

## Using of ATP-bioluminescence assay for hygiene-monitoring in dairy plants

ILIRIAN POSTOLI<sup>1</sup>

PhD, Faculty of Veterinary Medicine  
Agricultural University of Tirana, Albania

FATMIRA SHEHU

Department of Veterinary Public Health  
Faculty of Veterinary Medicine  
Agricultural University of Tirana, Albania

### Abstract:

*The efficiency of bioluminescence applied to monitor the state of hygiene in a dairy processing plant was assessed in relation to the results of contact slides method. ATP-bioluminescence assay was used to evaluate the cleaning and sanitizing procedures of stainless steel milk contact surfaces. The analyzed object was the surface of a raw milk transportation tank, a raw milk cooling storage tank, and milk centrifuge. The Relative Light Unit (RLU) is measured using a BioFix Lumi-10 and the Total Number of Bacteria was determined using "Contact Slides" in accordance with ISO 18593. For both methods, a set of 20 samples at each sampling point was taken in parallel, twice per each working day: at the start of the day when the surfaces had been cleaned and sanitized and at the mid of the day following the use of surfaces but before cleaning so as to compare hygiene conditions before and after the cleaning procedures. A total of 120 bacteriological samples and 120 samples for ATP assay testing were performed. ATP-bioluminescence method showed that 95% (114/120) of the surfaces were under inadequate hygiene conditions, while the Contact Slide detected only 55,8% (67/120), based on the APHA's recommendation, and 44,2% (53/120), based on the WHO's recommendation. High*

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<sup>1</sup> Corresponding author: [ipostoli@yahoo.com](mailto:ipostoli@yahoo.com)

*variations in RLU measurements were observed, suggesting the need for more than only one surface analysis.*

**Key words:** Dairy plant, ATP bioluminescence, Contact slide, HACCP

## **INTRODUCTION**

Microbial contamination of food increases the risk of illness and causes severe health consequences for people that could lead to death. Thus, the microbiological evaluation of food contact surfaces is important to assess the efficiency of cleaning systems on surfaces. A visual assessment for hygiene monitoring is insufficient in determining the possible risk posed by the contaminated food contact surfaces. The existence of foodborne pathogens on the surfaces of food-service establishments and their possibility to spread from surfaces to food are necessary reasons to implement effective cleaning regimes (Verran, J et al 2002).

All food businesses have a legal obligation to produce safe food. Food safety is primarily achieved through a preventative approach such as the implementation of a food safety management system based on the principles of Hazard Analysis and Critical Control Point (HACCP) and good hygiene practice (GHP) (Final Report., 2013). Both of these are legal requirements. In compliance with the Ordinance of the European Committee no. 1441/2007 of 5 December 2007 enterprises of the agro-food sector, including the dairy industry, are required to provide for foodstuffs to meet the respective microbiological criteria and to ensure the maintenance of respective hygienic criteria for the processes of their production. The development of new products and new food processing technologies, the increase in trade among countries, the concern about food safety and the increase in reports on food-borne disease outbreaks have shown that the implementation of more

effective and rigorous hygiene procedures is fundamental to the milk processing industry. Thus, the dairy food industry has been seeking rapid and sensitive techniques to assess hygienic conditions of milk processing contact surfaces. ATP bioluminescence assay may be used for hygiene monitoring in food industry (Kusumaningrum, H. D et al, 2003). The microbiological methods assessing cleanliness of the surfaces in contact with food, such as swabbing and contact plate method, are broadly used, but time-consuming due to the culturing stage. In manufacturing conditions, a method for rapid evaluation of microbiological cleanliness is sought for. This is associated with the requirements of the systems that assure safety of food production, which consist in rapid and efficient hazard detection and estimation in the control points of the HACCP system (Champiat et al., 2001). The technique of bioluminescence is applied in sectors producing animal products, in particular meat, and in farms with dairy and slaughter animals as well as in dairy industry (Finger and Sischo, 2001).

Not only does this technique allow microbial ATP to be measured, but also indicates the ATP level of food debris and other organic contaminants, which might become a culture medium for microbes. This enables to detect incorrectness in the process of cleaning and disinfection, but it requires establishing certain critical limits. Moreover, this method may be used on a continuous basis during the production process, which, if incorrectness has been detected, enables to undertake immediate action so as to make corrections and establish hazards in real-time (Aycicek et al., 2006)

The bioluminescence method uses the capacity of organic matter to release adenosine triphosphate (ATP), which is the principal energy source in all living organisms. This capacity is characteristic of bacteria, fungi and other microbes as well as food and food debris, including that on disinfected surfaces (Luo J et al.,2009). The principle of the assay is based on the

following enzymatic reaction:  $ATP + \text{Luciferin/Luciferase} \rightarrow AMP + PP + \text{light}$ .

Luciferase catalyses the oxidation reaction of luciferin to the form of higher energy state. The reaction can proceed properly only when energy carried by ATP is delivered. ATP breaks down to adenosine monophosphate (AMP) and phosphoric residues (PP). The oxidized form of luciferin returns to its primary energy state by emitting light with the wave of 562 nm, the precise measurement of which enables indirect assessment of ATP concentration. The amount of light emitted is directly proportional to the concentration of ATP. Thus, it is assumed that the amount of ATP in the sample is directly proportional to the amount of the microbial biomass. The amount of light emission, which results from the luminescence reaction, is measured with the use of a luminometer. This device contains a measuring chamber isolated from external light sources and a detector that processes the optic signal to the electrical one, which is expressed in relative light units (RLU).

## **MATERIAL AND METHODS**

### **Collection of samples**

The study was performed at 4 dairy plants in Tirana Region-Albania, through testing of 120 samples, respectively with 30 surface samples for the counting of Total Number of Bacteria. The object selected for analyses were surface samples for the counting of Total Number of Bacteria. The analyses were conducted on the surface of test from stainless steel surfaces from three pieces of equipment including raw milk transportation tank, raw milk cooling tank, and milk centrifuge. Cleanliness of adjacent surfaces in the object was analyzed after washing and disinfection processes by microbiological blotting tests and by bioluminescence. For both methods, a set of 20 samples at each sampling point was taken

in parallel, twice per each working day: at the start of the day when the surfaces had been cleaned and sanitized and at the mid of the day following the use of surfaces but before cleaning so as to compare hygiene conditions before and after the cleaning procedures. A total of 120 bacteriological samples and 120 samples for ATP assay testing were performed. According to recommendations of the ATP-bioluminescence equipment manufacturer, measurements lower than 150 RLU were considered clean, from 151 to 300 RLU were considered suspect, and values higher than 301 URL were considered inadequate hygienic conditions.

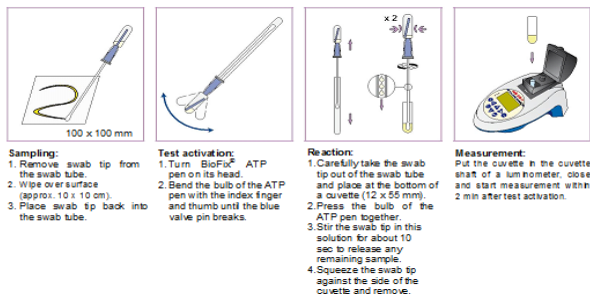
### **"Contact Slide TM" Method**

Microbiological test Envirocheck Contact DC by Merck KGaA (Darmstadt, Germany) were used to assess the efficiency of washing and disinfection of surfaces of technological line equipment at a dairy processing plant. The test is a rectangular plastic plate of approx. 9 cm<sup>2</sup>, covered on both sides with a layer of agar medium, and placed in a sterile vial. In the Envirocheck Contact DC test one side of the strip is covered by trypticase soy agar (TSA). It is an agar medium with casein peptone and soy flour peptone for the determination of microbiological load before washing and disinfection. The other side of the strip is covered with Agar with an addition of neutralizers. The application of neutralizers facilitates the growth of bacteria, which development was only inhibited as a result of washing and disinfection and which still remain capable of growing. The Agar medium with neutralizers is used to investigate microbiological load on the surface following washing and disinfection. The procedure was performed according to the manufacturer's recommendations. The cap on the tube was unscrewed and the Envirocheck slide was removed from the tube, taking care not to touch the agar surfaces. The slide was checked before use for any sign of dehydration or contamination. The terminal end of the paddle was held with

two fingers against the surface to be tested. The spike was pressed down to bend the paddle, while still holding the slide by the cap. With a firm and even pressure one medium was pressed against the surface to be tested. Care was taken not to smear the agar over the test area. The procedure was repeated with the other side of the paddle on an area adjacent to the initial test site. The slide was replaced back into the tube and closed tightly. Tests, after being pressed to the analyzed surface and replaced back to their tubes, were transported at a temperature up to 5°C. Tubes with tests were placed vertically in a mini incubator at 37°C for 48 h. Microbial counts were given in cfu/cm<sup>2</sup> area.

### ATP-bioluminescence

The total ATP on surfaces was detected by the Clean Test kit (5), following the same approach for mesophilic aerobics. Swabs were placed in a cuvette and the ATP sampled on surfaces reacted with the luciferin-luciferase enzymatic complex emitting light. The kits were transferred to the luminometer chamber and the bioluminescence measurements were determined after 10 seconds. The results were expressed as log<sub>10</sub> RLU released by the total ATP. According to recommendations of the ATP-bioluminescence equipment manufacturer, measurements lower than 150 RLU were considered clean, from 151 to 300 RLU were considered suspect, and values higher than 301 RLU were considered inadequate hygienic conditions.



**Fig. 1 Procedure for hygiene monitoring of surfaces: ATP-bioluminescence**

## INTERPRETATION OF RESULTS

According to recommendations of the ATP-bioluminescence equipment manufacturer, measurements lower than 150 RLU were considered clean, from 151 to 300 RLU were considered suspect, and values higher than 301 URL were considered inadequate hygienic conditions. The cleaning protocol used during this investigation ensured that, prior to inoculation, all traces of residual organic debris were removed from the test surface. After inoculation; if residual organic debris was detected on a surface, then that surface would be presumed dirty. This was the case if average ATP readings were >100 RLU (Clean-Trace/Uni-Lite), or if the color of the protein test differed from that indicated as clean by the manufacturer. The presence of microbial contaminants on a surface was presumed if the average number of microorganisms recovered from the surface was >1 CFU/100 cm<sup>2</sup> ( Moore G., et al 2001)

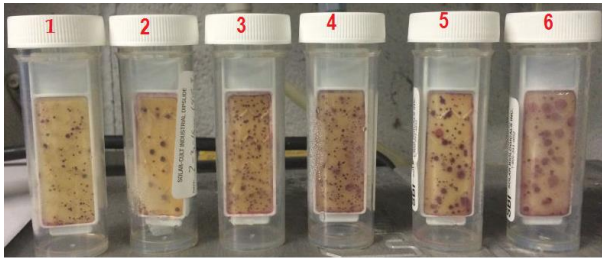
ATP Concentration (RLU)	Hygiene level
< 500	Good
501-1000	Average
> 1001	Bad

*Table 1: Scale of hygiene level depends on the manufacturer's choice of ATP concentration (RLU)*

## RESULTS AND DISCUSSION

A total of 120 bacteriological samples and 120 samples for ATP assay testing were performed. The ATP-bioluminescence method showed that 95% (114/120) of the surfaces were under inadequate hygiene conditions, while the Contact Slide detected only 55,8% (67/120), based on the APHA's recommendation (Evancho, G.M., et al, 2001) and 44,2% (53/120), based on the WHO's recommendation. High variations in RLU measurements were observed, suggesting the need for more than only one surface analysis.

The milk centrifuge, raw milk cooling tank surfaces showed the highest mesophilic aerobic counts, 2.24 and 1.19 log<sub>10</sub> respectively. Effectively cleaned milk contact surfaces will have low levels of ATP. The detection of higher levels of ATP on surfaces tested showed that milk or bacteria are remain on the surface.



**Fig. 2 Contact Slide after incubation**

## **CONCLUSION**

Although ATP bioluminescence technology cannot substitute traditional microbiological analyses for the determination of microbial load on food contact surfaces, it has proved to be a powerful tool for the real time monitoring of surface cleanliness at dairy plants, for verify the correct application of SSOP, and hence for their implementation/revision in the case of poor hygiene (Griffith, C. 2005). Contamination has always been a threat to public health, but the traditional testing methods have several drawbacks. It can be concluded that the implementation of the ATP-bioluminescence assay within food facilities is a reliable yet rapid detection technique for monitoring the cleanliness of surfaces and hygiene practices (Zubair M. Azizkhan, 2014). The ATP monitoring system helps to quickly identify contaminated areas so that corrective actions can be taken in a timely manner. Accordingly, this method can effectively enhance the assessment of sanitary conditions and, therefore, would support cleaning and sanitizing needs, assure safe operations and reduce interruption of processes.



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