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Short communication

Application of ATP bioluminescence for evaluation of surface cleanliness of milking equipment

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ABSTRACT

The ATP bioluminescence method was used to evaluate the cleanliness of milking equipment surfaces (teat cup rubbers, teat dip containers, milk receivers, and pipeline joints) in dairy farms in Galicia (northwest Spain) with parlour, pipeline tie-stall or bucket tie-stall milking systems. The cleanest surfaces were teat cup rubbers. The use of non-chlorinated water for cleaning, and of pipeline or bucket tie-stall milking systems, was associated with high ATP bioluminescence values. However, ATP bioluminescence values only explained 12% of the variability in bulk-tank bacterial count; this is attributable to the importance of other factors (notably the correct functioning of the tank cooling system) for maintenance of low bacterial count.

1. Introduction

The cleanliness and hygiene of dairy-farm milking equipment surfaces is one of the factors influencing the bacterial count (BC) of bulk-tank milk. Hence effective systems for ensuring the cleanliness of milking equipment are essential (Nieuwenhof, 1996; Slaghuis and Wiegersma, 1996).

Microbiological methods, including hygiene swabbing and agar contact methods, are widely used for assessment of the cleanliness of food contact surfaces, but require incubation periods of days. ATP bioluminescence methods are an attractive alternative because they provide a real-time assessment of surface cleanliness (Griffiths, 1993; Davidson et al., 1999; Samkutty et al., 2001). Thus these methods are well suited for monitoring cleanliness within hazard analysis critical control point (HACCP) systems (Seeger and Griffiths, 1994; Aycicek et al., 2006).

ATP bioluminescence has been used to assess the hygiene of food contact surfaces in diverse contexts, including care institutions (Seeger and Griffiths, 1994) and the dairy industry (Murphy et al., 1998), as well as to evaluate microbial contamination in foods like raw milk (Brovko et al., 1999), pork and beef carcasses (Siragusa and Cutter, 1995; Siragusa et al., 1995), and udder contamination of dairy cows (Finger and Sischo, 2001). This technique detects bacterial contamination, but also non-microbial sources of ATP, such as organic debris and food residues, which may indicate poor cleaning and be a source of nutrients for microbial growth (Corbitt et al., 2000; Champiat et al., 2001). The principal advantages of the technique are that it is rapid and relatively simple, facilitating use as a field test, and that it is repeatable (Finger and Sischo, 2001).

However, rather little information has been published on the use of ATP bioluminescence for evaluation of the surface cleanliness of milking equipment on dairy cattle farms, except for some short communications at conferences (Slaghuis and Wiegersma, 1996; De Celis, 2006). Furthermore, there have been no previous studies of relationships between ATP bioluminescence values and bacterial count in bulk-tank milk. Thus, the objectives of this study were i) to assess ATP bioluminescence on various types of milking equipment surface on dairy cattle farms, and to investigate whether ATP bioluminescence values are influenced by any of various milking and cleaning system practices; ii) to evaluate the relationship between ATP data and bulk-tank milk bacterial count; and iii) to suggest ATP bioluminescence values that can be taken as cut-offs for definition of adequate cleanliness of the different surfaces.

2. Materials and methods

2.1. Study design and data collection

The study was carried out between 2004 and 2006 on 106 dairy cattle farms in Galicia (NW Spain), where a certification programme using a HACCP methodology was being implemented. The mean number of animals per herd was 65.5 (8–480), and the mean number of lactating cows 40.9 (6–260). Of the 106 farms, 55.7% had a milking parlour, 32.1% pipeline tie-stall milking and 12.3% bucket tie-stall milking.

Every farmer was personally interviewed, using a questionnaire about their procedures for cleaning milking equipment, notably i) source of water used to clean the equipment, and ii) frequency with which acid

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Table 1
Relative luminescence unit (RLU) values for the 265 milking equipment surfaces analysed

	N[a]	Average	Minimum	Maximum
Teat cup rubbers	106	363.6	8	overload[b]
Teat dip containers	84	2197.1	0	overload[b]
Milk receivers	52	2929.7	0	overload[b]
Pipeline joints	23	8536.7	2	77,000

[a] N: number of surfaces assessed.
[b] Overload: RLU values >10^5, not used in the calculation of averages.

detergent was used. Of the 106 farms 31.1% used chlorinated water, while the remaining 68.9% used water from non-chlorinated wells. Acid detergent was used daily on 28.3% of farms, several times per week on 19.2%, weekly on 44.4%, and never on 8.1% meanwhile alkaline detergent was employed daily in all farms. Cleanliness and disinfection of dairy-farm milking equipment is always carried out in an automatic manner, except in bucket tie-stall milking systems in which cleanliness is manual.

In addition, BC values in bulk-tank milk were recorded for each farm, as assessed monthly by the Official Dairy Laboratory of Galicia. Farms were classified into 3 classes: A ($<20\times10^3$ cfu/ml), B (20–100×10^3 cfu/ml) and C ($>100\times10^3$ cfu/ml). Classification of the farms by bulk-tank BC indicates that 59.3% of farms produced class-A milk, 29.2% class-B milk and 11.5% class-C milk.

2.2. ATP bioluminescence assay

Various milking equipment surfaces were swabbed around 1 cm^2, and ATP amount was detected specifically by reaction with a luciferin–luciferase mixture in buffered solution. The light emitted in the process was measured and displayed in relative luminescence units (RLU) by a HY-LITE® portable luminometer (Merck KGaA, Darmstadt, Germany). The HY-LYTE® pen is a ready prepared test cuvette designed for use in a HY-LYTE® luminometer, according to the manufacturer's instructions. The milking equipment surfaces studied were teat cup rubbers from all farms, teat dip containers in 84 milking equipments, 52 milk receivers (parlour and pipeline tie-stall systems) and 23 pipeline joints (pipeline tie-stall systems). A total of 265 different surfaces were assessed.

2.3. Statistical analysis

Data were analysed using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, Illinois). RLU and BC data were analysed as $\log_{10}$ RLU (LRLU) and $\log_{10}$ BC (LBC). ANOVA was used to assess the effects of different hygiene-related factors on LRLU. A multiple linear regression was carried out to evaluate relationships between LRLU for each type of surface (independent variables) and LBC (dependent variable).

3. Results and discussion

3.1. Measurement of RLU on milking equipment surfaces

The measured values of RLU on the various types of milking equipment surface are shown in Table 1. The lowest arithmetic mean values of RLU were observed on teat cup rubbers, and the highest on pipeline joints. These results are as expected, since the measurements were made on the joints of pipelines in tie-stall milking systems, where environmental contamination and the difficulty of achieving proper cleanliness are greater.

Only in three measurements (one of each type of surface), the luminometer was overloaded (value greater than 10^5 RLU): this could be related to major cleaning system failures, such as using cold water or washing without detergent. These values were not included in the statistical calculations. Very low RLU values were observed in teat dip containers and milk receivers, indicating absence of contamination, and thus generally effective working of cleaning systems. Slaghuis and Wiegersma (1996) reported a very wide range of RLU values (9-38570) on several types of milking equipment surface, in a study of only 3 farms.

3.2. Associations between hygienic and milking factors and LRLU values

Table 2 summarizes the results of ANOVAs to assess the effects of hygienic and milking factors (cleaning water type, milking system, and frequency of use of acid detergent) on LRLU.

The hygienic quality of the water used for cleaning is important, because it may affect the BC in the milk; it is advisable to use drinking-quality water (Marco, 1998). In this study, most farms (68.9%) used water from private wells without any chlorination treatment. Mean LRLU values were significantly higher in teat cup rubbers and milk receivers from these farms than from farms using chlorinated water ($P=0.04$ for teat cup rubbers, $P=0.02$ for milk receivers). Mean LRLU was also higher in teat dip containers and pipeline joints from farms using non-chlorinated water, but these differences were not statistically significant.

LRLU in teat cup rubbers was likewise influenced by the milking system, though only 19.3% of variability was explained by this factor. Specifically, mean LRLU was significantly higher ($P<0.05$) in teat cup rubbers from farms using bucket tie-stall milking, as expected since cleaning in these milking systems is manual, and the temperature of

Table 2
Association between milking and cleaning practices and $\log_{10}$ relative luminescence units (LRLU) for each type of milking equipment surface

		Chlorinated water		Milking system			Use of acid detergent			
		Yes	No	Parlour	Pipeline	Bucket	No	Weekly	Several/week	Daily
Teat cup rubbers	Average	1.91	2.21	1.98	1.76	2.74	2.38	1.94	2.09	1.99
	SD[1]	0.66	0.65	0.61	0.58	0.64	0.47	0.68	0.69	0.66
	Sig[2]	0.04			0.00				0.35	
Teat dip containers	Average	2.19	2.23	2.22	2.26	2.02	2.43	2.18	2.23	2.32
	SD[1]	0.90	1.01	0.90	1.05	0.81	0.74	0.92	0.63	1.15
	Sig[2]	0.85			0.77				0.89	
Milk receivers	Average	1.86	2.66	1.76	2.33		0.78	1.82	2.55	2.67
	SD[1]	0.97	1.12	1.03	1.02			0.82	1.21	1.20
	Sig[2]	0.02			0.06				0.04	
Pipeline joints	Average	2.75	3.07		2.89			2.39	2.81	3.15
	SD[1]	1.14	1.37		1.23			1.37	1.03	1.13
	Sig[2]	0.55			0.06				0.87	

[1]SD: standard deviation.
[2]Sig: significance with ANOVA test.

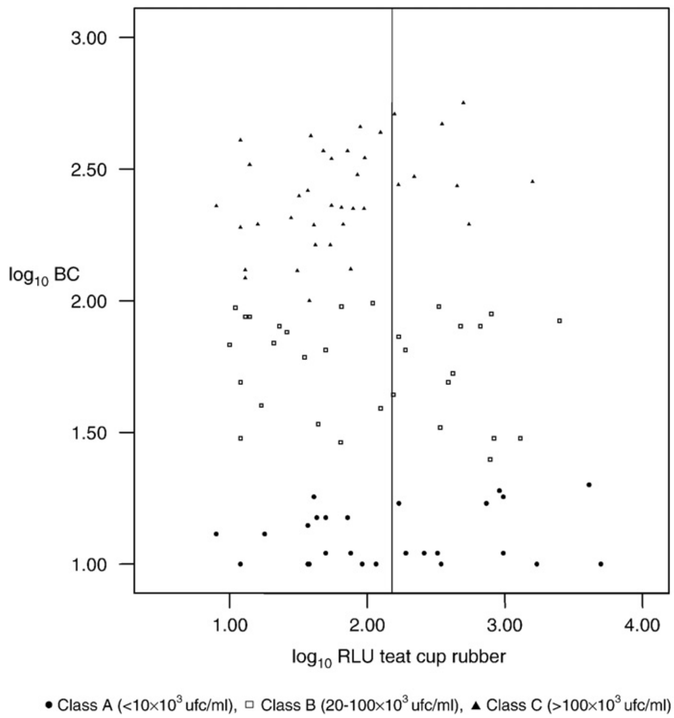

Fig. 1. Scatterplot of $\log_{10}$ bacterial count (BC) in bulk-tank milk *versus* $\log_{10}$ relative luminescence units (RLU) of 106 teat cup rubber surfaces, showing the cleanliness cut-off point of 2.18 $\log_{10}$ RLU (152 RLU). ● Class A ($<10\times10^3$ ufc/ml), □ Class B (20–100×10^3 ufc/ml), ▲ Class C ($>100\times10^3$ ufc/ml).

the cleaning water is often not appropriate (Marco, 1998). Mean LRLU in teat dip containers from farms with pipeline tie-stall milking was significantly higher than in teat dip containers from farms with parlour systems, in which the distance travelled by the cleaning

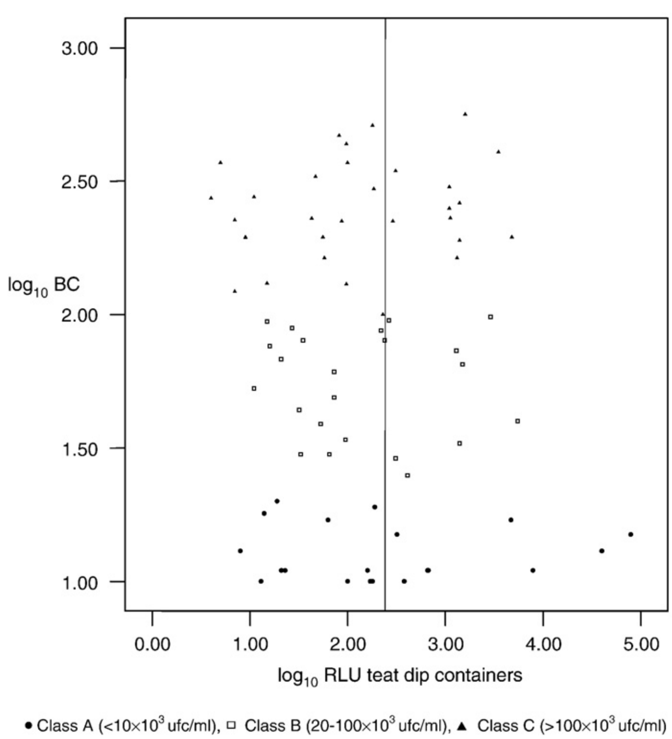

Fig. 2. Scatterplot of $\log_{10}$ bacterial count (BC) in bulk-tank milk *versus* $\log_{10}$ relative luminescence units (RLU) of 84 teat dip container surfaces, showing the cleanliness cut-off point of 2.38 $\log_{10}$ RLU (242 RLU). ● Class A ($<10\times10^3$ ufc/ml), □ Class B (20–100×10^3 ufc/ml), ▲ Class C ($>100\times10^3$ ufc/ml).

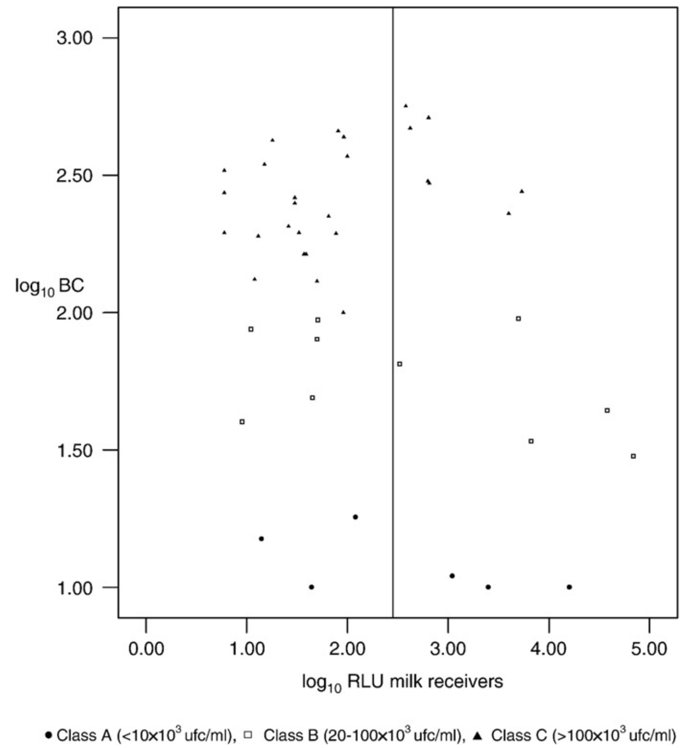

Fig. 3. Scatterplot of $\log_{10}$ bacterial count (BC) in bulk-tank milk *versus* $\log_{10}$ relative luminescence units (RLU) of 52 milk receiver surfaces, showing the cleanliness cut-off point of 2.45 $\log_{10}$ RLU (282 RLU). ● Class A ($<10\times10^3$ ufc/ml), □ Class B (20–100×10^3 ufc/ml), ▲ Class C ($>100\times10^3$ ufc/ml).

solution to the bulk tank is shorter, and thus the risk of contamination lower (notably because, when the pipeline is long, both temperature and pressure are lower at the end point) (Marco, 1998). Mean LRLU in

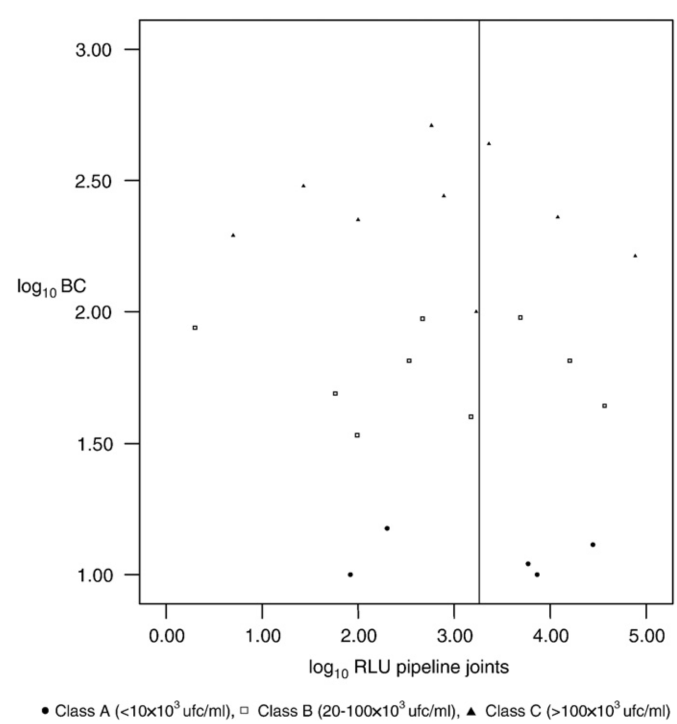

Fig. 4. Scatterplot of $\log_{10}$ bacterial count (BC) in bulk-tank milk *versus* $\log_{10}$ relative luminescence units (RLU) of 23 pipeline joints, showing the cleanliness cut-off point of 3.26 $\log_{10}$ RLU (1821 RLU). ● Class A ($<10\times10^3$ ufc/ml), □ Class B (20–100×10^3 ufc/ml), ▲ Class C ($>100\times10^3$ ufc/ml).

milk receivers were likewise higher in farms with pipeline milking than in farms with parlour systems ($P=0.06$).

The frequency of use of acid detergent to clean the inner surfaces of pipelines in contact with milk had a significant effect on LRLU values in milk receivers ($P=0.04$), but not in other surfaces. In teat cup rubbers and teat dip containers, LRLU was highest on farms where acid detergent was never used and lowest on farms where acid detergent was used weekly. By contrast, in milk receivers and pipeline joints LRLU was highest on farms where acid detergent was used daily, and lowest on farms where acid detergent was not used or was used only once a week. This difference could be explained by the thrust effect in milk receivers and pipeline joints when acid detergent was used daily. Alternatively, the difference might reflect some unknown difference in management practice between farms which could not be determined.

3.3. Associations between LRLU values and bulk-tank bacterial count

Figs. 1, 2, 3 and 4 summarize associations between LRLU values for the different surfaces and LBC in bulk-tank milk for the three classes of farm (A, B and C). Based on mean LRLU values observed in the farms class A, we have established reference values of RLU for each surface. Mean LRLU values were 2.28, 2.38, 2.45, and 3.26 respectively in teat cup rubbers, teat dip containers, milk receivers, and pipeline joints; i.e. 152, 242, 282, and 1821 RLU respectively. These values are achievable targets for all farmers. Using these values, 63, 63, 67 and 61% respectively of teat cup rubber, teat dip container, milk receiver and tie-stall pipeline joint surfaces considered in the present study showed acceptable cleanliness.

Table 3 shows the results of multiple linear regression for prediction of LBC bulk tank from the independent variables $\log_{10}$ teat cup rubber RLU (LRLUtcr), $\log_{10}$ teat dip container RLU (LRLUtdc), $\log_{10}$ milk receiver RLU (LRLUmr), and $\log_{10}$ pipeline joint RLU (LRLUpj), giving the following equation:

Eq. (1). Multiple linear regression equation.

$$\mathrm{LBC} = 1.93 + 0.30 \times \mathrm{LRLUtcr} - 0.09 \times \mathrm{LRLUtdc} - 0.08 \times \mathrm{LRLUmr} - 0.05 \times \mathrm{LRLUpj}$$

The correlation coefficient by this equation was $r^2=0.12$ ($P=0.78$); that is, milking equipment hygiene as assessed by ATP bioluminescence only explained 12% of the variance in BC in milk, as expected since BC is also affected by other factors such as cows' health status or cooling tank conditions. Moreover, most of the surfaces evaluated in the present study were on farms with mean BC below 20×10^3 cfu/ml.

Table 3
Multiple linear regression analysis for prediction of $\log_{10}$ bacterial count (BC) in bulk-tank milk from $\log_{10}$ relative luminescence units (RLU) values of each type of milking equipment superface

Model	Non-standardized coefficients		Standardized coefficients	t[a]	Sig.[b]	CI[c] of β (95%)	
	β	Typical error	β	–	–	lower Limit	Upper limit
Constant	1.93	1.15	–	1.68	0.12	−0.57	4.43
LRLUtcr[d]	0.30	0.33	0.26	0.91	0.38	−0.42	1.02
LRLUtdc[e]	−0.09	0.21	−0.12	−0.40	0.69	−0.55	0.38
LRLUmr[f]	−0.08	0.16	−0.14	−0.48	0.64	−0.43	0.27
LRLUpj[g]	−0.05	0.14	−0.09	−0.34	0.74	−0.36	0.26

Coefficients, dependent variable: $\log_{10}$ BC.
[a] t: Student–Fisher value.
[b] Sig.: significance with multiple linear regression analysis.
[c] CI: confidence interval of β.
[d] LRLUtcr: $\log_{10}$ teat cup rubber RLU.
[e] LRLUtdc: $\log_{10}$ teat dip container RLU.
[f] LRLUmr: $\log_{10}$ milk receiver RLU.
[g] LRLUpj: $\log_{10}$ pipeline joint RLU.

This correlation coefficient cannot be compared with previous studies, since as far as we are aware this is the first time that ATP bioluminescence values for milking equipment surfaces has been analysed in relation to total BC in bulk-tank milk.

The hygiene of internal surfaces of the pipelines and containers in which milk circulates reportedly accounts for 50–80% of total BC in bulk-tank milk (Marco, 1998); however, in the present study we have not been able to accurately predict total BC in bulk-tank milk from ATP bioluminescence values for milking equipment surfaces. Notwithstanding this, our findings are consistent with the accepted relationship between number of colony-forming units in pure culture and luminescence produced under conditions with relatively stable ATP background, when both are measured on the same surface. In any case, an acceptable hygiene of the milking equipment and bulk tank is of course essential (Poulis et al., 1993).

The presence of bacteria (mainly Gram-negative psychrotrophic bacteria) and other organic debris in milk pipelines or milk cooling/storage tanks is generally due to an inadequate programme of cleaning, sanitation, and/or maintenance (Marco, 1998; Murphy et al., 1998). Therefore, BC is considered a reliable indicator of the cleanliness with which milk is handled. It has sometimes been used as an indirect indicator of the cleanliness of milking equipment, but it is not a very accurate indicator because various other factors are important for the hygienic quality of raw milk, including correct udder preparation (teat cleanliness and fore-stripping), use of high-quality water, and notably the correct functioning of the cooling tank, and the length of the time during which the milk is kept in it (Nieuwenhof, 1996; Marco, 1998).

Despite the lack of a close relationship with bulk-tank BC in the present study, the use of the ATP bioluminescence method for monitoring the hygiene of surfaces in contact with milk certainly remains of value, principally within HACCP programmes, because it is a fast and simple method well suited for field use. It can also be used as an objective on-the-spot method to demonstrate the effectiveness of milking equipment cleanliness to farmers.

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References

Aycicek, H., Oguz, U., Karci, K., 2006. Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. International Journal of Hygiene and Environmental Health 209, 203–206.

Brovko, L.Y., Froundjian, V.G., Babunova, V.S., Ugarova, N.N., 1999. Quantitative assessment of bacterial contamination of raw milk using bioluminescence. Journal of Dairy Research 66, 627–631.

Champiat, D., Matas, N., Monfort, B., Frass, H., 2001. Applications of biochemiluminescence to HACCP. Luminescence 16, 193–198.

Corbitt, A.J., Bennion, N., Forsythe, S.J., 2000. Adenylate kinase amplification of ATP bioluminescence for hygiene monitoring in the food and beverage industry. Letters in Applied Microbiology 30, 443–447.

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, 33–38.

De Celis, P., 2006. Control de la limpieza de los equipos mediante bioluminiscencia. XI Congreso Anembe de Medicina Bovina. 5–6 May, Zaragoza, pp. 101–102.

Finger, R., Sischo, W.M., 2001. Bioluminescence as a technique to evaluate udder preparation. Journal of Dairy Science 84, 818–823.

Griffiths, M.W., 1993. Applications of bioluminescence in the dairy industry. Journal of Dairy Science 76, 3118–3125.

Marco, J.C., 1998. Mamitis bovina y calidad de la leche. Bovis 85, 45–71.

Murphy, S.C., Kozlowski, S.M., Bandler, D.K., Boor, K.J., 1998. Evaluation of adenosine triphosphate-bioluminescence hygiene monitoring for trouble shooting fluid milk shelf life problems. Journal of Dairy Science 81, 817–820.

Nieuwenhof, F., 1996. Raw milk, hygiene management and ATP bioluminescence. Proceedings, IDF Symposium on Bacteriological Quality of Raw Milk. 13–15 March, Austria, pp. 113–118.

Poulis, J.A., de Pijper, M., Mossel, D.A.A., Dekkers, P.P.A., 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. International Journal of Food Microbiology 20, 109–116.

Samkutty, P.J., Gough, R.H., Adkinson, R.W., McGrew, P., 2001. Rapid assessment of the bacteriological quality of raw milk using ATP bioluminescence. Journal of Food Protection 64, 208–212.

Seeger, K., Griffiths, M.W., 1994. Adenosine triphosphate bioluminescence for hygiene monitoring in health care institutions. Journal of Food Protection 57, 509–512.

Siragusa, G.R., Cutter, C.N., 1995. Microbial ATP bioluminescence as a means to detect contamination on artificially contaminated beef carcass tissue. Journal of Food Protection 58, 764–769.

Siragusa, G.R., Cutter, C.N., Dorsa, W.J., Koohmaraie, M., 1995. Use of a rapid microbial ATP bioluminescence assay to detect contamination on beef and pork carcasses. Journal of Food Protection 58, 770–775.

Slaghuis, B., Wiegersma, W., 1996. Use of ATP-technique for the control of the cleaning of milking equipment. Proceedings, IDF Symposium on Bacteriological Quality of Raw Milk. 13–15 March, Austria, pp. 173–174.