ROI CASE STUDY

Microbiological Monitoring of Paints Using 2nd Generation ATP

Tool: LuminUltra’s 2nd Generation ATP® - QG21S
Savings: $5,000 for preventing the loss of one batch of basic paint
**Problem:** Microbiological contamination of paints can cause major product defects, potentially leading to costly recalls and damage to the manufacturer’s reputation.

**Facility:** A paint manufacturer that produces more than 10 types of paint products for industrial and residential uses.

**Investment:** LuminUltra’s QuenchGone21™ Specialty (QG21S) test kit for measuring adenosine triphosphate (ATP).

**Economic Analysis:** Prior to the implementation of ATP testing, operators relied on plate tests to monitor the finished product for microbial contamination. This testing required up to two days incubation time and could not quantify the total microbial population. LuminUltra’s 2nd Generation ATP test kits provide rapid results and a measurement of total microbial content.

Ten paint samples were submitted for validation testing. Plate tests, performed by the manufacturer, indicated that three samples were contaminated, as visible colonies had formed on the plates. ATP measurements supported these results, as concentrations in each sample exceeded the preventive action limit of 100 pg/mL. The two methods also provided consistent conclusions for samples with low ATP concentrations. There was, however, one sample with a high ATP concentration that did not produce visible colonies during the plate test. This is because the plate tests only support the growth of specific microorganisms and are not capable of indicating total microbiological content. This means that microorganisms, which could contaminate and degrade the product, could go undetected by the manufacturer’s culture-based tests.

The manufacturer produces paint in large batches of up to 10 tons. Actual production costs were not provided by the manufacturer, but the value of a single batch is expected to be significant, particularly for some premium products. Assuming an average cost of $10 per gallon, which is a relatively conservative price for a basic paint, this would mean that a single spoiled batch would be worth up to $30,000. Annual ATP testing costs were anticipated to be approximately $25,000, so the prevention of only one spoiled batch would recover the testing costs and save the manufacturer an additional $5,000. Savings would be even greater for a premium product, which could be worth several times more than a basic product.

**Synopsis:** LuminUltra completed validation testing of the QG21S test kit using several paint products. The testing demonstrated that the kit was resistant to interferences, capable of producing repeatable results, and could accurately detect changes over a wide range of concentrations. ATP testing also provided a better indication of total microbiological content, as it was able to detect elevated ATP concentrations in samples that did not produce growth during plate tests. The full case study follows.
Case Study:
Microbiological Monitoring of Paints Using 2nd Generation ATP

A paint manufacturer produces a wide range of paint products for industrial, commercial, and residential applications. The products are manufactured in large batches of up to 10 tons. Before each batch is packaged and shipped, a number of tests are performed to ensure that the product meets strict quality control requirements. The testing program includes the use of culture-based plate tests to evaluate microbiological quality. Microbiological monitoring is an important component of the paint manufacturing quality control process, as microbiological contamination can cause a variety of serious product defects, which can lead to costly recalls and cause damage to the manufacturer’s reputation.

Microbiological growth at this facility is controlled by adding biocide in the initial mixing stage. The final product is tested using culture-based plate tests, and if growth is observed, additional biocide is added during the final let-down stage. While plate tests may provide helpful information about whether specific microorganisms might be present, they do not measure total microbiological content, as they are typically only capable of measuring a small fraction (typically less than 1%) of the total microbiological community. This is often because of selection pressures that are generated by the artificial growth environment. In this case, the medium only supports the growth of Pseudomonas. A number of other fungi and bacteria are capable of growing in water-based paints, so the existing test only provides a very limited assessment of microbial growth in the final product. The tests also require 48 hour incubation times, so each batch must be held in large storage tanks while waiting for results. Because of these limitations, the manufacturer contacted LuminUltra to evaluate the use of 2nd Generation adenosine triphosphate (ATP) testing for microbial monitoring of the final product. The results of this validation testing are summarized in the case study below.

Background

ATP is a molecule that is present in all living cells, so its concentration in a sample can provide a direct indication of its total microbial content. ATP testing offers several advantages over culture-based methods. It can be performed in only a few minutes, allowing operations staff to make immediate corrective actions at the time of sample collection. Unlike culture-based tests, ATP testing quantifies total microbial content, including both culturable and non-culturable cells.

In the paint manufacturing process, ATP monitoring can be used to test the process water, raw materials, tank and piping surfaces, and the finished product. For this application, the manufacturer was interested in monitoring the final product, so LuminUltra’s QuenchGone21™ Specialty (QG21S) test kit was selected. This 2nd Generation ATP test kit was specifically developed for complex chemical products, such as slurries, adhesives, paints and other coatings. Validation testing was conducted to demonstrate that the test kit could be used effectively for the manufacturer’s wide range of products.
The validation testing included repeatability, linearity, and inhibition testing. Repeatability testing is used to assess the precision of the test kit for a given sample. Linearity testing assesses the test’s response to changing microbial concentrations. Inhibition testing is used to determine if components in the sample interfere with the ATP-Luciferase assay. LuminUltra’s 2nd Generation test kits have been optimized to mitigate sources of inhibition as much as possible, but in some rare instances, inhibition may still occur. If inhibition occurs, then samples may require pre-dilution before processing according to the test kit instructions.

Methodology

Ten samples, representing seven different product types, were submitted to LuminUltra for analysis. Inhibition testing was performed with four paint products, repeatability testing was conducted with three products, and linearity testing was performed with two products.

Inhibition tests were performed by processing samples according to the standard QG21S test protocol and then performing two-fold serial dilutions on the final processed ATP solutions (i.e. UltraLute/Resin Tube for total ATP). A minimum of four serial dilutions were performed to obtain readings at five concentrations (undiluted, 1/2x, 1/4x, 1/8x, and 1/16x). The change in light output is monitored between each dilution. If the change in light output is not proportional to the change in the concentration, it means that inhibition is occurring. If the change in light output is proportional to the change in concentration, then inhibition is not occurring.

Repeatability tests were performed by testing each product in triplicate for total and dissolved ATP. Repeatability testing results were assessed by calculating the Coefficient of Variability (CV) and comparing it with the interpretation values listed in Table 1. Linearity testing was performed by spiking sub-samples of a given product type with different concentrations of ATP standard. The results were plotted, and the coefficient of determination was compared with the interpretation values listed in Table 1. Routine ATP tests were then performed on several samples to compare ATP results with the results of the plate test that were performed by the paint manufacturer.

<table>
<thead>
<tr>
<th>Performance</th>
<th>Repeatability</th>
<th>Linearity</th>
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<tr>
<td></td>
<td>Coefficient of Variability (%)</td>
<td>Coefficient of Determination (R²)</td>
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<td>&lt; 10%</td>
<td>&gt; 0.95</td>
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Results

The validation work was performed with several paint products. For demonstration purposes, validation results for one particular product, referred to as Product A, are shown in all tables and figures in this case study. Generally, the results were consistent for all products, but unique observations are highlighted in the text below. All results are shown for total ATP tests.
Inhibition and repeatability results for Product A are presented in Table 1. With each two-fold dilution, the light output from the sample decreased by approximately 50%, indicating that the product did not interfere with the enzyme or impede light output. This means that this product would not need to be pre-diluted before being processed according to the QG21S test kit method. A similar result was observed for all four products that were tested by LuminUltra. The test kit also produced excellent repeatability, with very consistent measurements that produced a low coefficient of variability.

Linearity test results are shown in Figure 1. As noted above, linearity testing is evaluated based on the coefficient of determination, where a value greater than 0.95 represents excellent response to changing concentrations. The QG21S test kit produced an excellent response to changing concentrations for both products that were tested by LuminUltra.

Routine ATP tests were also performed to help establish a baseline and compare the ATP concentrations with plate test data. These results are presented in Figure 2. The figure also indicates typical levels associated with good control, preventive action, and corrective action, using the green, yellow, and red backgrounds. These control levels are provided in the interpretation guidelines in the QG21S test kit instructions. Samples that produced growth during plate tests (performed by manufacturer) are also highlighted with red boxes around the respective data point.

The manufacturer’s plate tests detected growth in ‘Product A’, ‘Product A (inoculated)’, and ‘Product B’. ATP measurements supported these results, as ATP concentrations in these samples exceeded the preventive action limit of 100 pg/mL. This demonstrated that ATP testing provided an excellent indicator of potential risk. The two test methods also produced consistent results for Products C and E, where growth was not detected on the plates and the ATP concentrations were low. There was, however, disagreement between the two test methods for Product D, as the ATP concentration exceeded the preventive action limit, but growth was not detected by the plate test. This is because the plate tests only support the growth of specific microorganisms. The plate test will not detect other microbes that are present, which presents a risk of contaminating and degrading the product. It is also worth noting that several samples tested by the manufacturer contained elevated ATP concentrations without showing any growth on the plate tests, meaning our validation result was not an anomaly.
One item worth noting is that all results are presented as total ATP and not cellular ATP. With new users, we typically recommend testing for cellular ATP, because it specifically measures the ATP contained within living cells. However, in this case, it was found that total ATP was a more practical measurement. First, inhibition testing demonstrated that some products required pre-dilution in order to measure dissolved ATP. The manufacturer wanted to use a common pre-dilution factor for all products, so this meant that a relatively high pre-dilution factor would need to be used for all tests, even though it was only specifically required for a few products for dissolved ATP. Unfortunately, our testing demonstrated that a higher pre-dilution factor was associated with inferior repeatability, which was attributed to the high viscosity of the products. Greater pre-dilution also reduces the overall sensitivity of the method. There was also a consistent relationship between plate test growth and high total ATP concentrations. Therefore, we recommended the QG21St test kit for measuring total ATP. As an added benefit, the cost per test for total ATP is lower than for cellular ATP, as it does not require the measurement of dissolved ATP for each sample.

Economic Analysis

LuminUltra’s 2nd Generation ATP testing provided several advantages over the existing plate tests used by the manufacturer. First, ATP test results are available within only a few minutes, so monitoring, corrective actions, and follow-up testing can all be implemented immediately, rather than days after collecting the sample. Also, the plate tests cannot quantify a significant proportion of the total population, meaning the existing methods may not detect all instances of microbial contamination. High ATP concentrations were measured in several samples that did not produce any growth during plate tests. Without additional treatment, there is a risk that microbial contamination could degrade these products, requiring the manufacturer to issue a costly recall. While actual costs were not provided by the manufacturer, the paint is produced in large quantities, so the value of a single batch is expected to be significant. A relatively conservative cost estimate for a typical paint is $10/gal, which would mean that a single 10 t batch could have a value of up to $30,000. The annual cost for ATP test kits was anticipated to be approximately $25,000 per year. Therefore, by preventing the loss of one batch, the manufacturer would recover its annual costs for ATP testing and save an additional $5000. Given that premium paint products have a substantially higher value, the savings could actually be several times greater. Moreover, ATP testing helps to avoid the major headaches associated with recalls, including potential disputes with customers, damage to the manufacturer’s reputation, and costly disposal of spoiled product.

*Costs were estimated by assuming a production cost of $10/gal, batch size of 10 t, specific gravity of 1.2, and that the manufacturer would perform five ATP tests per day while operating five days per week.