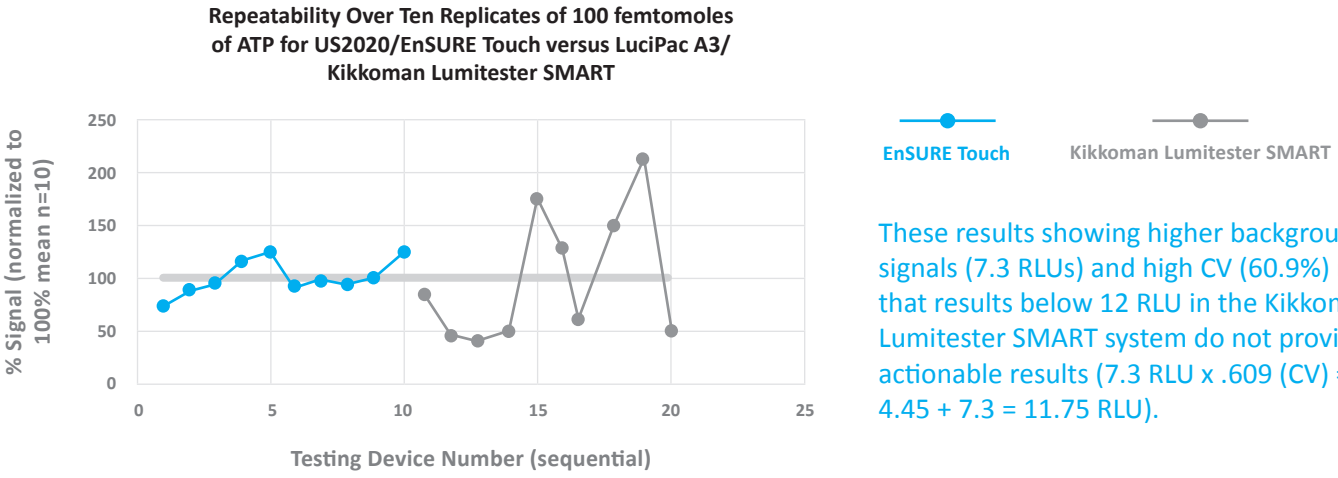
As the graph below shows, the relationship between RLUs and amounts of ATP detected (fmoles) should be completely linear. No linearity below 10 fmoles shows a lack of sensitivity of the Kikkoman system to low levels of contamination.



Repeatability at Typical Pass/Fail Threshold

Ten replicates (for each instrument) of 100 fmole ATP concentration showed CV values of 15.3% for UltraSnap versus 60.9% for LuciPac.(Fig. 2; CV = coefficient of variation, a way to measure the degree of divergent results).

Similar variation is also shown at 10 fmol of ATP.

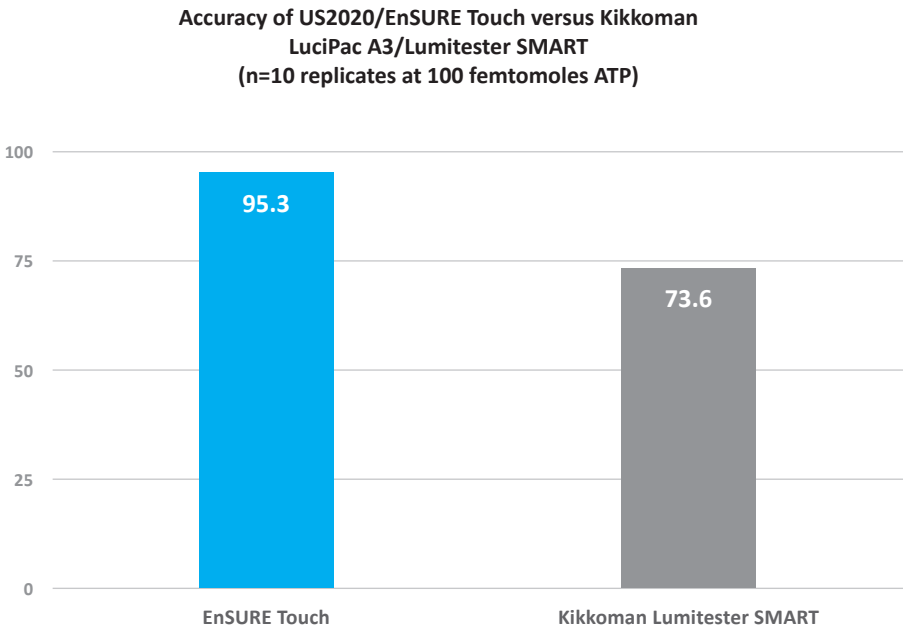


These results showing higher background signals (7.3 RLUs) and high CV (60.9%) mean that results below 12 RLU in the Kikkoman Lumitester SMART system do not provide actionable results (7.3 RLU x .609 (CV) = 4.45 + 7.3 = 11.75 RLU).

Accuracy

Detection accuracy was determined for each system at 100 fmol of ATP. While this level of ATP represents a typical fail level, the Hygiena system showed high reliability and consistency at 95.3% compared to 73.6% for Kikkoman.

Percent Recovery of ATP/AMP from Swab



The recovery from the Kikkoman Lumitester SMART test device was unacceptably low.

Performance in Specific Foods

Food residues on surfaces (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

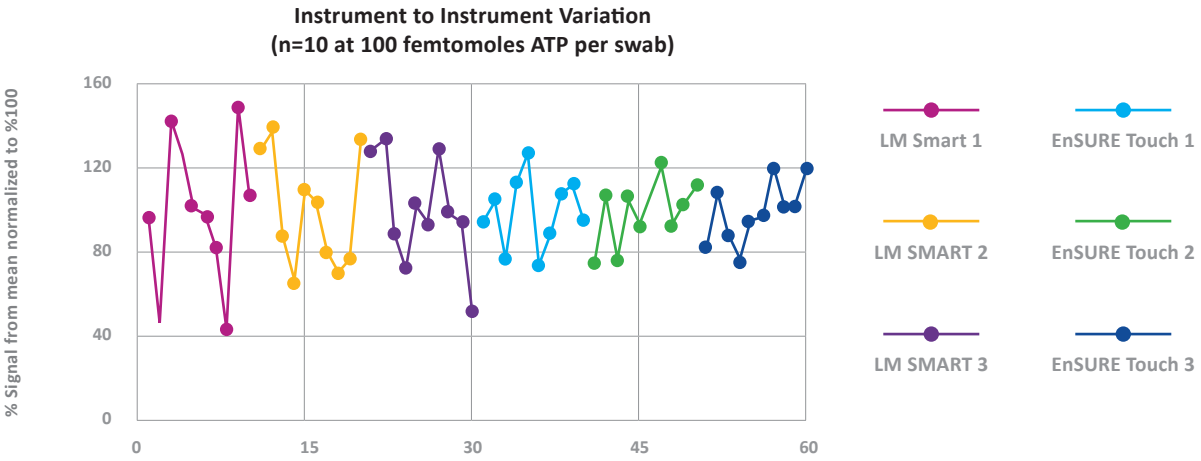
*Data provided by Hygenia AOAC certificate #101803 and Kikkoman AOAC certificate #051901

Note: Data here was obtained from published AOAC studies for the Kikkoman PD-30 and A3 swab (not the Lumitester SMART). The limit of detection for the PD-30 luminometer is ~ 3 fmol ATP which is appromimately 2X more sensitive than the Lumitester SMART, further impacting the reliability of the latter.

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). In fact, the background noise seen on clean, uninoculated surfaces was high and variable, ranging from 10 to 25 RLUs (10 - 25 fmol ATP).

Reproducibility

As seen with the repeatability assays, ten replicates (for 3 instruments) of 100 fmole ATP per swab showed high variability among the Lumitester Smart systems in comparison to the EnSURE Touch systems analyzed. The Lumitester Smart systems showed low detection values and high levels of variance (CV average of 29.2%). The EnSURE Touch System, on the other hand, was more able to accurately detect 100 fmol of ATP with a smaller CV (average of 15.2%).



Additional Notes/Discussion

Complex Chemistry

The Kikkoman LuciPac swab device has complex multi-component chemistry that requires lyophilization, which can introduce variability. In contrast, the Hygiena chemistry is liquid stable and requires only two chemical components, reducing variability. This may explain the variation seen in results when testing the addition of 100 fmol ATP per swab. This level of ATP is a typical fail level, data indicates that the Hygiena system will more reliably detect a fail (95.3% consistency vs. 73.6% for Kikkoman)

In addition, at lower levels of ATP (10 fmol), which are typically used to verify cleanliness, the Kikkoman system showed significant variation, indicating lower levels of reliability at critical levels.

In contrast, the Hygiena system showed low variation and high reliability due to its liquid stable chemistry. This allows for low background noise and high sensitivity and consistency.

Other Observations

Kikkoman LuciPac A3 Pens are delivered dry, with instructions to wet them “running under tap water.” This invites cross-contamination and can skew test results as tap water is not sterile. Instrument to instrument variation was also greater when testing the Kikkoman systems. Hygiena systems were 2X more reliable (15.2% variation) than Lumitester/LuciPac A3 system (29.2% variation).

Conclusions

Complex Chemistry

It is important to note that successful measurement and management of cleanliness depends on five integrated, optimized components:

- Light detector
- Instrument electronics and design
- Chemistry formulation and stability
- Swab device
- Data analysis software

The Kikkoman Lumitester SMART instrument has poor sensitivity (10 times weaker than EnSURE Touch with UltraSnap).

Key points are:

- No ATP system designed to test effectiveness of surface cleaning can directly identify bacteria.
- Background noise and variability of repeated tests are high enough that readings less than 12 RLUs are meaningless with the Kikkoman Lumitester Smart.
- Measuring AMP, ATP and ADP together effectively constitutes measuring the same molecule three times, because they are all involved in the same process (oxidative phosphorylation). It does not improve detection as indicated by the food performance testing results.
- The Kikkoman A3 LuciPac Pen is complex and offers no practical benefit to the end user.
- It's important to note that AMP and ADP do not exist without ATP, and ATP testing has been the gold standard as a reliable rapid sanitation monitoring method for decades.