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# Evaluation of the microbial parameters and hygiene status of dairy establishments in Tirana region

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**Abstract:**

*The most effective way to achieve food safety is to focus on prevention of possible hazards and to improve the process. Microorganisms in the milk processing plants environments may be found in the atmosphere (airborne), on food contact surfaces and/or employees hands. Airborne microorganisms from food handlers and in food products and raw materials (as part of bioaerosols) have in the past been implicated as having a potential to cause adverse health effects (especially in indoor environments) and therefore also to have economic implications. Recently their effect on food safety has received increased interest. The recent international interest in bioaerosols in the food industry has played a role in rapidly providing increased understanding of bioaerosols and their effects in different food processing environments. However, there is still a lack of research on the actual impact of bioaerosols over time in most of milk processing plants and the food premises in Albania. Also, special attention should be paid to hygienic testing of contact surfaces and employees hands, as possible ways of contaminating the product. Through this study, during a period of 12 months (from January to December 2016),*

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*we tested 24 environmental samples in 4 milk processing establishments in Tirana Region: 80 air samples, 80 contact surface and 80 from employees hands. Two methods for air sampling were used (impaction technique and sedimentation technique) and 2 methods for sampling surfaces and employees hands (contact slide and PRO-Clean test). The results demonstrated a poor hygienic situation in the samples being analyzed. For all the processing areas in the dairy plant, the numbers of mesophilic aerobic bacteria, yeasts and molds obtained by air samprer MAS-100 NT® were higher than 90 CFU $m^{-3}$, and according to culture settling plate technique higher than 30 CFU.$cm^{-2}$, the maximum value recommended by APHA's standard. Analysis of the Contact slide from employees hands revealed 5/80 positive sample for enumeration Enterobacteriaceae and 14/80 samples with higher total viable count. While the analysis of the contact slide from contact surfaces detected 17/80 samples positive for Enterobacteriaceae and 13/80 samples with higher total viable count. The swab samples (Protein Residue Test) taken from employees hands and contact surfaces, detected inadequate sanitary procedures during the phase of milk processing.*

**Key words:** Dairy plant, air, PRO-Clean, employees hands, Contact slide

## INTRODUCTION

Food production environments are considered critical factors in determining the quality and safety of food products and in recent years, the demand by consumers and retailers for the production of higher quality foods has increased. The dairy industry, which is associated with high-risk foods, is a major food industry that does not only produce dairy beverages but also raw materials for other food industries.

In the food processing establishments, there are many conditions that would provide a good environment for the attachment of bacteria and possible biofilm formation. In the dairy industry, raw milk is processed through a number of

steps such as chilling, pasteurization and homogenization, into a variety of milk (both liquid and dried) and milk products such as butter, cheese, ice cream, and yoghurt. Potential sources of contamination include both direct and indirect contact with contaminated water sources, unhygienic processing conditions and environmental surfaces, poor personal hygiene of food handlers, factory design, airborne contaminants, presence of animals and the efficacy of the cleaning procedures (Marchand, S., et al 2012).

The quality of the air in food processing plants remains a great concern, even though most plants strive to control it. Studies have indicated that air is one of the probable sources of contamination in various food processing environments, including those that process dairy products (Marchand, S., et al 2012, Sutton, 2004; Shale et al., 2007). It is as a result of the abovementioned potential contamination sources that the dairy environment is deemed a reservoir for foodborne pathogens (Oliver et al., 2005).

In the European Union, apart from systems of monitoring of the origin of food products, companies are also obliged to implement systems which ensure food safety such as HACCP and GHP/GMP. Also the Albanian food processing sector is obliged to implement the above-mentioned systems.

The HACCP system has become a synonym for sanitary security of food products. It is worldwide acknowledged as a systematic and preventive approach to control biological, chemical and physical dangers (hazards) (Panfiloiu et al. 2010). HACCP is a systematic method, preventive and science based, which first priority is the safety of the products through risk identification and risk management in the production process. It has a proactive, rather than reactive approach, emphasizing food hazard prevention rather than the detection of harmful defects in finished food products. Its main objective is to identify problems before they occur, establishing control measures that are critical to maximizing food safety at each

stage in the production process (Cannas and Noordhuizen 2008).

In developing countries, various factors combine to compromise the hygienic quality of milk products: the organization of milk supply chains themselves, dysfunctioning of the regulatory systems and quality control structures. In the European Union, apart from systems of monitoring of the origin of food products, companies are also obliged to implement systems which ensure food safety such as HACCP and GHP/GMP. Also the Albanian food processing sector is obliged to implement the above-mentioned systems.

Food products differ in their biochemical composition, they are also susceptible to contamination and/or spoilage by different microorganisms including airborne microbes. Some of these microbes can play a role in causing foodborne illnesses and foodborne outbreaks. The latter have increased notably over the past two decades in both developed and third-world countries (Rocourt et al., 2003).

The presence of airborne microorganisms in food processing plants represents a challenge due to the economic and health problems they may cause, as research has shown that processing plants are prone to indoor air contamination. Shale (2007) demonstrate that the presence of airborne contaminants can influence the quality of the food products, amongst others.

APHA recommends the following standards for aerobic plate count in the air of food processing areas 90 CFU $m^3$, when evaluated by the air sampler technique and 30 CFU$cm^{-2}$ $week^{-1}$ when evaluated by the culture settling plate technique, using plate count agar as culture medium (APHA, 2001).

Moreover, Jullien and co-workers (2002) report on pathogenic microorganisms' ability to contaminate surfaces as a serious concern in the food industry. Microorganisms are known to settle on and contaminate working surfaces, equipment and the hands of workers, which could lead to

contamination of milk and other dairy products (Cruz, P. et al 2007).

## MATERIAL AND METHODS

During a period of 12 months (from January to December 2016), 240 samples were collected and examined, 80 of samples were collected from air for the counting of Total Number of Bacteria (Impaction technique- air sampler MAS-100 NT®) , 80 from working surfaces (contact slide / PRO-Clean test) and 80 from employees hands (contact slide and swabs)

### Bioaerosol sampling

Microbial quality of air in a dairy processing plant was evaluated, by using a air sampler MAS-100 NT® (Merck Millipore) and Culture settling plate technique. Air sampling is useful for monitoring airborne biological agents and can be conducted qualitatively or quantitatively (Asefa D.T et al 2009). Quantitative methods include active air sampling (impaction technique) and passive air sampling (sedimentation technique) on solid surfaces (APHA 2001).

*Settling plate technique and isolation of microorganisms*

For the settling plate method, the aerosolised microorganisms were collected on an open petri dish containing suitable culture media. When the sampling session was over, the petri dishes were closed and incubated at 35°C for 48 hours, 25°C for 3-5 days and for 37°C for 24 hours for aerobic plate count, yeasts and moulds, and total coliform and *S. aureus* respectively (Salustiano *et. al.,* 2003). For the isolation of indicator organisms *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and the total viable aerobic organisms as well as the total viable fungi.

Plate Count Agar (PCA), Chromocult Coliform Agar (CCA), Baird Parker (BPA) and Potato Dextrose Agar (PDA)

(Merck, SA) with a pH=3.5 (tartaric acid) were used. Subsequent incubation of the plates was done at appropriate temperatures and incubation periods.

*Impaction technique*

All microbial samples were collected at a height of 1,5 m above the floor by means of impaction on soft agar plates. For the impaction technique, air sampler MAS-100 NT® (Merck Millipore) is used. The air sampler MAS-100 was calibrated , pre-autoclaved and disinfected with 70% ethanol between each sample run (Venter *et al.*, 2004; Coccia *et al.,* 2010). Active air sampling typically relies upon devices that draw a fixed volume of air at a specific speed over a specific period time for the assessment of viable airborne microorganisms (Andon B.M. et al 2006). Active air sampling is faster and recovers more airborne microorganisms than passive techniques. Additionally, active sampling methods are more sensitive in determining pathogenic contamination in specified areas (APHA 2001, Salustiano V.C et al 2003).

The impaction technique, volumes of 100 l of air were suctioned by air sampler and impressed on solid medium surface contained on Petri dishes, according to APHA's recommendation (Sveum W.H et al 2002). After microbial determinations, the Petri dishes were incubated in the same conditions as the culture settling plate technique. The results were expressed as CFU $m^{-3}$ of air (Kang Y. J,et al 1999). The final result in CFU/m3 of air is obtained using the formula:

$$X = \frac{CFU/plate\ x\ 100}{Volume\ of\ air}$$

**Protein Residue Test - Igienist – HACCP**

**Validation of hand cleanliness** – ATP testing was used to verify proper washing techniques and cleanliness of employees' hands used directly on skin. When doing this type of testing, it is important to identify appropriate pass/fail levels taking into

account naturally occurring ATP levels in skin cells. PRO-Clean quickly and accurately monitors the cleanliness of surfaces to help ensure product quality by detecting protein residues left behind after cleaning. The test is simple to use. Swabbing a surface, release the reagent, and if protein residue is present the reagent will turn purple. The color change provides a semi-quantitative measure of the surface cleanliness. Detects down to 20 ug of protein and requires no instrumentation.

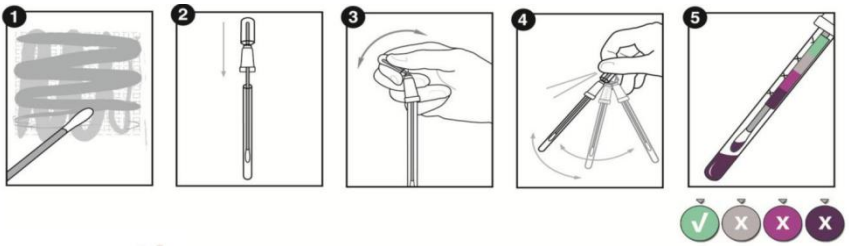


**Fig. 1 PRO-Clean Procedure**

The more contamination present, the quicker and darker the color change to purple. PRO-Clean quickly validates the hygiene of a surface, allowing immediate corrective action to be taken if necessary. The use of PRO-Clean has these benefits: Results in 1-10 minutes, easy to interpret color change, no instrumentation required, Unique liquid stable chemistry produces consistent results, Pre-moistened swab provides reliable collection, recovery, and detection to ensure consistent results.

**"Contact Slide TM" Method - employees hands**

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 $cm^2$, 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$^2$ area.

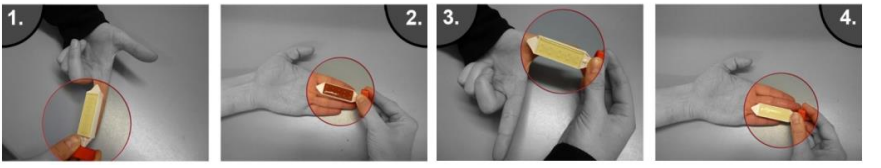


**Fig.2 Sampling Procedures employee hands using Contact Slide TM**

## RESULTS AND DISCUSSION

The results demonstrated a poor hygienic situation in the samples being analyzed. For all the processing areas in the dairy plant, the numbers of mesophilic aerobic bacteria, yeasts and molds obtained by air samprer MAS-100 NT® were higher than 90 CFU $m^{-3}$, and according to culture settling plate technique higher than 30 CFU.$cm^{-2}$, the maximum value recommended by APHA's standard. Analysis of the Contact slide from employees hands revealed 5/80 positive sample for enumeration *Enterobacteriaceae* and 14/80 samples with higher total viable count. While the analysis of the contact slide from contact surfaces detected 17/80 samples positive for *Enterobacteriaceae* and 13/80 samples with higher total viable count (Table.1). The swab samples (Protein Residue Test) taken from employees hands and contact surfaces, detected inadequate sanitary procedures during the phase of milk processing.

### Air samples

For all the processing areas in the dairy plant, the numbers of mesophilic aerobic bacteria, yeasts and molds obtained by air samprer MAS-100 NT® and according to culture settling plate technique where higher than 90 CFU.$m^{-3}$, the maximum value recommended by APHA's standard (30 CFU.$cm^{-2}$. $week^{-1}$) (APHA 2001, Downes F. P et al 2001)

The microbiological numbers in the air of the processing areas obtained by air sampler were between 15 CFU $m^{-3}$ and 1400 CFU $m^{-3}$, and are similar to the results reported in other studies (Radha K 2014, Salustiano V.C 2003). The culture settling plate technique was not able to detect *coliforms* and *Staphylococcus aureus* in the evaluated processing areas. The results of compact technique showed a difference ($p<0,05$) for the *Staphylococcus aureus* numbers at processing areas.

The number of microorganisms recovered by impaction technique were about 2 to 9 times higher than by sedimentation technique, of mesophilic aerobic bacteria. The increase of temperature at processing areas did not seem to affect the numbers of airborne microorganisms. On the other hand, the increase of air humidity showed a relation with the increase of microorganisms numbers. The impaction technique should be chosen since it is better to recover airborne (Kang Y. J et al 1989)

**Surface samples (Protein Residue Test)**

Analysis of the Contact slide from employees hands revealed 5/80 positive sample for enumeration *Enterobacteriaceae* and 14/80 samples with higher total viable count. While the analysis of the contact slide from contact surfaces detected 17/80 samples positive for Enterobacteriaceae and 13/80 samples with higher total viable count. The swab samples (Protein Residue Test) taken from employees hands and contact surfaces, detected inadequate sanitary procedures during the phase of milk processing in 20 samples.

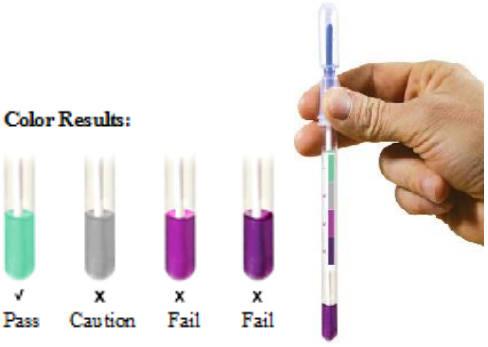


**Fig. 2 Color Reults - PRO-Clean test**

**Table 1. Results for employee hands and working surfaces from all four dairy premises that do not comply with the legislative**

| Sample type | *Enterobacteriacae* (>1 cfu/cm2) | Total viable count (> 10 cfu/cm2) |
|---|---|---|
| **Employee hands** | 5 (4 %) | 14 (11,2 %) |
| **Contact surface** | 17 (13,6%) | 13 (10,4 %) |

## CONCLUSION

The results are indicating that although there is a high level of appropriate hygiene practice in all of the food productio premises, there is still a percent of unacceptable results, which suggests a lack of hygiene and can emphasis the need for further improvement of the cleaning and disinfecting techniques especially for the surfaces and an improvement in the personal hygiene in the dairy industry.

High levels of air contamination on plant production/processing of food can pose a risk to food safety, or can influence the reduction of commercial life. It is therefore necessary periodic monitoring of microorganisms in the environment where food is produced/processed. Raw milk has been a known vehicle for pathogens for more than 100 years. Outbreaks associated with the consumption of raw milk and raw milk products were the major causes of food borne morbidity and mortality (Douwes, J., et al 2003, Gillespie I.A, et al 2003)

Air is known to contain dust which can comprise of microorganisms and other airborne contaminants which may possibly contaminate food and beverages during processing and packaging. There is a wide range of airborne contaminants found in food processing environments, but microbial particles are considered more important because of their ability to cause infections.

Proper hand washing is a critical but often overlooked intervention step in the prevention of foodborne illness. When the entire workforce is knowledgeable about and committed to

proper hand washing, will avoid costly food safety problems. Educating the consumer in the utilization of proper hand washing is a critical, but very difficult, goal to achieve.

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