ATP BIOLUMINESCENCE CAN EVALUATE CLEANING AND SANITIZING EFFECTIVENESS IN THE MILKING PARLOR[1]

M. J. Meyer and K. A. Schmidt

Summary

Four areas of the milking parlor were evaluated for effective cleaning and sanitation using total aerobic counts (standard plate count) and ATP bioluminescence (ATPB) techniques. Whereas the plate counts only monitor bacterial numbers, the ATPB results (reported as relative light units, RLU) also indicate residual soil or food residue on the surface. Results showed little correlation between the RLU values and the aerobic plate count data; however, the ATP bioluminescence technique detected the presence of soil residue on the contact surface. The ATP bioluminescence system is a fast (<2 min) and simple method that evaluates the effectiveness of cleaning and sanitation procedures employed.

(Key Words: Milking Parlor, HACCP Plan, Sanitation, ATP Bioluminescence.)

Introduction

Cleanliness of the milking parlor is very important in maintaining high quality raw milk. Although most people think of bacterial as being the main determinants of raw milk quality, other factors, such as cleanliness and protein quantity, can have an effect. Generally, as raw milk quality decreases, shelf life and usefulness also decrease. Because milk from a healthy animal contains little, if any, microbial contamination, any surface that milk contacts is a potential contaminating source.

The typical way to monitor the cleanliness of an area is to swab its surface and then use plating and incubation techniques to enumerate the number of microorganisms on the surface (TPC or total plate count). These values are reported as colony forming units per area or volume (CFU/cm^2 or ml). The downfall of this technique is that it only measures the number of aerobic microorganisms and not the presence of soil or food residue. This microbial technique is time consuming (24 to 48 hr before results are available), requires a fair amount of knowledge, and is expensive (both reusable and nonreusable equipment and resources are necessary).

The ATP bioluminescence (ATPB) system is relatively new. Currently, this technology is used to monitor sanitation effectiveness in food processing plants. The ATPB monitors both microbial loads and food residue but fails to distinguish between the two. An effective sanitation program relies on the cleanser to remove soil and food residue and the sanitizer to kill microorganisms. The ATPB is relatively simple (training time of 30 min) and produces results within 2 min of swabbing a contact surface. The downfall of the ATPB is that nebulous values are generated and referred to as relative light units (RLU). Each user must develop his or her own RLU limits to designate "clean", "warning" (values are elevated and may indicate some contamination), and "dirty" zones (values are too high and the surface needs to be recleaned).

A milking parlor environment is very different from a food plant environment. But with the increased concern for food safety, consumers and legislators have suggested that HACCP (Hazard Analysis Critical Control Point) plans be considered and possibly established to start at the "farm" and end at the "plate". In this situa-

[1]The authors thank Biotrace LTD (Brigend, UK) for use of the instrument and Kansas State University College of Agriculture Honors Program for partial funding of this project.

tion, it will be important that sanitation procedures can be verified in a milking parlor, so that milk contact surfaces do not contaminate the milk. As with all verification procedures, obtaining results quickly and accurately is important. Thus, the question was asked, can the ATPB be used to ascertain cleaning and sanitation effectiveness in the milking parlor?

Procedures

Four milk contact areas were identified in the milking parlor located at the Kansas State University Dairy Teaching and Research Center. Location A was the inside of a rubber inflation liner on the milker claw. Location B was the inner surface of the milk filter canister. Location C was the inside of the milk line going into the milk tank, and location D was the interior of the refrigerated bulk tank. For locations A, B and C, swabs were taken after running the 7-minute sanitizing cycle using common Clorox® bleach (500 mL) as the sanitizing agent. Swabbing of these locations was done 10 min after the sanitizing cycle was completed. Location D was cleaned independently, by an automatic bulk tank cleaning system. On two sample dates, swabbing was done 15 min after the tank had been sanitized on the hot acid wash cycle. On the other sample date, the bulk tank contained raw milk at 2.8°C or 37°F.

Over a 17-day period, the four locations (either 2.5 cm^2 or 5 cm^2) were swabbed with a sterile cotton swab moistened with sterile peptone broth. These broth samples were refrigerated, transported to the KSU Dairy Plant Laboratory, and analyzed for total number of aerobic microorganisms (TPC) following standard procedures using Petrifilm®. The TPC values were standardized and reported as the number of colony forming bacteria/ml of sample (CFU/ml).

No accept or reject limits exist for TPC values for food contact surfaces; however, the general rule is the lower, the better. For a dairy processing plant, TPC values of greater than 100 CFU/ml are potentially problematic and require recleaning.

To evaluate the ATPB system, the Biotrace Uni-Lite™ Xcel Luminometer (Biotrace, Ligend, England) was used. For these samples, Biotrace Uni-Lite™ swabs were used on adjacent areas of the microbial swabs. These Uni-Lite™ swabs were placed back into their carriers, activated by an enzyme solution. The end products of this reaction produce light, which is sensed by the hand held Uni-Lite™ Xcel Luminometer, generating the RLU value within 45 seconds. The higher the value, the more contamination (microbes, food residue, or soil) is present on the food contact surface.

Biotrace designates the following ranges: acceptable--less than 250 RLU (clean surface) and unacceptable--greater than 300 RLU (dirty surfaces). Values between 250 to 300 RLU would be in the questionable zone. These limits adequately evaluate sanitation in a food processing operation.

Results and Discussion

Preliminary work showed that we could obtain accurate and precise results. For swabs from clean, sanitized surfaces, RLU values were low, and microbial counts generally were not detected. In addition, the results agreed with previous research. No correlation was detected between the microbial counts and RLU values. The only apparent trend was that swabs from dirty surfaces had higher RLU values and CFU/ml (in certain circumstances) counts than did swabs from clean surfaces.

Thus, three different scenarios from the milking parlor are shown and discussed. Because the experimental conditions vary, results are shown independently and not combined. Results of our three trials are shown in Tables 1, 2, and 3.

Results varied considerably. Table 1 depicts the results of cleaning and sanitizing before swabbing. All RLU values are less than 250, indicating a thorough cleaning and sanitizing. The TPC results produced no growth, indicating an effective sanitation program. Considering both sets of data, we concluded that the milking equipment and raw milk bulk tank had been cleaned and sanitized adequately and should not add contaminants to the raw milk.

Note that the two different tests produced different information.

In Table 2, a different situation is shown. On this date, the bulk tank contained raw milk. When the tank was sampled, swabbing occurred close to milk line and tried to incorporate some milk residue (from splashing) in the swabbed samples.

Results in Table 2 indicate that locations A and B would pass a cleaning/sanitation inspection from either a TPC count or an RLU value. Location C would not pass an inspection from either test, but location D would pass by the TPC count, but not by the RLU value. This will be explained further.

When these two situations are considered independently, the RLU value at location C indicates that this surface is not clean and should be recleaned before using. The TPC data indicate that the counts are less than 250 CFU/ml. Microbial counts between 100 to 250 CFU/ml would warrant that this piece of equipment be recleaned before milk runs through this pipe. The TPC results required 48 hours to obtain. Obviously, milk would have run through this pipe before the results were available. Quick turn-around of cleaning might have prevented contamination of raw milk.

Location D produced mixed results. TPC results show a sanitized milk tank, whereas the ATPB results indicate dirty surfaces in the bulk tank. This scenario illustrates that milk residue is measured by the ATPB system, but not the TPC. The TPC results show only microbial contamination, but the RLU value indicates microbes (apparently minor) and residual dirt or milk left on the surface. Based on both sets of results, we could conclude that sanitation may have occurred, but the cleaning step was omitted.

Table 3 shows the third scenario. Locations A and D would pass inspection, whereas locations B and C would fail inspection by either technique. The logical conclusion would be that surfaces A and D are cleaned and sanitized; locations B and C would need to be recleaned and resanitized before use.

The results for locations B and C (Table 3) show a strange relationship. In location B, the TPC count is higher and RLU value lower than the comparable results from location C. This is contrary to what would be expected. This situation shows the lack of a linear relationship between TPC counts and RLU values. The TPC results are real numbers. Higher TPC counts mean more microbes present per unit surface area. A surface with 1000 CFU/ml is more contaminated than a surface with 100 CFU/ml. The same cannot be said about RLU values. A surface with 900 RLU is not necessarily more dirty than a surface with a 350 RLU reading.

This technology still can be used to distinguish between clean and dirty surfaces. At this time, RLU values are only “relative” and cannot be used to quantitate the amount of contamination or microbes on a surface. In this case, if the RLU values were over 300, the TPC counts either indicated that poor sanitation occurred, or we knew that it was a ”dirty” surface. Thus, we conclude that the ATPB can be used to evaluate the sanitation effectiveness in the milking parlor.

Conclusions

This work indicates that the ATPB system is useful to monitor appropriate cleaning and sanitation programs. If either step is overlooked, RLU values are elevated. With the Biotrace unit, guidelines of <250 as acceptable and >300 as unacceptable seem to hold true for the milking parlor as well as a food processing plant. The advantages of the ATPB method are its speed (less than 5 min) and ease (minimal instructional time). As HACCP farm to plate plans are realized, this technology may provide a viable, easy method to verify adequate cleaning and sanitation procedures.

Table 1. Standard Plate Counts (TPC) and ATP Bioluminescence (ATPB) Results (November 15, 1996)

Location	ATPB	TPC
	RLU	CFU/ml
A - milk claw	81	NG[1]
B - milk filter canister	173	NG
C - milk line	41	NG
D - raw milk tank	20	NG

[1]NG = no growth.

Table 2. Standard Plate Counts (TPC) and ATP Bioluminescence (ATPB) Results (November 22, 1996)

Location	ATPB	TPC
	RLU	CFU/ml
A - milk claw	45	NG[1]
B - milk filter canister	136	41
C - milk line	319	NSG[2]
D - raw milk tank	2279	NG

[1]NG = no growth.
[2]NSG = no significant growth, in this situation, <250 CFU/ml estimated, as defined by *Standard Methods for the Examination of Dairy Products*.

Table 3. Standard Plate Counts (TPC) and ATP Bioluminescence (ATPB) Results (December 1, 1996)

Location	ATPB	TPC
	RLU	CFU/ml
A - milk claw	39	NG[1]
B - milk filter canister	325	2760
C - milk line	935	270
D - raw milk tank	18	NG

[1]NG = no growth.