

**AN INVESTIGATION OF ATP BIOLUMINESCENCE AND QUANTITATIVE BULK  
TANK CULTURES TO ASSESS CLEANLINESS OF MILKING MACHINES**

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**Summary:** A series of experiments were performed to evaluate the performance of experimental detergents. Initial assessment of cleaning efficacy was done using a standardized milk soil (whole milk inoculated with bacteria) sprayed onto stainless steel test chips and allowed to incubate. These test chips were cleaned under controlled conditions in the laboratory. The residual milk soil was assessed using ATP bioluminescence techniques. Subsequent testing involved a switchback experiment in the UW milking parlor with a control detergent and the experimental product. Cleaning efficacy was evaluated using ATP bioluminescence methods as well as the following bacterial assessment of bulk tank milk: Standard Plate Count (SPC), Preliminary Incubation Count (PI) and Laboratory Pasteurized Count (LPC). The relationships between the various tests of cleaning performance were investigated. Recommendations for field tests of milking machine sanitation are presented.

**Keywords: Milking Machines, Cleaning and Sanitation, ATP, Test Methods**

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# AN INVESTIGATION OF ATP BIOLUMINESCENCE AND QUANTITATIVE BULK TANK CULTURES TO ASSESS CLEANLINESS OF MILKING MACHINES

## INTRODUCTION

The two main sources of bacteria in raw milk are organisms transported from the environment into the milking machine and mastitis organisms from within the udder. Bacteria deposited in milk handling equipment will multiply and become a major source of contamination if this equipment is not cleaned and sanitized properly. Some form of testing for bacterial contamination is done periodically on all farms to assure compliance with national, state, and local milk plant requirements. The most common method used to assess the bacterial quality of raw milk in the United States is the standard plate count (SPC). The SPC is a broad-spectrum test does not identify the types of organisms present. Likewise, the most common method to assess udder health on farms is the somatic cell count (SCC). These tests provide an overall measure of milk quality but they have little diagnostic value in determining the source of bacterial contamination. Some dairy plants also include the Preliminary Incubation count (PI), Laboratory Pasteurized Count (LPC) or other tests of specific bacterial populations (Reinemann et al, 1997). A method for diagnosing cleaning failures using the relative relationships between the SPC, SCC, LPC, and Coliform count have been presented by Guterbach and Blackmer (1984). An example of implementation of this method can be found in Britt et al, (1997).

Quantitative bulk tank cultures (QBTC), which enumerate the various types of organisms, are commonly used in mastitis control programs to identify the type of mastitis organisms in bulk milk. Organism types identified in the QBTC are typically:

<i>staph. aureus</i>	<i>strep. agalactiae</i>	<i>coliforms</i>
<i>staph. non-aureus.</i>	<i>strep. non agalactiae.</i>	<i>other</i>

The “other” category can include a variety of organisms including:

<i>pseudomonas</i>	<i>bacillus cereus</i>	<i>klebsiella sp.</i>
<i>c. pyogenes</i>	<i>candida</i>	<i>prototheca</i>

The quantitative bulk tank culture yields a Total Bacteria Count (TBC) that should correspond closely to the SPC. All of these bacterial tests rely on culture media and incubation from two to three days.

Recent developments of ATP detection methods using a bioluminescence have been proposed as a rapid method for assessing the effectiveness of sanitation in the dairy industry (Griffiths, 1993). ATP bioluminescence is a rapid detection method suited for on-site sampling and takes less than five minutes to perform. Much attention has focused on the effectiveness of CIP systems in dairy farm and dairy processing operations. Dunsmore, et. al, (1981) using a simulated CIP system, conducted several studies on the effectiveness of CIP procedures. With the use of ATP bioluminescence results can be obtained on-site within 5 minutes of sampling and as opposed to plate count methods which must be done in the laboratory and do not provide results for at least 24-48 hours. Plate count methods also only detect the presence of bacterial contamination on equipment surfaces, whereas ATP bioluminescence can detect both bacterial contamination and non-microbial contamination such as milk soil.

A study was performed to evaluate the use of ATP detection methods to assess sanitation of a typical milking parlor. The ATP detection methods used in the milking parlor were compared to QBTC, LPC, PI and Coliform Count of raw milk in the bulk tank of the milking parlor and the results of control testing in the laboratory

## MATERIALS AND METHODS

### *Laboratory Tests*

A commercially available milk meter and milking unit were tested in the laboratory using ATP detection methods. Before each experiment the components were disassembled and washed thoroughly with Haemo-Sol laboratory detergent solution (Haemo-Sol, Inc., Baltimore, MD), rinsed several times with distilled water and air-dried. A culture of *Lactobacillus brevis*, originally isolated from raw milk was suspended in 3000 ml of 0.01M phosphate-buffered saline (PBS, pH 7.2). The milking components were filled with the suspension and left stationary for 1 h at room temperature to allow bacteria to attach to the interior surfaces. After 1 h, the bacterial suspension was poured out of the milking components and the components rinsed with distilled water to remove non-adhering bacteria.

The milk meter and milking unit were cleaned by circulating both plain water and detergent solutions. Swabs from the LUMAC<sup>R</sup> Hygiene Monitoring Kit (Perstorp Analytical, Landgraaf, Netherlands) were used to swab the surfaces of the milking equipment after cleaning. The Biocounter<sup>R</sup> M 1500 Light (LUMAC<sup>R</sup>) and the Hygiene Monitoring QM Kit (cat no 9330-5) was used to measure ATP in the milk soil residue. The light produced was determined and recorded as Relative Light Unit (RLU) with the Lumac Biocounter M1500 M Light.

Test areas of 10 cm<sup>2</sup> were used for each swab. For every test location sampled using the ATP assay, an equivalent area was swabbed and sampled using traditional plate count techniques. The surface was swabbed with a calcium alginate swab and the tip of the swab broken into a tube containing 0.1% peptone. The appropriate dilutions were then plated on APT agar containing 0.02% bromocresol purple and incubated anaerobically for 48 hr at 30°C. Samples were also plated on plate count agar (PCA, Difco, Detroit, MI) to check for background contamination. The results obtained using ATP bioluminescence and the plate count technique for all the experiments were compared using linear regression analysis.

A spraying apparatus was constructed to deliver multiple, uniform layers of a milk solution to the surface of stainless steel test chips (Muljadi et al, 1996). Test chips were cleaned with hot alkaline detergent, rinsed with double distilled water and air-dried prior to spraying. Chips were placed on a disk that rotated once per minute under a stationary spray nozzle. Air at 37.8°C was circulated through the chamber during spraying. Raw milk was taken from the bulk tank at the UW milking parlor and allowed to stand at room temperature for 24 hours to increase the bacterial population of the milk. This incubated milk solution was sprayed on the chips and incubated for 24 hours at room temperature prior to cleaning. Twenty-eight chips were placed on the rotating disc and sprayed for four hours to obtain approximately 240 soil layers per chip. Four sprayed chips were used for each cleaning experiment. Two replicates were conducted of each experiment.

A rectangular stainless steel (type 304) test section (151x3.5x8 cm) with the internal surface honed to a #4 finish was placed 1 m from the receiver on one leg of the looped milkline. The test

section was fitted with six removable stainless steel chips with surface area of 10 cm<sup>2</sup>. When mounted, the surface of the chips was flush with the inner surface of the test section. The test chips were removed to apply milk soil before cleaning and refitted in the test section for a cleaning experiment. Upon completion of an experiment, test chips were removed from the test section and rinsed with water to remove any cleaning solution residue on their surface and then processed to measure the remaining milk soil residue.

The temperature of the cleaning solutions was adjusted to the desired level by mixing hot and cold water in the wash vat before addition of any cleaning chemicals. Cleaning solutions were circulated through the test chamber for 25 seconds followed by five seconds of air injection. This process was repeated using the positive controlled conditions (a standard alkaline detergent) until the chips were visibly clean. The tests were done using experimental cleaning solutions for the same number of liquids/air cycles as used for the control. The LUMAC<sup>R</sup> Hygiene Monitoring Kit described above used to remove milk soil residues from the test chips.

### *Milking Parlor Tests*

Additional testing was done using the experimental cleaners in the UW milking parlor. The performance of experimental cleaners was compared to the standard alkaline detergent used normally. Cleanliness was assessed using the ATP bioluminescence method. The locations most sensitive to cleaning failure were chosen as test points. This included 4 locations in each of 4 milk meters and 5 locations in the receiver for a total of 21 swabs. This series of tests was done 3 times per week (Monday, Wednesday and Friday) about 4 hours after the cleaning procedures were completed. This allowed surfaces to drain completely. Past experience indicated that water residuals on surfaces typically resulted in high variability of the ATP readings.

Sterile milk samples were taken from the bulk tank 3 times per week during testing. The milk sample was split into 3 parts. One sub sample was used to run a QBTC. The second sub sample was incubated according to the standard procedures for the PI test (13 C for 18 hours). Following this incubation a QBTC was performed. The third sub sample was pasteurized according to the standard method for the LPC test (63 C for 30 minutes). Following this laboratory pasteurization a QBTC was performed.

A standard cleaning cycle incorporating a warm water prerinse, alkaline detergent wash, acid rinse and sanitize cycles was used for some tests. A combined acid/sanitize cycle was used for some tests. Bulk tank samples and ATP testing were performed for a period of at least two weeks using a standard alkaline detergent as the cleaning agent. Testing continued for 1 to 2 weeks after switching to an experimental cleaning product. An additional 1 week of testing was performed after switching back to the standard alkaline cleaner.

## **RESULTS AND DISCUSSION**

### *Laboratory Tests*

The correlation of RLU's obtained using ATP bioluminescence to colony forming units (CFU's) obtained using the plate count method for the laboratory testing of the milk meter is shown in Figure 1. The coefficient of determination ( $R^2$ ) was 0.73. The correlation between RLU and CFU obtained in the experiments reported here was higher than that reported by Poulis, et. al. (1993) ( $R^2 < 0.40$ ). One possible reason for this difference is that Poulis used

environmental samples as opposed to the standardized milk/bacteria mixture used in these studies.

The results of the laboratory testing of an experimental detergent using the sprayed on milk/bacteria mixture and ATP assessment of the residuals after cleaning are presented in Figure 2. Two experiments were performed; One with the experimental product at a low concentration and a second with a higher concentration. The error bars on the chart indicate the 95% confidence interval of the mean RLU value with a sample size of 8 for each. In the first experiment the experimental detergent had a higher residual RLU ( $p < 0.05$ ) than the control detergent used at the same temperature and cleaning time. The mean residual milk soil for the experimental detergent in the second experiment was higher than, but not significantly different than that for the control.

### *Milking Parlor Tests*

The results of ATP testing of the milking parlor are shown in Figure 3. The control detergent was used from day -17 to -1. The experimental detergent was used from day 0 to 15. The control was used again from day 16 to 40. The experimental detergent was used at a higher concentration from day 41 to day 55. The control was used again from day 56 to 64. The y error bars are the 95% confidence interval of the mean of the 21 ATP measurements for each day. There was considerable variation in the measurements between the different test sites. A t-test of the means of the control period (mean = 1.92) compared to the means of the treatment period (mean = 2.22) approached significance at the 95% confidence level ( $p = 0.058$ ). When a pair t-test was performed using the control period measures paired with the treatment measures for the same test location the difference between the control and treatment periods was highly significant ( $p = 0.0019$ ). The same tests showed no significant difference between the experimental detergent at the higher concentration and the control periods. These results agree with the testing of this detergent in the laboratory using the sprayed on milk/bacteria culture.

The results of QBTC performed simultaneously with the ATP testing are shown in Figure 4 for the first part of the experiment. There were no significant differences in any of the bulk tank culture measures (TBC, LPC, or PI) between the control period and the period during which the experimental detergent was used at the lower concentration. There was also no correlation between any of the bulk tank culture measures and the ATP(RLU) measures. This may be because the difference in cleanliness detected with the ATP method was not sufficient to cause a major cleaning failure or significant change in the bacterial populations in the bulk tank. No indications of visual build up was observed on any of the milking machine components.

## **CONCLUSIONS**

ATP bioluminescence has the potential to be a useful tool to evaluate the effectiveness of cleaning procedures used on the milking machines. The ATP method appeared to be a more sensitive method to detect differences in cleaning effectiveness than bulk tank culture method in this study. There is considerable variation in the data collected, however, and the method must be used carefully to obtain meaningful results. It is essential to perform a sufficient number of ATP swabs to obtain meaningful results. The required sample size will depend on the skill of the user and the stability of the system being monitored. Care must be taken to avoid contaminating the inner surfaces of components as they are opened for swabbing. It was suspected that some of

the very high RLU numbers may have been caused by this type of contamination. The variability in the ATP data can be reduced significantly by using the same measurement locations over time.

## REFERENCES

- Britt, J.S., F. Hartmann and D.J. Reinemann, 1997. Use of Microbiology and Strategic Sampling at strategic times to solve High Bacteria Count Problems in bulk Tank Milk. Proc. 36<sup>th</sup> annual meeting of the National Mastitis council, 16-19 Feb. 1997.
- Dunsmore, D. G., Twomey, A., Whittlestone, W. G., and H. W. Morgan. 1981. Design and performance of systems for cleaning product-contact surfaces of food equipment: a review. *J. Food Prot.* 44: 220-240.
- Griffiths, M. W. 1993. Applications of bioluminescence in the dairy industry. *J Dairy Sci.* 76: 3118-3125.
- Guterbach, W.M., and P.E. Blackmer, 1984. Veterinary Interpretation of Bulk Tank Milk. *Veterinary Clinics of North America: Large Animal Practice*, Vol. 6, No. 2, July 1984. Pp257-268.
- Muljadi, A., D.J. Reinemann, and A.C.L. Wong, 1996. Air injected Clean-In-Place for Milking systems: Development of a Study Method and Characterization of Chemical, Mechanical and Thermal Factors. ASAE paper No. 963019.
- Poullis, J. A., de Pijper, M., Mossel, D. A., and P. A. Dekkers. 1993. Assessment of cleaning and disinfection in the food industry with the rapid ATP-bioluminescence technique combined with the tissue fluid contamination test and a conventional microbiological method. *Int. J. Food Microbiol.* 20: 109-116.
- Reinemann, D.J., G.A. Mein, D.R. Bray, D Reid and J.S Britt, 1997. Troubleshooting High Bacteria counts in Farm Milk. Proc. 36<sup>th</sup> annual meeting of the National Mastitis council, 16-19 Feb. 1997.

Figure 1. milk meter lab testing with ATP and SPC

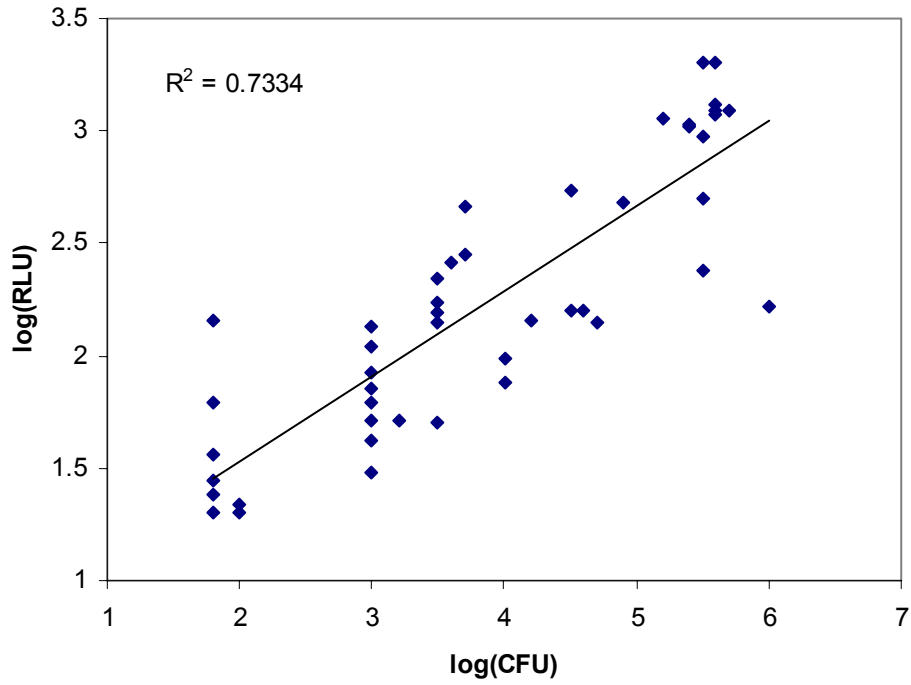


Figure 2. Laboratory tests of sprayed chips using ATP/RLU assessment of residuals.

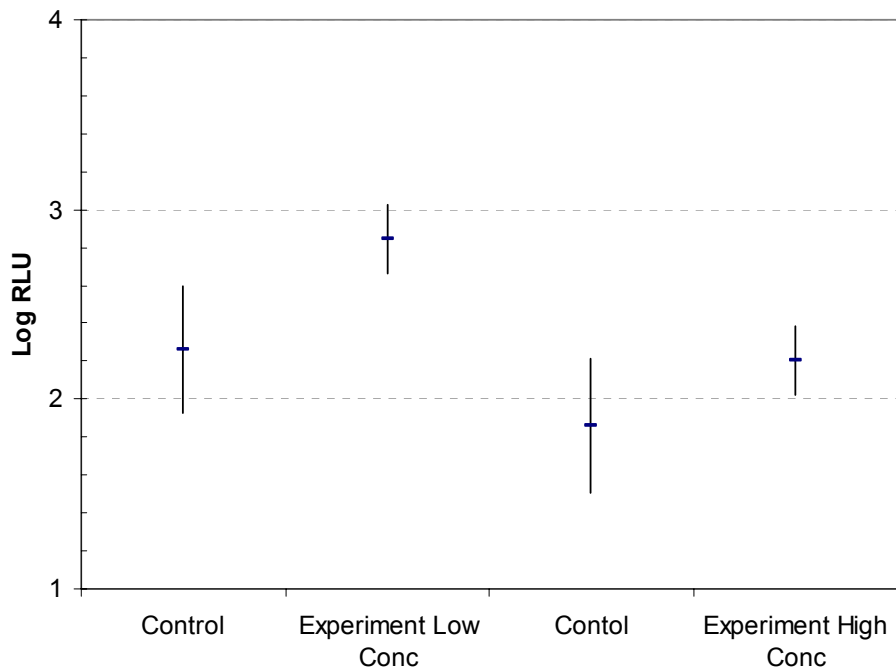


Figure 3. ATP of experimental detergent in the milking parlor.

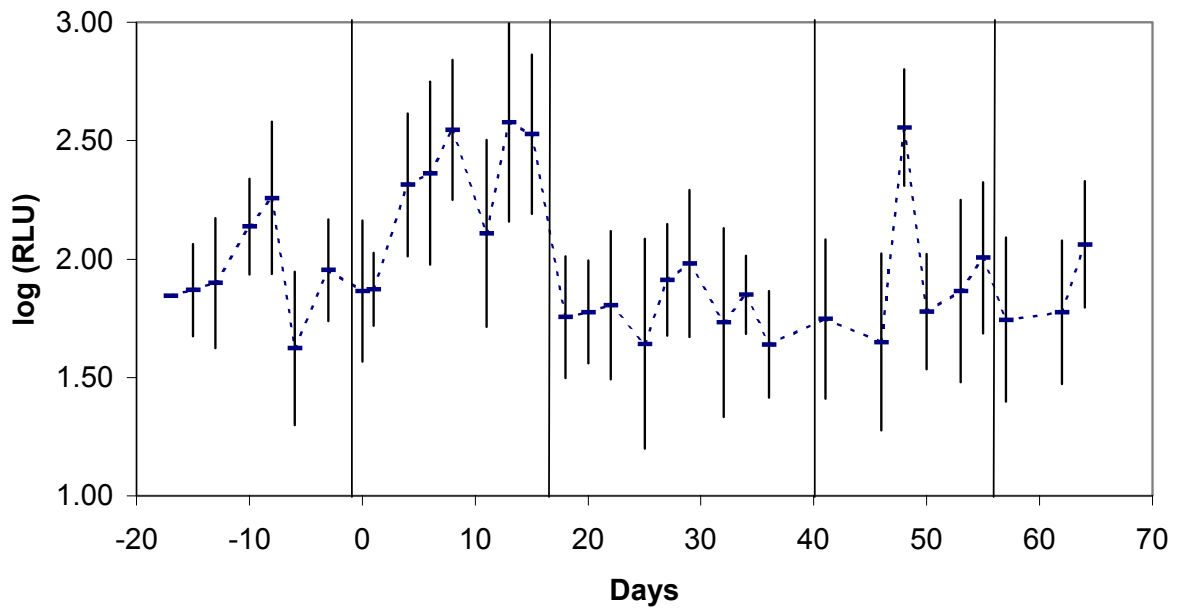


Figure 4. Bulk Tank Culture and ATP results for Experimental Detergent.

