An Example of the Adoption of Prompt, Simplified Product Inspections Using ATP Measurements

Aseptic Dairy Product Inspections Using the "CheckLite AT" Microbial Test Kit

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This article is a summary of a special talk delivered by Mr. Yoshitaka Haijima of Moriyama Milk Industry Co., Ltd. (headquarters: 9-32, Miyanomae, Hiratsuka City, Kanagawa Prefecture; President: Naoto Ohtsuka), to the 84th Lumitester Seminar held by Kikkoman Biochemifa Company on June 25 at the Tsukishima Hall of Social Education in Chuo Ward, Tokyo.

Business Summary of Moriyama Milk Industry Co., Ltd.

Founded in 1918, Moriyama Milk Industry Co., Ltd. celebrates its 95th anniversary this year as dairy products manufacturer. The first company in Japan to produce coffee milk, Moriyama improved the product to extend its shelf life and began to sell it via the shops operated by Tetsudo Kosaikai (predecessor to the "Kiosk" chain of shops found on the premises of Japan Railways train stations). Since those days, we have manufactured numerous aseptic products.

Moriyama currently operates two factories—Hiratsuka plant located in Kanagawa Prefecture and Kuzumaki plant in Iwate Prefecture. The aseptic products is the main product produced at the Hiratsuka plant including "Soft Mix" (liquid packages of soft-serve ice cream), single-serve milk portions, and soft drinks such as cocoa beverages, as well as beverages sold in cup-style packaging (including dairy-based beverages). There also have been increasing production of liquid foods (fluid types, dessert types) that are supplied to hospitals and similar facilities. Aseptic desserts (almond jelly, panna cotta, others), cream for coffee, bag-in-box packaged (10-liter containers) whipped cream, milk shakes, butter, gum syrup, and canned foods (fresh cream) are the main products produced in Kuzumaki plant.

Photo 1: A selection of Moriyama products (products in cup-style packaging).

Photo 1 shows some of our own brands (products in cup-style packaging). Aside from our own branded products, Moriyama also does consignment manufacturing of brands from other major companies.

Using ATP Measurements to Inspect Aseptic Products

I would now like to introduce you to some case examples of product tests utilizing the ATP tests that our company has adopted.

First of all, food residues left behind due to insufficient washing have been spotlighted as one source of food-related incidents caused by microbes in food-manufacturing facilities and similar sites. The ATP swab test uses as indicators of the amounts of adenosine triphosphate (ATP) found in food contaminants (food residue) and of the adenosine monophosphate (AMP) that is one of ATP's degraded products. The test is used to ascertain the cleanliness of food manufacturing environments and is employed by many food companies for the means of checking how clean equipment and utensils are after they have been washed or how clean employee hands and fingers are after hand washing.

As Figure 1 shows, food residue includes both food itself and the microbes (germs) that adhere to the food. For that reason, when you measure how much ATP and AMP are present in food residue, you are testing for both the amount of ATP and AMP in the food itself as well as the amounts in the microbes adhering to the food.

As I mentioned earlier, our company mainly manufactures aseptic products. Accordingly, we use testing methods that is capable to check whether there

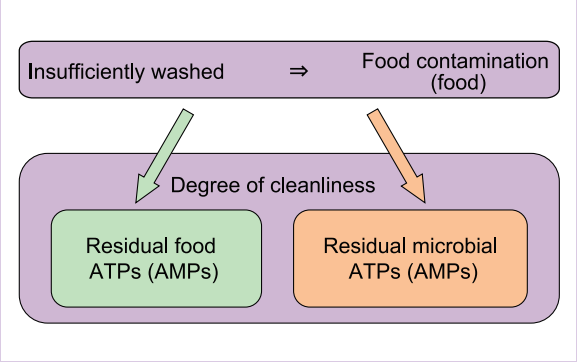


Figure 1: The ATP in food residue includes both ATP that comes from the food itself as well as ATP that comes from the microbes that adhere to the food.

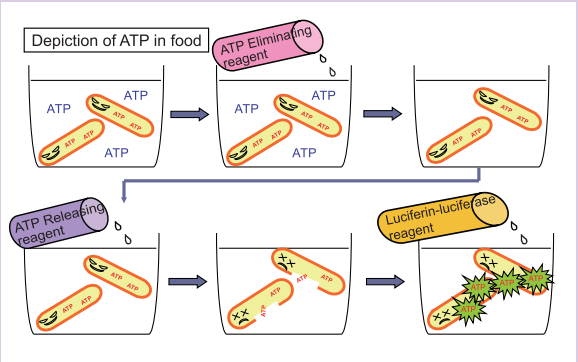


Figure 2: Illustration of test procedures using the CheckLite AT kit for measuring ATP levels.

is any microbial contamination in our products by measuring the volume of ATP they contain. Today I would like to discuss the actual testing cases which the dairy product conducted at Moriyama, and possibly explain that this method can be applied for testing in other types of business.

Correlation between ATP measurements (measured light output) and microbial count measurements attendant with culturing

First, I would like to explain the basic principles of the ATP measurement approach. Kikkoman Biochemifa Company currently sells an inspection kit called CheckLite AT (Photo 2, the AT stands for "Aseptic Test"). This kit was developed as a joint research project between Moriyama and a unit of Kikkoman Biochemifa Company (at that time Kikkoman Corporation). The test reagents include a component that eliminates the ATP that originates in food or food products and another component that detects the ATP that originates in the microbes or germs.

Figure 2 presents an illustration of testing procedures using CheckLite AT. Generally, food products contain 2 kinds of ATP. First is the ATP that is present in the food itself and the second is the ATP that is present in microbes (please refer to illustration in the upper left of Figure 2). As shown in the upper right of Figure 2, processing them with a component that eliminates the ATP originating from the food results, resulting it with no ATP from the food present. Next, please refer to the lower left of Figure 2. Processing them with the component that extracts the ATP breaks down part of the cell walls of the microbes. The ATP in the microbes is then extracted and released outside of the cell walls (lower center of Figure 2). Finally, they are processed with a luminescent reagent so that we can measure the amount of ATP that originates with the microbes (lower right of Figure 2). The amount of ATP (light output) will be low if there are no microbes present. This is the principle used in our company when we check to make sure that no microbes remain in our aseptic products.

Photo 2: The CheckLite AT microbial biomass test kit, which measures ATP levels.

As for the bacteriological examinations, we used to conduct examinations using the culturing approach. In relation to this, we looked at the correlation between the ATP measurements and those examinations using the method shown in Figure 3 to examine whether the ATP measurements used with CheckLite AT could be used interchangeably with the conventional bacteriological examinations. The testing to examine the correlation was conducted in following procedures. First, we began by injecting bacteria into our aseptic products. After that, we then conducted a pre-incubation procedure (done for 72 hours at 37° C in our verification experiment). We then serially diluted the test specimen and compare the bacterial counts when cultured using the pour culture method with the

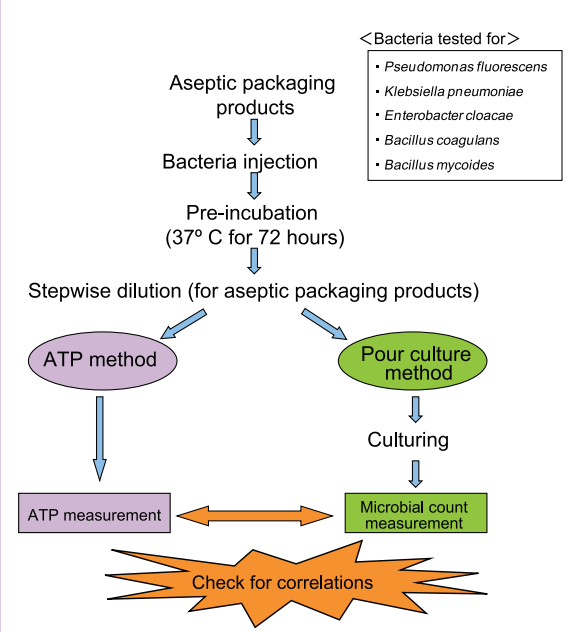


Figure 3: Identifying the correlation between the ATP measurement method (measured light output) and microbial count measurements performed in bacteriological examinations

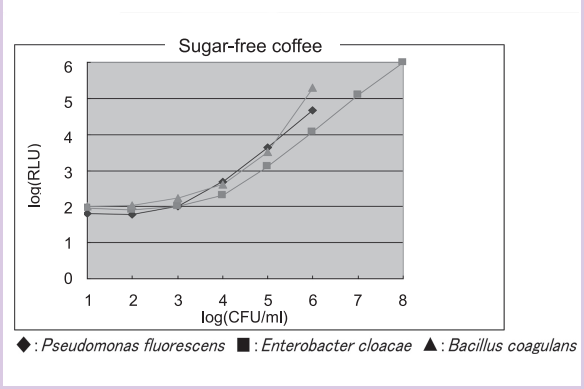
amount of ATP measured using the ATP test method. These results are shown in Figure 4.

In the figure 4, *Pseudomanas fluorescens*, *Enterobacter cloacae*, and *Bacillus coagulans* were cultured in aseptic coffee, and *P. fluorescens, Klebsiella pneumoniae*, and *B. mycoides* were cultured in cream, single-serve cream portions, and cocoa (note: in this article, while we present examples with the five types of bacteria mentioned above as test specimens, we usually check into correlations with a variety of other germs as well). From this, we can say that there is a correlation between the ATP measurement readings (light units) and the bacterial counts on just about any product.

Presently, there are a variety of simple and speedy test kits sold on the market. When a company adopts one, there is a need of establishing standards for the test that are appropriate to that company. However, it is necessary to collect and analyze data on a considerable number of specimens and do repeated testing in order to complete establishing the standards. We at Moriyama had to do tests on quite a few specimens before we obtained the test results you see in Figure 4. It was through that process that we established our current testing method of Moriyama Milk Industry. The testing process required considerable time and effort, but now we have also gained the benefit of having adopted tests that are swift and convenient (I will go on to explain the benefits of measuring ATPs in the next section).

Benefits from Adopting the ATP Measurement Method

What, then, are the benefits from using ATP measurements as alternative method to doing bacteriological examinations?



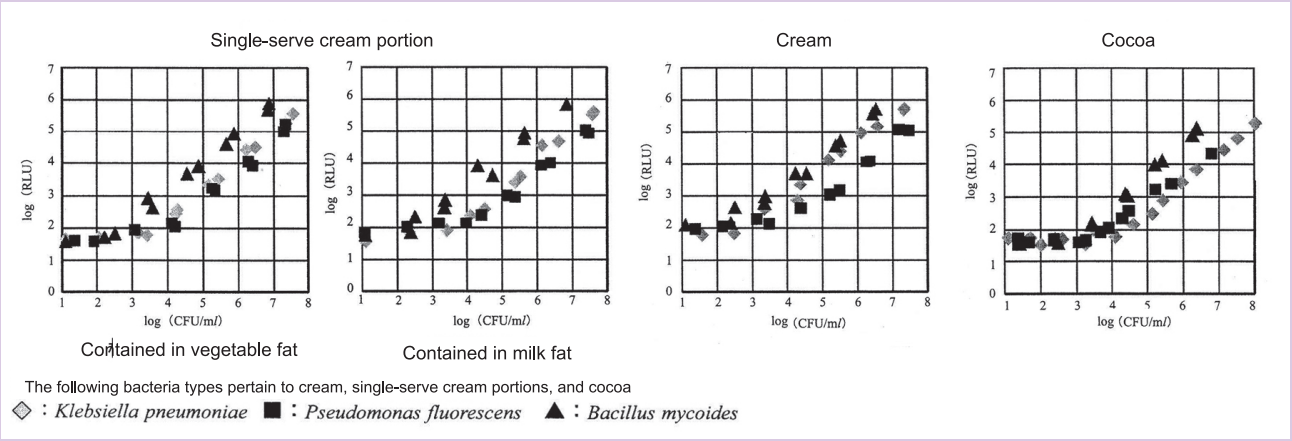


Figure 4: Correlation between ATP measurements (light output) and microbial counts

Benefit 1: Shortening of the time need for testing

Firstly, the first benefit is connected to shortening the time needed for testing (the number of days and culturing temperatures shown are nothing more than one example from the testing methods used at Moriyama) as shown in Figure 5. The products that we handle are processed to make them germ-free. Any germs that may remain behind in our products are damaged. Accordingly, we need to perform pre-incubation in order to resuscitate germs. This is a job that needs to be done whether conducting bacteriological examinations or measuring ATPs, and as shown in Figure 5 a three-day static culture period is required after the date of manufacture. Because dairy products contain abundant amounts of nutrients, if they settle for three days at a temperature (37° C) suitable for culturing the germs can revive and propagate.

Next, we start the testing on the fourth day for bacteriological examinations, using the pour culture method and the pour plates are subjected to keeping incubation on the following day. Accordingly, it takes six days to give a passing or failing grade. On the other hand, when using ATP measurements after pre-incubation is finished we can conduct the test right away and get the results back about one hour later. In short, the time needed before making a pass-fail assessment can be shortened by two days. Moreover, using ATP measurements doesn't require aseptic techniques for bacteriological examinations and the expense for setting up a clean room, which is required in conventional examination. Also, the operation is simple compared to a bacteriological examination and can be done by anyone.

Benefit 2: Reduce in Time and Costs Associated with Tests

Furthermore, since the ATP test can reduce the time needed prior to shipment you can also expect the advantage of having reduced warehouse expenditures due to cutting down on inventory. What's more, since the work hours required for preparing to do measurements and cleanup is greatly shortened for ATP measurements compared to conventional method. This, in fact, leads to a reduction in labor costs as well.

Let me give you an example of our single-serve cream portions to show how much testing costs can be reduced. The filling machines used for single-serve cream portions have 70 nozzles, so 70 portions of cream can be made in a single filling procedure. To note, different companies has different approaches when they schedule product inspections in their manufacturing plans. Some will test at the start of filling, some during, and some after. Others might take samples once an hour, while still others might do intensive testing at the outset. At Moriyama, we test samples at the start of filling to make sure that there is no product residue leftover following the clean-in-place (CIP) procedure performed the day before and that sterilization has been adequately performed.

So, when using the pour culture method we conduct tests on 70 shots from the nozzles. To calculate the labor costs bacteriological examination requires, let's do a rough estimate of the work hours that testing takes. For instance, if we assume the dispensing operation takes 50 minutes, the sensory test takes 10, washing up takes 30, getting visual observation of petri dish and findings takes 20, prepa-

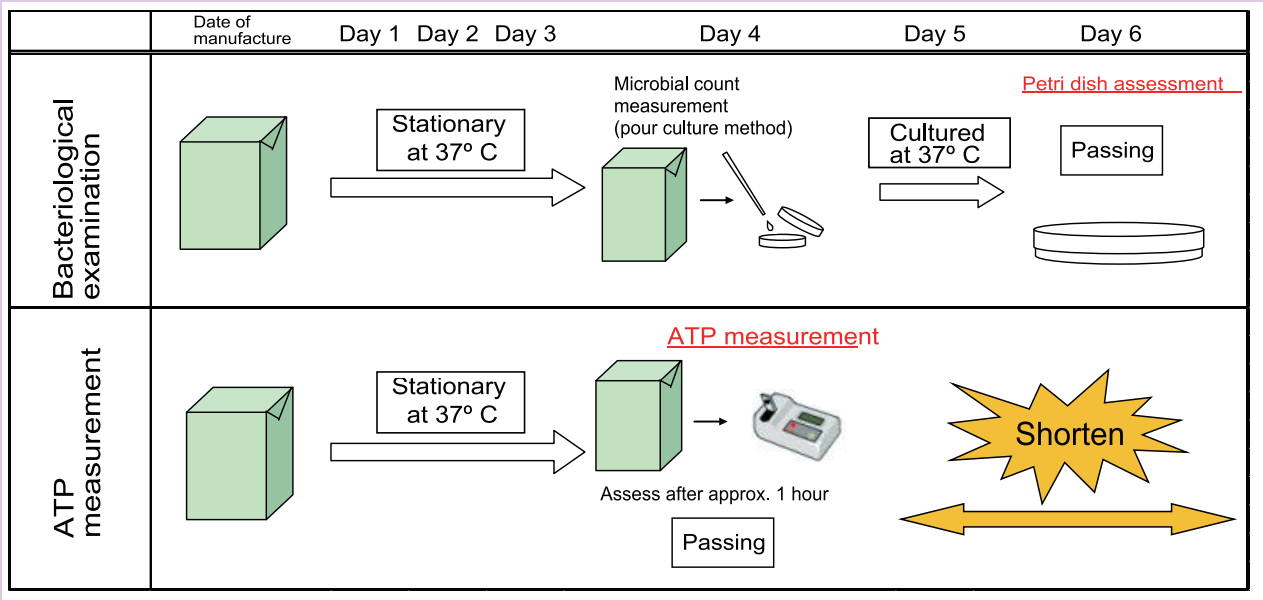


Figure 5: Comparison of the testing times needed for ATP measurements and bacteriological examinations (examples from Moriyama Milk)

ration of the culture madium takes 25, the time needed for the work totals 135 minutes (2.25 hours). Assuming labor costs to be 2,000 yen per hour, the cost required will be 4,500 yen (2,000 yen x 2.25 hours). Turning to testing costs, assuming 50 yen per test specimen (which includes the culture medium, peptone water, and sterile petri dishes and pipettes), and the total here is 3,500 yen (50 yen x 70 specimens). In sum, doing the test one time costs 8,000 yen (4,500 yen plus 3,500 yen).

On the contrary, at Moriyama, we put 35 shots into one beaker and treat that as a single test specimen (in brief, this means 70 shots can be divided up into two test specimens) with the ATP method. Just as we did before, let's do a rough estimate of the work hours required for testing in order to calculate the labor costs that doing ATP measurements requires. Assuming, for example, that lining up the portions takes 6 minutes (lining up 70 shots on a tray), the sensory test takes 10, prep work takes 8 (transferring the portions into 35-shot beakers), doing the measurements takes 8, washing up takes 3, and preparing the pipette tip takes 10, we get a total of 45 minutes (0.75 hours). Providing that labor costs once again are 2,000 yen per hour, the total here is reduced to 1,500 yen (2,000 yen x 0.75 hours). With respect to testing costs, assuming these to be 480 yen per test specimen (including the LumiTube, pipette tips, and reagents), these are reduced to 960 yen (480 yen x 2 test specimens). In sum, the costs required for doing one test fall to 2,460 yen (1,500 yen plus 960 yen). Moreover, since no culturing is done, equipment like incubator is unnecessary so we can save there, too.

As discussed above, we have realized that adopting the ATP measurement approach is connected to cutting down on testing costs compared to bacteriological examinations.

Benefit 3: Faulty products can be found more quickly

There is also a benefit that is difficult to clearly state in terms of cost being able to reduce the time required to make a decision about shipping. The benefit is that it is quicker to discover abnormalities in the products using ATP measurements (as opposed to the pour culture method). As stated in the Figure 5, two days at minimum are required after testing has begun to discover abnormalities using pour culture method. Because of this, the compenstation issue arises of products that have been manufacturing during those two days. What if, for example, the test results show that there is an abnormality in the products and the root cause of that abnormality is thought to be microbial contamination along the production line? Depending on the situation, the decision may be made to dispose the products that were manufactured during that two-day period. However, thecompensation issue and losing two days of production can be avoided if we do ATP measurements, which can provide us with results on the day of the test. Also, if abnormalities are discovered at an early stage then it is also possible to respond more quickly in searching the root cause.

Furthermore, as for the aseptic products, there are some cases bacteria are still growing while they are in a state when there are no pH fluctuations and a sensory test shows no problems. Abnormalities cannot be found during handling of the pour culture method on a test specimen in this condition (because neither a visual inspection nor a sensory test reveals any differences from a normal product). However, using ATP measurements any abnormalities can be found promptly since the readings can be obtained that same day.

Issues with Product Inspections Using ATP Measurements

There are many benefits to be obtained as the foregoing shows, but there also are downsides to ATP measurements as well.

For example, with products that contain maccha (a type of Japanese green tea) powder or black tea (plain teas, milk teas with a large tea leaf component, etc.) the readings will sometimes jump since the ingredients in maccha and black tea may have some effects on measurements. There are some cases results of the ATP measurements of specimens differ greatly from the pour culture method. Some show the value that greatly differs from the pour culture method where the result was sterile. Therefore, doing such measurements on such products is considered difficult. However, ATP testing can be used without any particular problem on items other than those with maccha or black tea, so at this point we do our testing of such products using the pour culture method.

Furthermore, since ATP measurements can be taken easily, there is a tendency to want to increase the number of specimens used regardless. However, perhaps because the unit costs of the reagents are high one gets the sense that if the number of test samples is increased the apparent test costs will correspondingly increase. Of course, one cannot say without reservation that this is a downside. I think of tests as being the same thing as insurance. In the case of insurance, if you buy a costly policy the guarantee you have gets that much bigger. Food inspections are the same. If you increase the sample size then the guarantee of safety gets that much greater.

However, your costs will rise by the increased sample size. The question of how frequently to conduct tests is something that every company worries about.

However, at Moriyama we don't look at tests as being something that costs a lot of money. We do our testing with the attitude of wanting to guarantee process controls through testing as much as possible rather than having to spend a lot of money to deal with some problem that has come up.

Conclusion: Preventing Risks from Food Incidents in Advance through Voluntary Testing

The products we handle at Moriyama are aseptic products. Accordingly, microbial counts are irrelevant. What we want to determine is whether germs are present or not. That is why we have implemented product inspections that use the CheckLite AT ATP measurement kit. This testing method makes it possible to eliminate the ATP that originates in foods while drawing out and measuring only that which originates with microbes. For that reason, when microbes are left behind in one of our products, those products are abundant in nutrients that will make it easy for germs to grow. Accordingly, we increase the microbial count through propagation by pre-incubation and as a result the ATP readings will jump.

Incidentally, in the case of beverage products with headspace, when you pre-incubate using a shake culture the microbes can readily make use of the dissolved oxygen in the product. This effect makes it possible to shorten the time required for pre-incubation.

At Moriyama, we believe that doing ATP measurements using CheckLite AT is quite effective as a method for replacing bacteriological examinations to determine whether a product can be shipped or not. However, this is not to say that we can decide on our own what product inspection method to use when doing consignment manufacturing. There are the opinions of the brand owner to take into account, and furthermore there is no guarantee that all brand owners will show us they understand about product testing using ATP measurements.

Currently, a considerable track record has been built up at food companies and public health centers for the method of using ATP readings from swab tests, and I believe its use is spreading widely. At Moriyama, we do ATP swab tests using the Lumitester PD-20 (see photo 2) as our method for checking cleanliness levels after washing. The ATP swab test can provide us with measurements in the space of 10 seconds to give a passing or failing grade. When the readings shown exceed the reference value, we can show the results to the operator on site, explain them, and action can be taken to make improvements. We think this makes it an extremely effective testing method.

However, I believe that applying ATP measurements to product inspections as discussed in this article has yet to penetrate fully as a testing method. Having said that, the responsibility for guaranteeing product safety ultimately rest on the shoulders of the manufacturers themselves. In that sense, we have to think about how quickly they can detect or how they might forestall the possibility of problems or incidents occurring. I believe that doing swab tests based on the Lumitester or on product inspections done using the CheckLite AT is effective as one measure for handling this.

I hope that the data we have collected as shown in Figure 4 will be useful for other food companies, and believe it would beneficial to collect data from other, similar verification tests.

Photo 2: Lumitester PD-20 ATP swab test kit

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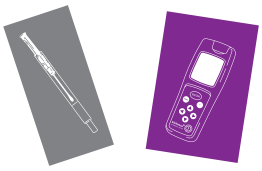
Website: http://www.fujimilk.co.jp



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