kikkoman
Kikkoman Biochemifa Company

The Utilization of ATP Test (Kikkoman A3) for Allergen Control in Poultry and Meat Industry

Presenter

Kikkoman Food Products Company
Product Development Division,
Processed Food Development

Mr. Takayasu Watanabe

Introduction

This is a summary of the 129th Lumitester Seminar (Webinar) by Mr. Takayasu Watanabe from Kikkoman Corporation, entitled "The Utilization of the ATP Test (Kikkoman A3) for Allergen Control in Poultry and Meat Industry".

The seminar highlighted (1) the importance of allergen control in food production facilities, (2) the verification of the effectiveness of cleaning for allergen control, (3) the effectiveness of the use of the ATP Test (Kikkoman A3) for this cleaning verification application, and (4) a case study of Kikkoman Corporation's use of the ATP Test (Kikkoman A3) for this type of allergen control testing.

Contents

1. The Importance of Cleaning Assessment for Allergen Control

(1) Risk of food allergen in food production facilities

In the past few years, regulatory efforts have been increased to reduce the incidence of allergen cross-contamination in foods. In Japan, for instance, numerous violations for allergen cross-contamination have been made public and, in 2021, more than one hundred recalls due to allergens were reported. Not only can food allergen cross-contamination cause serious health problems for consumers, but these incidents can cause significant damage to a company, including costs for recalls and damage to their brand.

Food allergens are "substances in foods that can cause allergic reactions in sensitive individuals." Most of these allergens are known to be proteins naturally found in certain foods. The main causes of incidents caused by food allergens is “mislabeling of raw materials” or “unintended cross-contamination during food processing.”

The latter of these causes, unintentional allergen cross-contamination, is typically caused by contamination of a product through the use of equipment or utensils that have not been sufficiently cleaned following their use on another allergen-containing product. This can often be the case in meat processing factories when multiple types of meats are processed, each with their own allergenic proteins, and cross-contamination of one type with another can easily occur. Special attention to cleaning and hygiene must be taken to avoid cross-contamination from insufficiently cleaned tools and surfaces.

(2) Handling food allergens in food factories.

Kikkoman Food Products Company offers a number of products designed for consumers' convenience in cooking that contain a wide variety of ingredients that enhance the value and convenience of the products. Many of these products – like the “*Uchinogohan*” and “*Gumen*” branded meal kit series - contain meat as an ingredient, so, at the production sites where these are produced, it is essential to correctly handle any meat-derived allergens.

To prevent cross-contamination of allergens at these production sites, some of the mitigations used include, (1) Segregation – the use of separate processing lines, workspaces, and tools for each food product with a specific allergen, (2) Scheduling - avoiding productions of products with different allergens on the same day of production, and, (3) thorough cleaning of production lines during product changeovers. Depending on the site area and operating conditions of the manufacturing plant, measures such as segregation and scheduling may not always be feasible making the third option - very effective cleaning processes - ever more important.

(3) Cleaning verification tests

Various types of “swab” tests are generally used to verify the effectiveness of cleaning. These tests can be useful, but each has different advantages and drawbacks that need to be understood when selecting which to use. (Table1)

Enzyme-linked immunosorbent Assays (ELISA) are highly sensitive and accurate methods that can directly measure the concentration of residual allergens remaining on surfaces and equipment after cleaning. ELISA tests are, however, more complicated to use, typically require longer to produce a result and often need to be evaluated by a trained analyst. Another potential disadvantage is, because ELISA tests are highly specific for only a single allergen, a separate test is required for each allergen being controlled – This adds complexity and cost to the control program and, in some cases, there are a limited number of test kits available for allergens other than the higher-volume varieties (e.g., milk, egg, wheat, buckwheat, peanut, shrimp, crab).[1]

Tests that detect the presence of protein in general are also available. These protein swab tests have the advantage of being low cost, being able to produce a rapid result onsite, and as they detect protein in general they are not limited to being specific to only one allergen. The drawbacks to these tests, however, include that most are qualitative and provide only a positive or negative result for the presence of protein (above a certain detection level) and are unable to quantify the amount of residual protein present. The selection of such tests for each operation should be made with an understanding of the impact of these limitations on an allergen control program.

Due to these shortcomings of other available tests, in our research, we focused on the ATP Test (Kikkoman A3). ATP tests are able to quantify the level of soil on a surface or equipment in general, but do not directly measure proteins or specific food allergens. "Do these attributes make ATP an effective cleaning verification tool for allergen control?" To answer this question, we conducted a verification of this method.

[1] For some allergens there are no commercially available test kits.

Swab method	Function	Merits	Demerits
ELISA	Detect and quantify allergen	•Able to quantify allergen •High sensitivity and high accuracy	•Complex •Takes time to get results •Expensive
PCR	Detect DNA	•High accuracy	•Operation complex •Takes time to get results
Protein swab	Detection of protein residues	•Results on site •Cost effective	•In many cases, there is no quantifiability.
Kikkoman A3	ATP (+ADP, AMP)	•Results displayed numerically •Results in 10 seconds •Cost-effective	•Correlation of ATP measurements to protein is not clear.

Table 1. Characteristics of various swab methods

(4) The ATP test and the ATP Test (Kikkoman A3) for allergen control testing

An ATP test uses the principle of firefly luminescence, that is, the biochemical reaction used by a firefly to produce light. The compound luciferin in the presence of the enzyme luciferase reacts in the presence of adenosine triphosphate (ATP) to produce light. Using this mechanism, an ATP test that contains luciferin and luciferase can quantify the amount of ATP present on surfaces by measuring the amount of light produced (or "luminescence"). (Fig.1)

ATP is a critical metabolite and energy source for all living organisms. This makes ATP ubiquitous and present virtually everywhere on surfaces, making an ATP tests an effective indicator verification of cleaning effectiveness (i.e., verifying if food residue has been removed completely). ATP Test (Kikkoman A3) (Photo 1) is a unique ATP test in that it measures not only the ATP present but also the commonly found ATP degradation products ADP (adenosine diphosphate) and AMP (adenosine monophosphate). Degradation of ATP to ADP and/or AMP can occur by heating, pH, and various enzymatic reactions and environmental factors. ATP Test (Kikkoman A3) tests for ATP·ADP·AMP and this larger detection target allows for a more effective verification of cleaning effectiveness than ATP tests that can only measure ATP. ATP tests and ATP Test (Kikkoman A3) has been widely used in food factories, restaurants, medical fields, and public facilities.

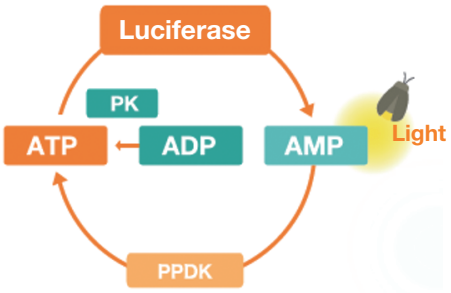

Figure 1. Principle of ATP+ADP+AMP measurement

Photo 1. Lumitester Smart meter (Left)
LuciPac A3-Reagent (Right)

2. The effectiveness of ATP test (KikkomanA3) in allergen control

(1) Summary of validation experiment

In the manufacturer's instructions for use of the ATP Test (Kikkoman A3), a pass/fail benchmark of <500 RLU is recommended for use as a "passing" result indicating effective cleaning. The goal of this project was to examine whether such a < 500 RLU level is appropriate as a pass/fail criterion for allergen control in a meat processing plant. Generally, a concentration of allergens at a level of "a few ppm or more" may cause meat allergy reactions to occur. For example, the Consumer Affairs Agency of Japan states that "if the concentration of allergen protein is less than a few ppm, the labeling can be exempted.

Based on the above, it is judged that if the "*heterologous meat protein contamination rate*" is less than 1 ppm when the ATP content is 500 RLU, then a pass/fail limit of 'less than 500 RLU' is appropriate as a cleaning pass/fail limit for allergen control purposes. We will further explain the concept of "heterologous meat protein contamination rate" in more detail in the next section.

The validation study was conducted in 3 steps:

①Meat solutions were prepared from various types of protein (chicken, pork, beef) and the amount of ATP in each was measured.

②The total protein content of each sample (i.e., protein content of meat) of each solution as adjusted in Step 1, was determined.

③The level of meat protein present when the ATP level was measured at 500 RLU*[2] was calculated along with the "heterologous meat protein contamination rate"

(2) Calculation of "heterologous meat protein contamination rate"

For this verification experiment, we defined the "*heterogeneous meat protein contamination rate*" as "the value calculated for the concentration of protein of any meat species remaining after equipment cleaning that could cause cross-contamination of a different product produced on that same equipment. For example, Fig.2 shows processing chicken on equipment that was cleaned after previously processing pork. If the cleaning is insufficient and residual pork protein is still present on the equipment, it can contaminate the chicken products (i.e., heterologous meat).

The contamination rate of pork protein in the chicken (as ppm), can be calculated, by dividing "amount of pork protein(μg) present in the measurement space S" by "weight of pork(g) per measurement space S" (Table 2).

Explanation of the validation study to calculate "different meat contamination rate" below.

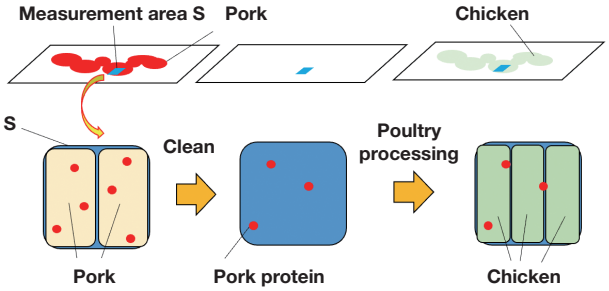

Figure 2. Example of pork protein contamination in chicken processing

$$\text{Contamination rate of protein in chicken (ppm)} = \frac{\text{Pork protein present in S (μg)}}{\text{Weight of chicken per S (g)}}$$

Table 2. Calculation of contamination rate for protein

(3) Result of Validation

①Correlation between the amount of each meat type and ATP measurements

Meat solutions prepared from each type of protein (chicken, poultry, and pork) were subjected to ATP measurement, and the correlation between the weight of each type of meat and the amount of ATP was measured.

Procedure: Remove the fat and skin of chicken, beef and pork and mince using a meat chopper set for the minimum texture (3.2mm). For each type of protein, parts with the highest protein content were selected (i.e., chicken: breast, pork: shoulder loin, beef: thigh). 10 g of each meat sample was mixed well with 90 g of purified water (1 g/10 ml = 1 × 10^5 μg /ml), and 100 μl samples of the sample liquid was used for the ATP Test (Kikkoman A3)[3] using serial dilutions of the sample after the meat residue was removed.

Fig.3 shows the test results for chicken. The data shows that a proportional relationship exists between the weight of meat and the ATP measurement. (the graph to the right of Fig.3 focuses on measured figures only around 500 RLU). Also, a similar proportional relationship was found to exist for the pork and beef samples. (Figs. 4 and 5).

From these results, we can conclude that "the residual weight of meat" can be calculated based on this relationship with ATP measurements.

2 RLU: "Relative Light Unit" – the unit of measure of the amount of ATP luminescence in the sample.

3 Measurement taken 30 seconds after the reagent reaction

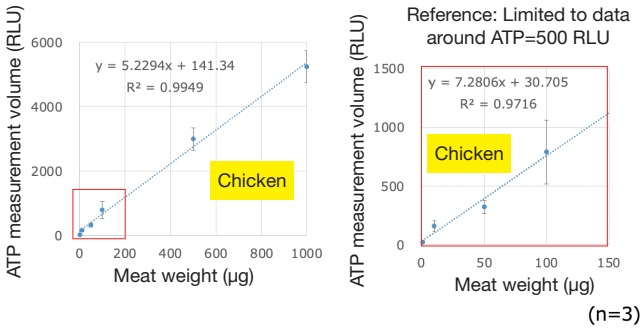

Figure 3. Meat weight (chicken) and ATP measurement

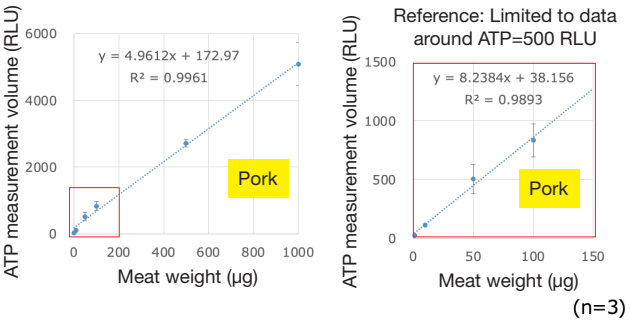

Figure 4. Meat weight (Pork) and ATP measurement

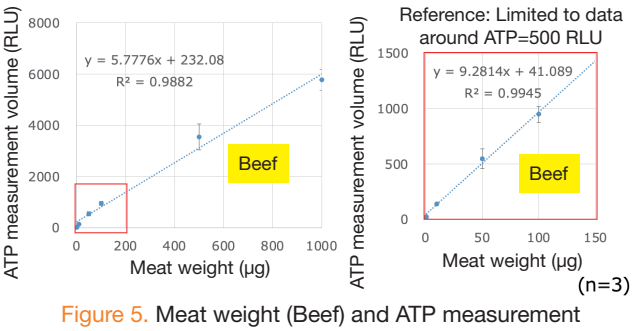

Figure 5. Meat weight (Beef) and ATP measurement

②Quantification of the total protein content

Using the meat solution from the experiment in ① above, we attempted to determine the total protein content of each type of meat using the Bradford method, and were able to determine the total protein content of each meat sample. (Fig.6).

Note: The values of total protein in the various meat solutions obtained by this experiment are lower than the literature values for the protein content of various types of poultry meat. This may be due to the removal of meat residues during the preparation of the solutions. Since this experiment was conducted using solutions assumed to be representative of the residual meat residue present prior to equipment cleaning, we do not believe that such a difference is problematic.

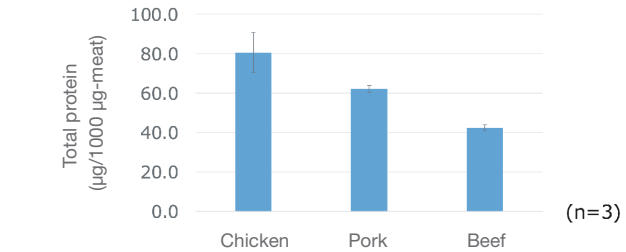

Figure 6. Determination of total protein content in meat (chicken, pork, beef)

③Correlation between the RLU values and total protein content

Based on the results, a correlation graph between RLU values and total protein content for each type of poultry meat was created. A proportional relationship was observed between ATP measurements and total protein content for both chicken, pork, and beef (Fig. 7).

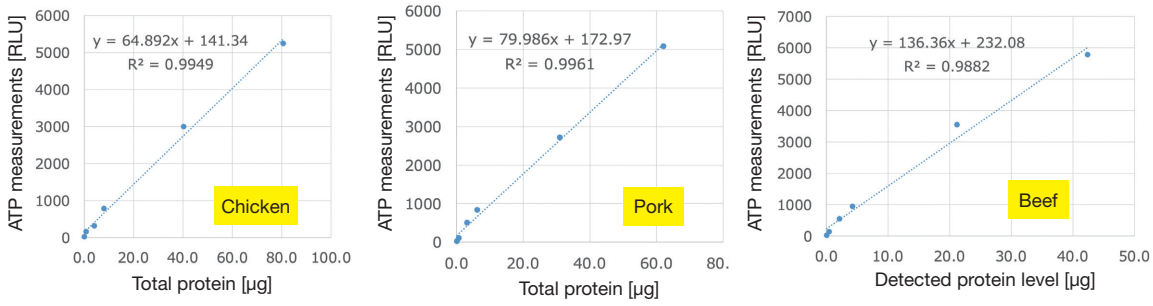

Figure 7. Correlation between ATP level and total protein level

④The adeadequacy of pass/ fail limit "500 RLU"

From the results, the detected protein level at 500 RLU was calculated to be 5.53µg for chicken, 4.09µg for pork, and 1.96 µg for beef for each meat species.

The recommended swab area for an ATP test is 10cm × 10cm , therefore, we measured the weight of each type of poultry meat in the sample in a 100cm² as the measurement area. The results were 21.03g for chicken, 20.72g for pork, and 24.31g for beef. (Table 3). Therefore, we calculated the "*heterologous meat protein contamination rate*" by dividing the amount of protein in each carcass by the weight of the meat, and found that the contamination rate was well below 1 ppm for all meat types (Table 3).

We were able to determine that a pass/fail limit of "500 RLU or less" is appropriate as an indicator of effective cleaning for preventing protein cross-contamination of meat. In the earlier section it was mentioned that "differences were observed and in the literature values of total protein content in various meat solutions and protein content of various types of poultry meat." Even when this variability was taken into consideration, it was confirmed that the "heterogeneous meat protein contamination rate" was less than 1ppm for all combinations of meat species.

Type of meat	**Detected protein level [µg]**	Livestock weight per S [g]	Contamination rate [ppm] (Protein/ chicken weight)	Contamination rate [ppm] (Protein/ chicken weight)	Contamination rate [ppm] (Protein/ chicken weight)
Chicken	**5.53**	21.03	-	Chicken protein contamination in pork **0.267**	Chicken protein contamination in beef **0.227**
Pork	**4.09**	20.72	Pork protein contamination in chicken **0.194**	-	Pork protein contamination in beef **0.168**
Beef	**1.96**	24.31	Beef protein contamination in chicken **0.093**	Beef protein contamination in pork **0.095**	-

Table 3. Percentage of heterologous meat protein contamination at ATP measurement = 500 RLU, S = 100cm².

⑤Summary and Discussion

Based on the above verification results, the ATP Test (Kikkoman A3) can be shown as an effective allergen cross-contamination control method using this indirect measurement of protein cross-contamination. Using a pass/ fail limit of "500 RLU or less per 10cm² sampling area equates to "less than 1 ppm of protein contamination for the meat species combinations tested. Using this data, we can conclude that if the production surfaces are cleaned such that the ATP Test (Kikkoman A3) is less than 500 RLU following cleaning the risk of protein cross-contamination sufficient to present a risk for food allergen contamination using the definitions in Japanese Law (and perhaps other jurisdictions), is extremely low and, therefore it is safe to consider that "*allergen labeling is not necessary*". Please note that the verification method and threshold values provided are for reference purposes only.

3. A Case Study of the use of the ATP Test (Kikkoman A3) by Kikkoman Corporation

The following is a case study for the use of this allergen control process by a raw meat supplier for the Kikkoman Food Products Company.

(1) Daily hygiene management by ATP Test (Kikkoman A3)

The company produces a variety of raw meats that are used as raw ingredients for various Kikkoman products. As a condition of being a supplier, Kikkoman Foods requests that their meat suppliers verify the cleanliness of their processing equipment after cleaning using the ATP Test (Kikkoman A3) applying a pass/ fail limit of "500 RLU or less."

If the value is 500 RLU or less, cleaning is considered to be verified. If the value exceeds 500 RLU, the production surface is re-cleaned. After re-cleaning, the ATP Test (Kikkoman A3) is performed again, and this process repeated until the value is 500 RLU or less. The inspection and recording of the results are done by the supplier itself.

This testing is conducted on a frequency of at least once per month. In determining the sampling frequency, the first criteria is "*Is the existing cleaning method appropriate for allergen control?*" This is determined by first conducting sampling at an accelerated sampling frequency for several weeks to confirm that the currently used cleaning methods are effective for allergen control. After this initial evaluation period, and once it has been confirmed that 500 RLU can be repeatedly achieved by the prescribed cleaning method, and no other evidence is found that cross-contamination has occurred (by testing raw materials/end products using ELISA) the test frequency is then set to once a month or a more appropriate frequency.[4]

(2) Verification Analysis Using the ELISA Method

In addition to the cleanliness verification using ATP Test (Kikkoman A3) mentioned above, independent meat species analysis using the ELISA method may also be conducted to confirm that cross-contamination from raw meat materials has not occurred.

(3) Summary

As described above, by combining "routine rapid tests" such as the ATP Test (Kikkoman A3) and "highly specific verification analysis" such as ELISA, we strive to thoroughly prevent allergen contamination from the raw material level and ensure product safety (Refer to Figure 8).

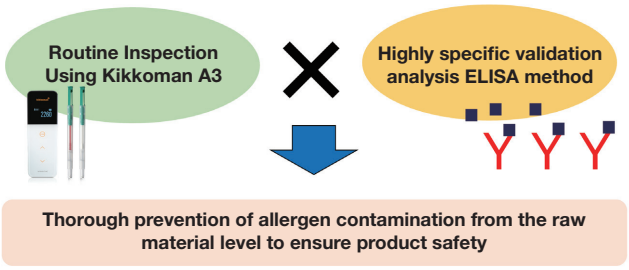

Figure 8. Kikkoman A3 for routine inspection and ELISA method for highly specific verification analysis

4. Conclusion

Although the ATP Test (Kikkoman A3) is not a method for direct detection of allergenic proteins, it can detect the presence of food residues with high sensitivity to quantify the effectiveness of cleaning practices. This makes it an excellent test method in terms of objectivity and simplicity. It is an effective daily management tool for preventing allergen cross-contamination, and further expansion of its use is expected in the future.

We hope that the ATP Test (Kikkoman A3) will help you to establish an effective production process - including an allergen control system - that can help you assure your food safety.

[4] Verification and analysis by ELISA - in addition to the "cleanliness confirmation by ATP Test (Kikkoman A3)" mentioned above, "voluntary analysis by ELISA" may be conducted on meat ingredients to confirm that no cross-contamination from the ingredients has occurred.

Kikkoman Biochemifa Company

2-1-1 Nishi-Shinbashi, Minato-ku,
Tokyo 105-0003, Japan

E-mail : biochemifa@mail.kikkoman.co.jp
URL : https://biochemifa.kikkoman.com/e/