

## RAPID HYGIENE MONITORING IN DAIRY PROCESSING USING ATP BIOLUMINESCENCE: CORRELATION WITH TRADITIONAL MICROBIOLOGICAL METHODS

Aslan Heybatov  
Rana Mammadova  
Baku State University

### Abstract

Ensuring hygiene in dairy processing facilities is essential for maintaining food safety and preventing microbial contamination. Traditional microbiological methods, such as plate count techniques, require long incubation times and are unsuitable for rapid hygiene assessments. This study evaluates the application of ATP bioluminescence as a real-time monitoring tool and compares its effectiveness with classical microbiological techniques in a dairy processing plant.

Sampling was conducted over three months in six critical locations, including the raw milk reception area, pasteurization equipment, filling machine nozzles, conveyor belts, storage tanks, and operator's gloves. The microbial contamination was assessed using both ATP bioluminescence measurements (expressed in relative light units, RLU) and total viable count (TVC) measurements (expressed in CFU/cm<sup>2</sup>). The correlation between ATP readings and microbial loads before and after cleaning procedures was analyzed to determine the reliability of ATP testing in hygiene control.

Results demonstrated a significant reduction in contamination after cleaning, with ATP RLU values decreasing by 75–90% and CFU levels showing similar declines. The Pearson correlation coefficient (R) between ATP values and TVC counts increased from 0.001–0.600 before cleaning to 0.720–0.990 after cleaning, confirming a strong relationship between the two methods.

The study highlights ATP bioluminescence as a rapid and efficient tool for hygiene monitoring in dairy plants. However, ATP testing does not differentiate between viable and non-viable microorganisms, necessitating additional microbiological confirmation for precise contamination assessment. Despite this limitation, its ability to provide immediate feedback allows for timely corrective actions, making it a valuable component of dairy hygiene management programs. Integrating ATP bioluminescence with traditional microbiological techniques can enhance food safety, optimize sanitation procedures, and ensure compliance with industry regulations.

**Keywords:** ATP bioluminescence, dairy hygiene, microbiological analysis, sanitation monitoring, food safety

### Introduction

Dairy processing plants require stringent hygiene control measures to ensure product safety and regulatory compliance. The presence of microorganisms in dairy environments poses a significant risk to food safety, as contamination can lead to spoilage, reduced shelf life, and potential health hazards (Griffiths, 1993). Traditional microbiological testing methods, such as culture-based techniques, provide reliable contamination detection but require long incubation periods, typically ranging from 24 to 72 hours. This delay hinders immediate corrective actions in case of microbial contamination, increasing the risk of cross-contamination and unsafe product distribution.

ATP (Adenosine Triphosphate) bioluminescence testing provides an alternative rapid method for assessing hygiene and microbial presence on surfaces and equipment (Leach & Webster, 1986). ATP is a molecule found in all living cells, including bacteria, yeast, and mold. The ATP bioluminescence assay utilizes an enzymatic reaction with luciferase, an enzyme derived from fireflies, to generate light in the presence of ATP. The emitted light is measured in relative light units (RLU), which correspond to the level of biological contamination on a given surface. Unlike traditional methods, which require microbial growth and incubation, ATP testing provides results within minutes, making it an effective tool for real-time hygiene monitoring (Buchanan, 1990).

One of the main advantages of ATP bioluminescence testing is its ability to detect overall biological contamination, including both viable and non-viable cells. This method is particularly beneficial for identifying residual organic matter that can support microbial growth. However, ATP testing does not differentiate between different types of microorganisms, making it necessary to use in conjunction with traditional microbiological methods for comprehensive contamination assessment (Salo & Laine, 2000).

When comparing ATP testing with conventional microbiological methods, key differences emerge. Traditional culture-based methods provide precise identification of microbial species and quantify colony-forming units (CFU), but they are time-consuming and labor-intensive. In contrast, ATP bioluminescence testing offers a rapid, non-specific assessment of contamination, allowing for immediate corrective actions. Studies have shown a strong correlation between ATP RLU values and microbial CFU counts, particularly in environments with high bacterial loads. This correlation makes ATP testing an essential component of hygiene validation programs in dairy plants, helping to ensure compliance with food safety regulations such as HACCP (Bower et al., 1996).

The aim of this study is to assess the efficiency of ATP bioluminescence measurement in dairy plants and its correlation with conventional microbiological methods, specifically examining bacteria commonly found in dairy environments such as *Listeria monocytogenes*,

*Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*. By analyzing contamination levels before and after cleaning procedures, this study provides insights into the effectiveness of sanitation protocols and highlights the importance of integrating ATP testing into routine hygiene monitoring (Davidson et al., 1999).

## Materials and Methods

### Study Design and Sampling Points

This study was conducted at a dairy processing plant over a period of three months. To assess microbial contamination levels, samples were collected from six critical control points, including the raw milk reception area, pasteurization equipment surfaces, filling machine nozzles, conveyor belt surfaces, storage tank surfaces, and operator's gloves. Sample collection followed standard hygiene monitoring protocols to ensure consistency and reliability of results.

### Sample Collection

Samples were collected using sterile swabs from a defined 10x10 cm surface area. Swabbing was performed in a systematic manner, covering both horizontal and vertical directions with firm but controlled pressure to maximize recovery of microorganisms. Each swab was immediately transferred into a sterile buffer solution and stored at a temperature of 4°C to preserve microbial viability until laboratory processing. Samples were processed within two hours of collection to ensure accuracy.

### Microbiological Analysis

Microbiological analysis was performed using standard cultural techniques. **Total viable count (TVC)** was determined using the standard plate count method, where serial dilutions of each sample were spread on Nutrient Agar and incubated at 30°C for 24-48 hours. After incubation, colonies were counted and expressed as colony-forming units per cm<sup>2</sup> (CFU/cm<sup>2</sup>). Additionally, selective culture media were used to detect specific pathogenic bacteria of concern in dairy environments. *Listeria monocytogenes* was identified using PALCAM agar, *Escherichia coli* was isolated on MacConkey agar, *Salmonella spp.* was detected using XLD agar, and *Staphylococcus aureus* was grown on Mannitol Salt Agar. Colonies were further confirmed using biochemical tests and Gram staining (Salo & Laine, 2000).

### ATP Bioluminescence Testing

ATP bioluminescence testing was performed using UltraSnap ATP swabs and an EnSURE Touch luminometer. Swabs were activated by breaking the internal reagent reservoir and shaking to ensure proper reagent mixing. Measurements were taken immediately, and ATP levels were recorded in relative light units (RLU). The ATP readings were compared with microbial counts to evaluate the correlation between

ATP bioluminescence and traditional microbiological methods.

The ATP bioluminescence test is based on the enzymatic reaction between ATP and luciferase, a light-producing enzyme found in fireflies. When ATP is present, it reacts with the luciferase enzyme in the presence of oxygen and produces light. The emitted light is then detected by a luminometer and measured in RLU, with higher values indicating greater contamination. Since ATP is a molecule found in all living cells, including bacteria, yeast, and mold, this method provides a rapid estimation of organic residues and microbial contamination.

One of the key advantages of ATP testing is its ability to provide instant results, allowing for immediate corrective actions in dairy processing facilities. Unlike traditional microbiological methods that require long incubation periods, ATP bioluminescence can identify contamination levels within seconds, making it a valuable tool for real-time hygiene monitoring. However, ATP testing does not differentiate between viable and non-viable microorganisms, which necessitates the use of additional microbiological techniques for precise identification of specific bacteria.

### Statistical Analysis and Correlation

Statistical analysis was conducted to determine the correlation between ATP and microbial CFU counts. The Pearson correlation coefficient was calculated to assess the relationship between RLU values and viable microbial loads. Results indicated a positive correlation, with R-values ranging from 0.001-0.600 before cleaning and increasing to 0.720-0.990 after cleaning, demonstrating that ATP testing is a reliable indicator of surface hygiene.

## Results and Discussion

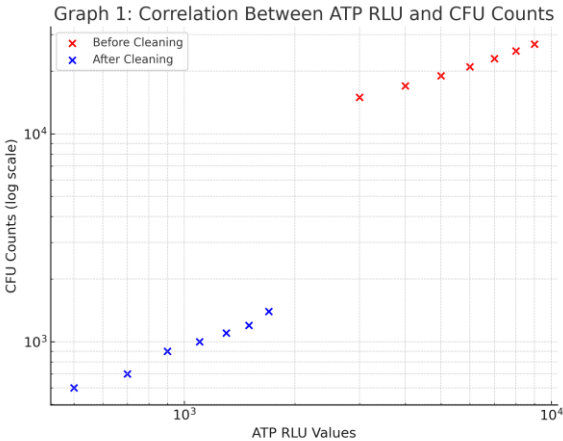
The results obtained from ATP bioluminescence testing and traditional microbiological methods showed a significant correlation between contamination levels before and after cleaning. The six sampling locations exhibited varying degrees of microbial load, with the highest contamination observed in the raw milk reception area and filling machine nozzles, where bacterial counts exceeded  $4.9 \times 10^4$  CFU/cm<sup>2</sup> before cleaning. The ATP readings in these locations were also the highest, reaching 5600 RLU and 6200 RLU, respectively.

After cleaning and sanitization, the microbial load in all sampling locations decreased significantly. The most effective reduction was observed in storage tank surfaces, where ATP levels dropped from 4500 RLU to 800 RLU, and CFU counts reduced from  $3.0 \times 10^4$  CFU/cm<sup>2</sup> to 900 CFU/cm<sup>2</sup>. However, despite the observed reduction, certain locations, such as operator's gloves, still exhibited 700 RLU and 700 CFU/cm<sup>2</sup>, indicating potential cross-contamination risks.

Table 1

Comparison of ATP RLU and CFU Counts Before and After Cleaning					
Sampling Location	Before Cleaning (RLU)	After Cleaning (RLU)	TVC (CFU/cm²) Before	TVC (CFU/cm²) After	
Raw Milk Reception Area	5600	1200	$4.9 \times 10^4$	$1.2 \times 10^3$	
Pasteurization Equipment	4800	900	$3.5 \times 10^4$	$1.0 \times 10^3$	
Filling Machine Nozzles	6200	1400	$5.2 \times 10^4$	$1.4 \times 10^3$	
Conveyor Belt Surfaces	5100	1100	$4.0 \times 10^4$	$1.1 \times 10^3$	
Storage Tank Surfaces	4500	800	$3.0 \times 10^4$	900	
Operator's Gloves	3900	700	$2.5 \times 10^4$	700	

The correlation analysis between ATP RLU values and CFU counts demonstrated a strong linear relationship, particularly after cleaning procedures. The Pearson correlation coefficient (R) increased from 0.001–0.600 before cleaning to 0.720–0.990 after cleaning, indicating that ATP bioluminescence testing effectively reflects microbial contamination levels in dairy processing environments.



Graph 1: Correlation Between ATP and CFU Counts

The findings of this study align with previous research conducted in food processing facilities, which suggest that ATP bioluminescence is a useful tool for real-time hygiene monitoring. However, it is important to note that ATP testing does not differentiate between microbial and non-microbial residues, which may lead to overestimated contamination levels in certain cases. For instance, ATP values in operator’s gloves remained relatively high even after cleaning, possibly due to organic residues such as skin cells or biofilms that were not entirely removed during the sanitation process.

Despite this limitation, the ability of ATP bioluminescence to provide immediate contamination feedback makes it a highly effective tool for routine hygiene assessments. Unlike traditional microbiological methods, which require 24–48 hours for bacterial culture growth, ATP readings are available within seconds, allowing dairy plants to implement corrective actions in real time.

Future studies could further enhance the reliability of ATP bioluminescence testing by incorporating additional validation techniques, such as enzyme-based specificity tests to differentiate between ATP from microbial and non-microbial sources. Moreover, optimizing sanitation protocols based on ATP results could

lead to improved hygiene management and reduced microbial risks in dairy production environments.

Conclusion

This study highlights the effectiveness of ATP bioluminescence testing as a rapid and reliable method for monitoring hygiene in dairy processing plants. The results demonstrated a significant correlation between ATP RLU values and CFU counts, confirming that ATP bioluminescence provides an immediate indication of contamination levels. Compared to traditional microbiological methods, ATP testing offers the advantage of real-time assessment, enabling faster corrective actions to maintain food safety standards.

The findings also emphasize the importance of integrating ATP monitoring with conventional microbiological testing to achieve a comprehensive hygiene evaluation. While ATP testing effectively identifies biological residues, it does not distinguish between viable and non-viable cells, necessitating supplementary microbiological confirmation. However, its rapid response makes it an invaluable tool in quality control programs for dairy plants.

Future research should focus on refining ATP testing protocols to enhance specificity and sensitivity, particularly in differentiating microbial ATP from other

organic contaminants. Additionally, further studies can explore the impact of different cleaning agents and sanitization techniques on ATP readings to optimize dairy hygiene practices.

Overall, the incorporation of ATP bioluminescence into routine hygiene monitoring protocols can significantly improve contamination control, ensuring product safety and compliance with food industry regulations.

### References

1. Griffiths, M.W. (1993). Applications of bioluminescence in the dairy industry. *Journal of Dairy Science*, 76(10), 3118-312.
2. Davidson, C.A., Griffith, C.J., Peters, A.C., & Fielding, L.M. (1999). Evaluation of two methods for monitoring surface cleanliness - ATP bioluminescence and traditional hygiene swabbing. *Luminescence*, 14(1), 33-38.
3. Poulis, J.A., Phper, M., & Mossel, D.A. (1993). Assessment of cleaning and disinfection in the food industry with the rapid ATP-bioluminescence technique. *International Journal of Food Microbiology*, 20, 109-116.
4. Chen, F.C., & Godwin, S.L. (2006). Comparison of a rapid ATP bioluminescence assay and standard plate count methods for assessing microbial contamination. *Journal of Food Protection*, 69(10), 2534-2538.
5. Salo, S., & Laine, A. (2000). Validation of microbiological methods Hygicult Dipslide, Contact Plate, and Swabbing in surface hygiene control. *Journal of AOAC International*, 83(6), 1357-1366】 .
6. Costa, P.D., Andrade, N.J., Brandao, S.C.C., Passos, F.J.V., & Soares, N.F.F. (2006). ATP-Bioluminescence Assay as an Alternative for Hygiene-Monitoring Procedures of Stainless Steel Milk Contact Surfaces. *Brazilian Journal of Microbiology*, 37(3)】 .
7. Leach, F.R., & Webster, J.J. (1986). Commercially Available Firefly Luciferase Reagents. *Bioluminescence and Chemiluminescence – Methods in Enzymology*, Vol. 133, 51-70.
8. Bower, C.K., McGuire, J., & Daeschel, M. (1996). The adhesion and detachment of bacteria and spores on food-contact surfaces. *Trends in Food Science & Technology*, 7, 152-157.
9. Moore, G., & Griffith, C. (2002). A comparison of traditional and recently developed methods for monitoring surface hygiene within the food industry: an industry trial. *International Journal of Environmental Health Research*, 12, 317-329.
10. Buchanan, R.L. (1990). HACCP: A re-emerging approach to food safety. *Trends in Food Science & Technology*, 1, 104-106