Correlating the contamination of milking cups with cell count and mastitis prevalence by measuring ATP

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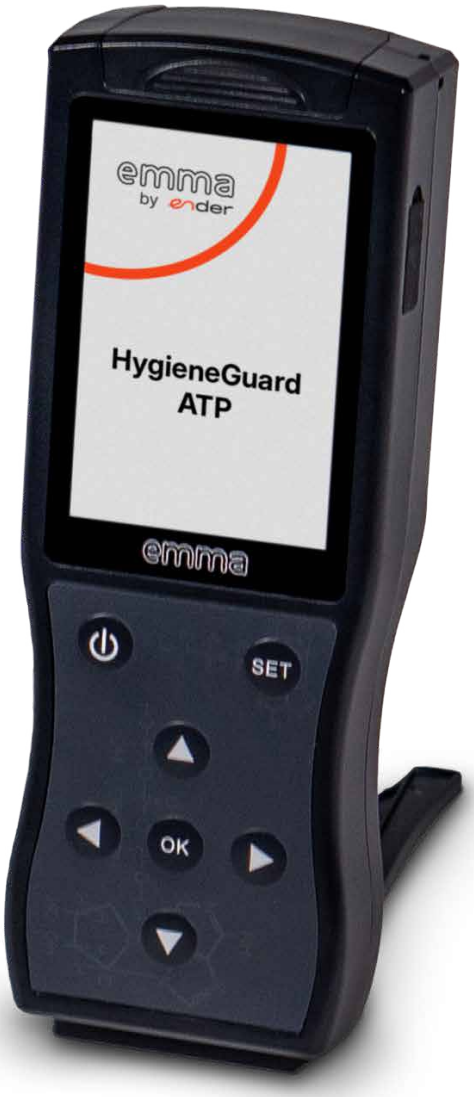
ender diagnostics ag, 2024



1. Summary

Effective hygiene protocols are essential to prevent disease transmission and control antimicrobial resistances, particularly on dairy farms where improper handling during the milking process can contribute to an increased incidence of mastitis.

In this study, hygiene levels across three dairy farms were measured using the emma HygieneGuard ATP, with a focus on the cleaning of milking machine cups. The test, measured in relative light units (RLU), is directly correlated with the presence of microorganisms and therefore a suitable solution for verifying the effectiveness of cleaning protocols for milking equipment. One of the farms was known to have ongoing hygiene-related issues, resulting in higher cell count and more frequent cases of mastitis. This was also visible in the RLU measurements, as the pre- and post-wash test results were higher than on the other two farms. After the wash, the RLU values decreased on all farms. These results demonstrate that the emma HygieneGuard ATP is an effective tool for identifying contamination sources, improving hygiene protocols, and verifying cleaning effectiveness, thereby helping to prevent the spread of pathogens and reduce mastitis.

2. Introduction

To prevent disease transmission and reduce mastitis prevalence, effective hygiene protocols are essential, particularly on dairy farms, where improper handling during the milking process can increase the incidence of mastitis. ATP (adenosine triphosphate), the fundamental energy molecule in all living cells, serves as a reliable indicator of microbial presence on surfaces (Vilar *et al.* 2008). Its detection can therefore be used to verify hygiene protocols and identify contamination at various stages of food processing and in health care. This verification has become crucial in times where new diseases and antimicrobial resistances are spreading with increased speed.

The presence of ATP is measured through bioluminescence, the light emission occurring when the enzyme luciferase catalyzes a reaction between ATP and luciferin, producing AMP and oxyluciferin (Hysert *et al.* 1976). Bioluminescence is detected and quantified by the emma HygieneGuard ATP and expressed in relative light units (RLUs), that is directly proportional to the amount of ATP. As ATP is found in all living cells, the RLU measurement reflects the concentration of living cells, providing a practical method for assessing cleanliness and verifying hygiene protocols.

In the dairy industry, proper hygiene during milking is critical. Contaminated milking machine cups can lead to a higher incidence of mastitis on farms. This is not only a problem for animal welfare, but also contributes to a lot of direct and indirect economic losses in dairy industry (Bennett *et al.* 1999; Hogeveen *et al.* 2011; Das *et al.* 2018, Aghamohammadi *et al.* 2018; Hadrich *et al.* 2018).

In this study, the emma HygieneGuard ATP test was used on three dairy farms and the resulting measurements of RLU compared with the differences in hygiene protocols.

3. Material and methods

ATP swabs were done on three different farms located in Switzerland. The selection of the farms was based on cell count: Farm A and C had <100k/ml and farm B >350k/ml with a higher prevalence of mastitis. Two farms were equipped with classical milking machines (Farm A, B), while farm C was equipped with a milking robot. Swabs were taken before and after washing the milk cups, whereas one swab was used for two cups. In total, there were 12 milk cup pairs tested per visit on 12 different occasions. For farm A and B, the washing step was conducted after the whole herd was milked and took about 40 min. On farm C the robot cleaned the cups after each cow for 3-4 minutes. The ATP levels

were measured on site, right after the 12 samples were taken. The measurements were performed on the emma HygieneGuard ATP, currently under development by ender diagnostics ag, for environmental and liquid testing.

4. Results

The RLU measurements for the milk cups are arranged in pre- and post-wash measurements, while for each timepoint the mean of the 12 measurements was calculated (see Figure 1). The bars show the standard deviation. The mean values of farm A and C are around 2000 RLU prewash and 200 RLU post-wash. For Farm C, the mean values were around 8000 RLU pre-wash and around 2000 RLU post-wash. The difference in RLU of farm B to farm A and C is significant, except for the first two timepoints in the pre-wash condition.

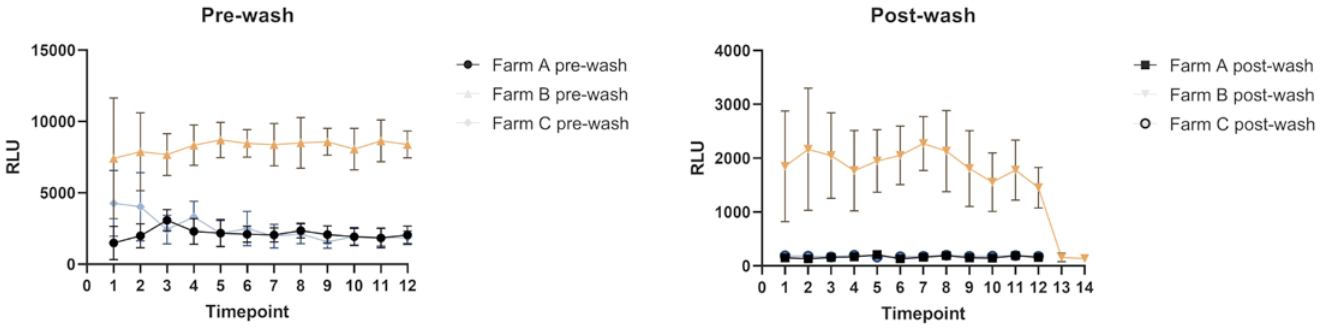


Figure 1: ATP measurements on three farms (A, B and C). The milk cups of the milking machines were measured for ATP. One measurement was taken before the washing (pre-wash) and one after (post-wash). For each timepoint, the mean (N=12) and the standard deviation are displayed. After a replacement of milking cups between timepoint 12 and 13, a decrease in measured RLU after washing was observed.

5. Discussion

The RLU measurements of the milk cups clearly indicate which farm has hygiene-related issues. On farm B, the RLU values before the wash were approximately four times higher than those on farms A and C. After the wash, the RLU levels of farm B decreased but remained within the same range as the pre-wash values on farms A and C. This suggests that while the washing process on farm B reduces contamination, it does not achieve the same level of cleanliness as observed on the other farms. After replacing the milking cups, the RLU values finally matched the lower levels observed on farms A and C, indicating a significant improvement in hygiene.

High RLU measurements correlate with the microbiological contamination in the milking cups, which poses a constant infection pressure for the whole herd. There could be multiple reasons why the cleaning is insufficient. The rubbers of the milking machine become porous if they are not changed frequently and therefore serve as perfect ground for microbes to grow and not being washed away during the cleaning cycle. Furthermore, it is possible that the water used for cleaning may be contributing to the hygiene issue. If the cleaning water itself is contaminated, it can reduce the effectiveness of the cleaning process.

The results of this study show a correlation between elevated cell counts, incidence of mastitis and high RLU measurements, indicating inadequate hygiene during the milking process on farm B. Based on these results, the rubber components of the milking machine were replaced, and in a continuation of this study, the effectiveness of these changes will be monitored. Additionally, the water on farm B was tested for microbiological contamination, and all values were found to be within acceptable limits, excluding the water as a source of the elevated ATP levels. If these measures are successful, a reduction in mastitis cases and a general decrease in the spread of microorganisms among the cows on farm B are expected.

In conclusion, the use of the emma HygieneGuard ATP on dairy farms provides a quick and reliable tool for evaluating hygiene protocols. When issues are identified, veterinarians and farmers can work together to develop targeted solutions. ATP measurements provide immediate action steps and locations that deliver direct benefits to the farmer, such as the timely replacement of milking cups to improve overall cleanliness.

6. Usability in routine

Whether you are a veterinarian, dairy advisor, or farm manager, the emma HygieneGuard ATP testing offers a fast, reliable way to monitor hygiene across the practices and farms. In just 10 seconds, it provides real-time data on surface cleanliness at a microbiological level, helping to refine cleaning protocols, verify biosecurity measures and identify potential contamination risks precisely. Being able to test and show results right on site presents a powerful tool in communication with the farm manager – identifying critical control points and presenting effects (or lack of effect) of cleaning protocols is a powerful way for explaining and establishing necessary adjustments.

References

Aghamohammadi, M., Haine, D., Kelton, D. F., Barkema, H. W., Hogeveen, H., Keefe, G. P., Dufour, S., 2018. *Herd-level mastitis-associated costs on Canadian dairy farms.* Frontiers in Veterinary Science, 5, 100.

Bennett, R., Christiansen, K., Clifton-Hadley, R., 1999. *Preliminary estimates of the direct costs associated with endemic diseases of livestock in Great Britain.* Preventive Veterinary Medicine, 39(3), 155–171.

Das, D., Panda, S. K., Jena, B., Sahoo, A. K., 2018. *Economic impact of subclinical and clinical mastitis in Odisha, India.* International Journal of Current Microbiology and Applied Sciences, 7(03), 3651–3654.

Hadrich, J. C., Wolf, C. A., Lombard, J., Dolak, T. M., 2018. *Estimating milk yield and value losses from increased somatic cell count on US dairy farms.* Journal of Dairy Science, 101, 3588–3596.

Hogeveen, H., Pyorala, S., Waller, K. P., Hogan, J. S., Lam, T. J. G. M., Oliver, S. P., Schukken, Y. H., Barkema, H. W., Hillerton, E. J., 2011. *Current status and future challenges in mastitis research.* In Proceedings of the 50th Annual Meeting of the National Mastitis Council, 23–26 January, 2011, Arlington, USA (pp. 36–48).

Hysert, D. W., Kovecses, F., Morrison, N. M. 1976. *A Firefly Bioluminescence ATP Assay Method for Rapid Detection and Enumeration of Brewery Microorganisms.* Journal of the American Society of Brewing Chemists, 34(4), 145–150.

Vilar, M. J., Rodriguez-Otero, J. L., Dieguez, F. J., Sanjuan, M. L., Yus, E., 2008. *Application of ATP bioluminescence for evaluation of surface cleanliness of milking equipment.* International Journal of Food Microbiology, 125, 357–361.

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