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Method for Assessing Teat and Udder Hygiene

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Abstract. *A new method was developed to quantify bacteria on bovine teats prior to milking. Previous methods using swabs to recover bacteria from teat skin have shown a high degree of variability in the amount of bacteria recovered depending on the amount of pressure applied to the teat/swab interface, the variability in the surface area of the teat swabbed, and the choice of the area to swab as the entire teat surface cannot be practically swabbed. This new method uses a single towel moistened with water to recover soil and bacteria from all four teats of each individual cow. Bacteria are then recovered from the towel and suspended in a sterile water solution. This solution is then cultured and tested using direct microscopic methods. Data are presented from several case studies which were designed to detect differences between different bedding management strategies on the bacteria population on the teats of cows as they entered the milking parlor as well as to detect the efficacy of pre milking teat sanitation in both conventional and automatic (robotic) milking facilities.*

Keywords. pre-milking teat sanitation, bacteria counts, milking management, milk quality, test methods, robotic milking unit, automatic milking,

Introduction

The over-riding causal factor in the development of mastitis is the population of bacteria at the opening of the teat canal. The major exposure to environmental bacterial occurs in the cows bedding / housing area. Neave et al. (1966) stated that the rate of new intra-mammary infection is related to the number of bacteria that the teat end is exposed to, and several studies have made associations between clean housing, clean cows and lower bulk tank somatic cell counts (Bodoh et al., 1976; Barkema et al., 1998; Barkema et al., 1999). In addition, Bartlett et al. (1992) found that an index of environmental sanitation based on the amount of manure on the cow and in her environment was a predictor of the occurrence of coliform mastitis, and Ward et al. (2002) noted that in four study herds, the lowest incidence of mastitis occurred in the herd with the cleanest cows and the most satisfactory beds. In addition to the increased risk of environmental mastitis, dirty teats and udders can also be a significant source of bacteria into bulk tank milk through the cleaning action of the milking machine and transport of bacteria from teat skin to the bulk tank. Methods to quantify bacteria populations on teat skin have therefore been of interest for some time.

Several different methods of visual assessment of udder hygiene have been documented (Cook, 2002; Schreiner and Ruegg, 2003; Reneau et al., 2005). Schreiner and Ruegg (2003) showed that cows with 'dirty' udders (score 3 or 4 in their visual assessment system) were 1.5 times more likely to be infected with a major mastitis pathogen than cows with 'clean' udders (scores of 1 or 2). They also reported a weak association between leg hygiene score and the prevalence of a major mastitis pathogen isolated from the udder. Reneau et al. (2005) used a more complex visual scoring system and documented a significant association between udder and lower leg hygiene and individual cow linear somatic cell score measured within 2 days of recording.

Teats can also be exposed to contagious and environmental organisms by contact with contaminated teatcup liners. Pre-milking teat and udder sanitation is an important step in reducing the number of bacteria at the teat end during milking as well as the number of bacteria residing on teatcup liners and thus, transferred from cow to cow by the milking machine. Proper teat end disinfection can reduce teat surface bacteria by 75% (Ruegg et al., 2000; Galton et al., 1984; Galton et al., 1986).

Several attempts have been made to quantify bacteria numbers on teats before milking using swabs or rinses combined with subsequent plate culture methods. More recently, bioluminescence assessment methods have been described. The need to assess automated teat cleaning in robotic milking systems has spurred activity in this area. Slagjuis et al. used both a cobalt tracer (2004a) and poppy seeds (2004b) mixed with manure and manually applied to teats before cleaning to assess efficacy of teat cleaning. Melin et al. (2004) used *Clostridium tyrobutyricum* spores added to a manure slurry and applied to teats before cleaning to assess removal rates. Knappstein et al. (2004) reported on the use of both total bacteria counts and ATP measurements of teat swabs for assessing teat-cleaning efficacy. They recommended an ATP based method as a pragmatic evaluation of teat cleanliness on farms with either automatic or conventional pre-milking preparation. To date, these methods have not found wide application for routine field use because of their cost, complexity, large cow-cow variability and/or considerable variability introduced by small changes in sample technique.

The objective of this study was to develop a method of quantifying the bacteria population on cows' teats that is simple, effective and inexpensive enough to be widely used on farms without requiring special test equipment.

Materials and Methods

Our first attempt at a new method used a single high-quality paper towel (similar in texture to a cloth towel, Kimberly-Clark L40 All-Purpose Wipers, 12.5 x 14.5 inches). The appropriate number of towels for one test session were moistened enough to make entire towel wet or damp and placed in individual plastic "zip-loc" bags. During the test session experimenters put on a pair sterile rubber gloves, removed the moistened towel from its plastic bag, thoroughly wiped the entire surface of all four teats from one cow and placed the towel back in the sealed plastic bag. Care was taken to touch only the teat surface (not the base of the udder) and also to remove as much of the debris on the teat barrel and end surface as possible. The individual towel bags were frozen immediately and kept frozen until processed.

The individual towels were processed further in the UW milking lab by thawing at room temperature for about 30 minutes. A thawed towel was removed from its plastic bag and place in a sterile plastic 300 ml vial with 225 ml of sterile water and vigorously shaking for one minute. Duplicate 30 ml samples of the bacteria solutions were taken for some of the tests to evaluate the repeatability of bacteria recovery methods and bacterial enumeration methods. Some of the bacteria tests were performed on composite samples in which 10 to 20 towels were combined into a single 2-liter vial and shaken to suspend bacteria in water.

Samples were assessed for standard plate count (SPC). Some of the tests also enumerated bacteria in following ways: preliminary incubation count (PI), thermoduric or lab pasteurized count (LPC), coliform, *strep. species, staph. non-ag*., and mold bacteria counts using traditional plate culture methods. Samples were also assessed using the Bentley direct bacteria counter to enumerate bacteria in the raw sample (DBC) as well as samples that were incubated at room temperature as in the preliminary incubation count method (PI-DBC). The DBC technology uses a reagent to dye bacteria and then performs a macroscopic image analysis to count the number of dyed cells. This method typically enumerates many more bacteria than plate count methods. Plate count methods rely on the recovery of viable bacteria which form colonies on growth media, whereas DBC technology can enumerate both viable and killed bacteria. Reduction in DBC were therefore used as an estimate of the effectiveness removing solids from the teat skin in a similar way to the previous studies which used various types of tracer materials. The comparison of viable to viable + dead bacteria reductions also allowed for an estimate of the killing action of pre-milking teat disinfection. All values were log transformed and a student T-test was performed for significance of comparisons.

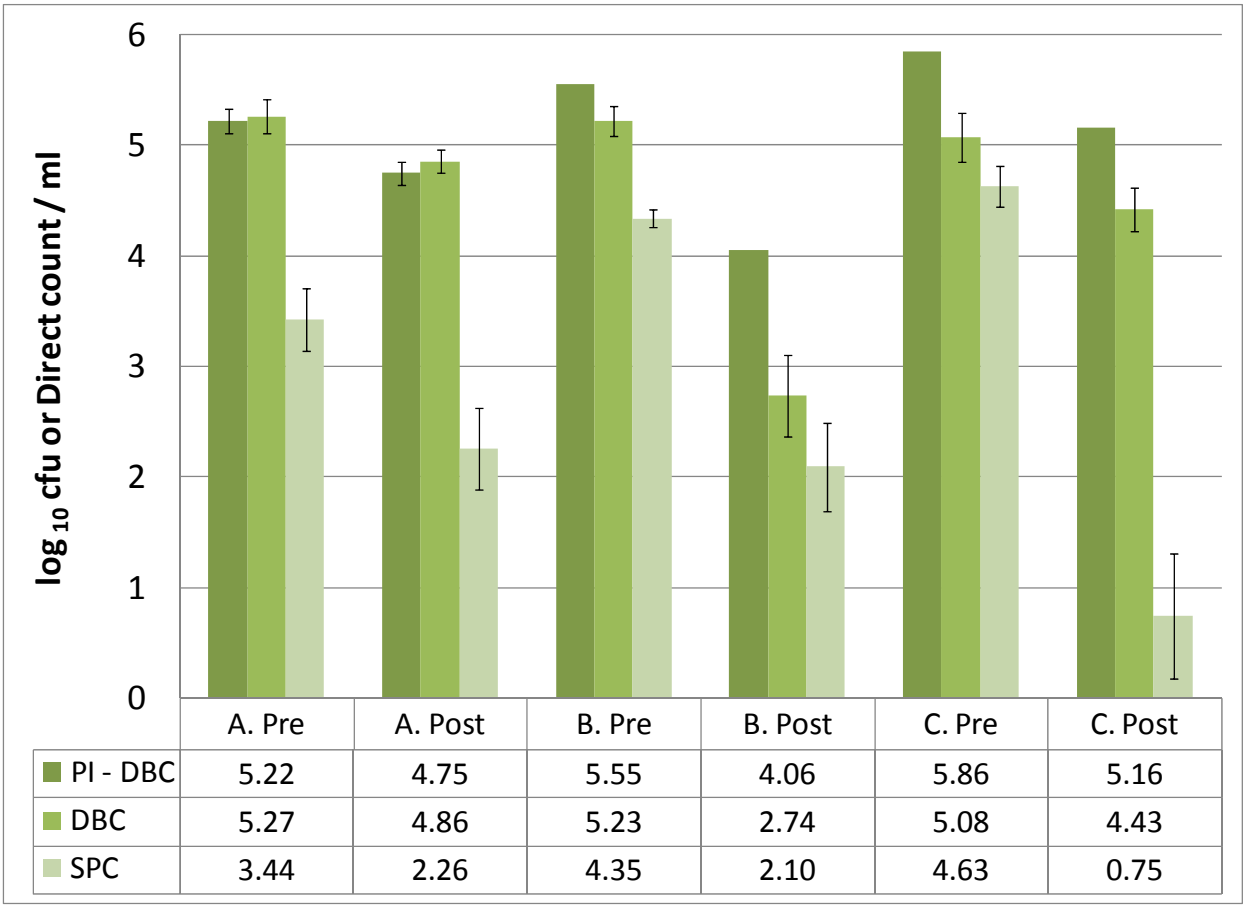
Study farm A was located in New York State and used an automatic milking unit (AMU) with sand bedded free-stalls. A total of 50 samples were collected prior to automatic teat cleaning and an additional 50 samples taken after automatic teat cleaning had been implemented. Pre and post cleaning sampling was alternated as cows entered the automatic milking machine (a total of 100 cows). The primary objective of this test was to determine the effectiveness of automatic teat cleaning.

Study farm B was located in Wisconsin and used a conventional double-20 milking parlor with human cow prep and a free-stall barn bedded with dried manure solids from and anaerobic digester. A total of 58 pre-cleaning and 58 post-cleaning samples were taken at this farm. The pre-milking preparation procedures at this farm were considered by the investigators to be excellent and above norm for Wisconsin dairy farms.

Study farm C also located in Wisconsin and used a conventional double-16 milking parlor with human cow prep and a free-stall barn bedded with sand. A total of 28 pre-cleaning and 28 post-cleaning samples were taken this farm, also considered by the investigators to have excellent pre-milking cow preparation procedures.

Results and Discussion

The results of the SPC, DBC and PI-DBC data are presented in Figure 1. There was no signficant difference in the pre-sanitation DBC between any of the farms, while the pre-sanitation level of SPC was lower for farm A than for farms B and C. All methods of teat sanitation showed a signficant reduction in SPC and DBC. Post-sanitation DBC values were lower for farm B than for farms A and C, while post-sanitation SPC values were lower for farm C than for farms A and B. The reduction in SPC was greater than the reduction in DBC for the farms using san bedding (A and C). This would be expected because of the reduction of viable organisms killed by pre-milking sanitizing solution. The reduction of SPC and DBC were of similar magnitude on the farm using dried manure solids as bedding.

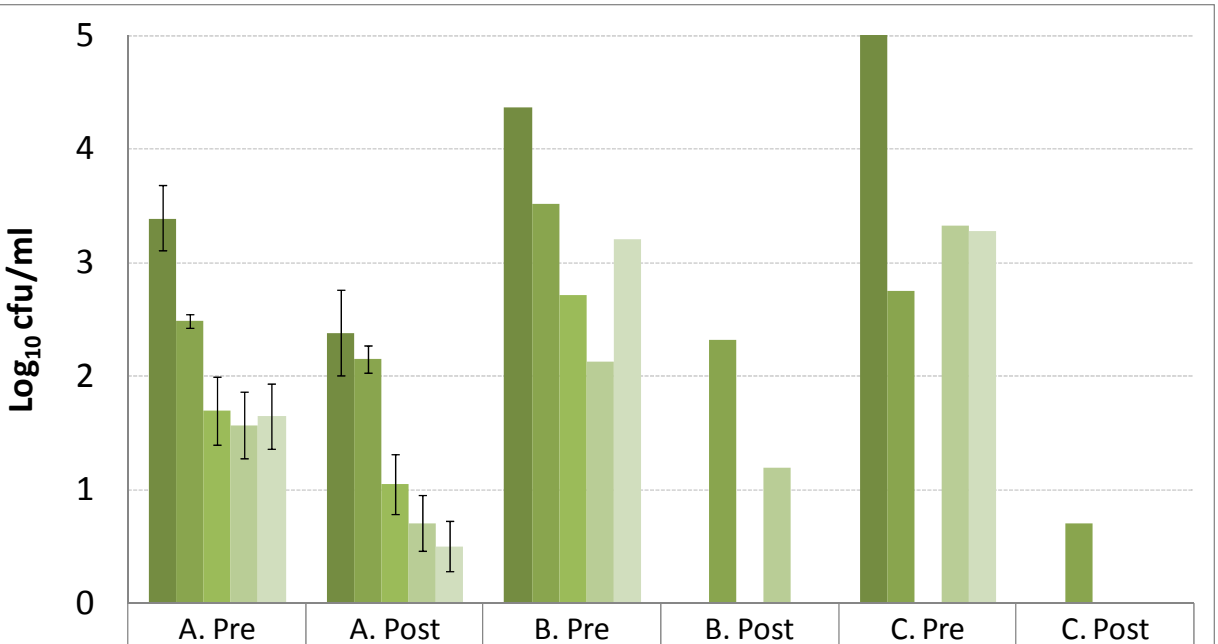


	A. Pre	A. Post	B. Pre	B. Post	C. Pre	C. Post
PI - DBC	5.22	4.75	5.55	4.06	5.86	5.16
DBC	5.27	4.86	5.23	2.74	5.08	4.43
SPC	3.44	2.26	4.35	2.10	4.63	0.75

Figure 1. Results of SPC, DBC and PI-DBC for Farms A, B, and C pre and post teat sanitation. Error bars indicate 95% confidence intervals of the mean. Columns with no error bars are geometric means of 2 or 3 composite samples.

The results of the remaining bacteria speciation tests are presented in Figure 2. There was a significant reduction in all bacteria types on farm A (automatic teat sanitation). The data from farms B and C are composite samples with only 2 or 3 bacteria counts. While claims of significance are more difficult to substantiate, a reduction in all bacteria types was also seen on these farms. The reduction in LPC was smaller than for other bacteria types on farm A and was the most prevalent type of bacteria after teat sanitation on farms B and C, probably because pre-milking teat sanitizing solutions are less effective at killing thermoduric organisms than common environmentals such as coliform, strep and staph organisms. Thermoduric organisms were also among the most prevalent type of bacteria in the pre-sanitation samples that were not

pre-incubated. This indicates that thermoduric organisms recovered from teat skin could be a contributor to LPC in bulk tank milk especially when poor pre-milking teat sanitation is used and may still be a contributor when good pre-milking teat sanitation is used; and that failure of the milking machine cleaning and sanitation systems are not the only contributor to LPC in bulk tank milk.



	A. Pre	A. Post	B. Pre	B. Post	C. Pre	C. Post
PI	3.39	2.38	4.37	0.00	5.16	0.00
LPC	2.49	2.15	3.52	2.32	2.75	0.71
coli	1.70	1.05	2.72	0.00	0.00	0.00
staph.	1.57	0.70	2.13	1.20	3.32	0.00
strep	1.65	0.51	3.21	0.00	3.27	0.00

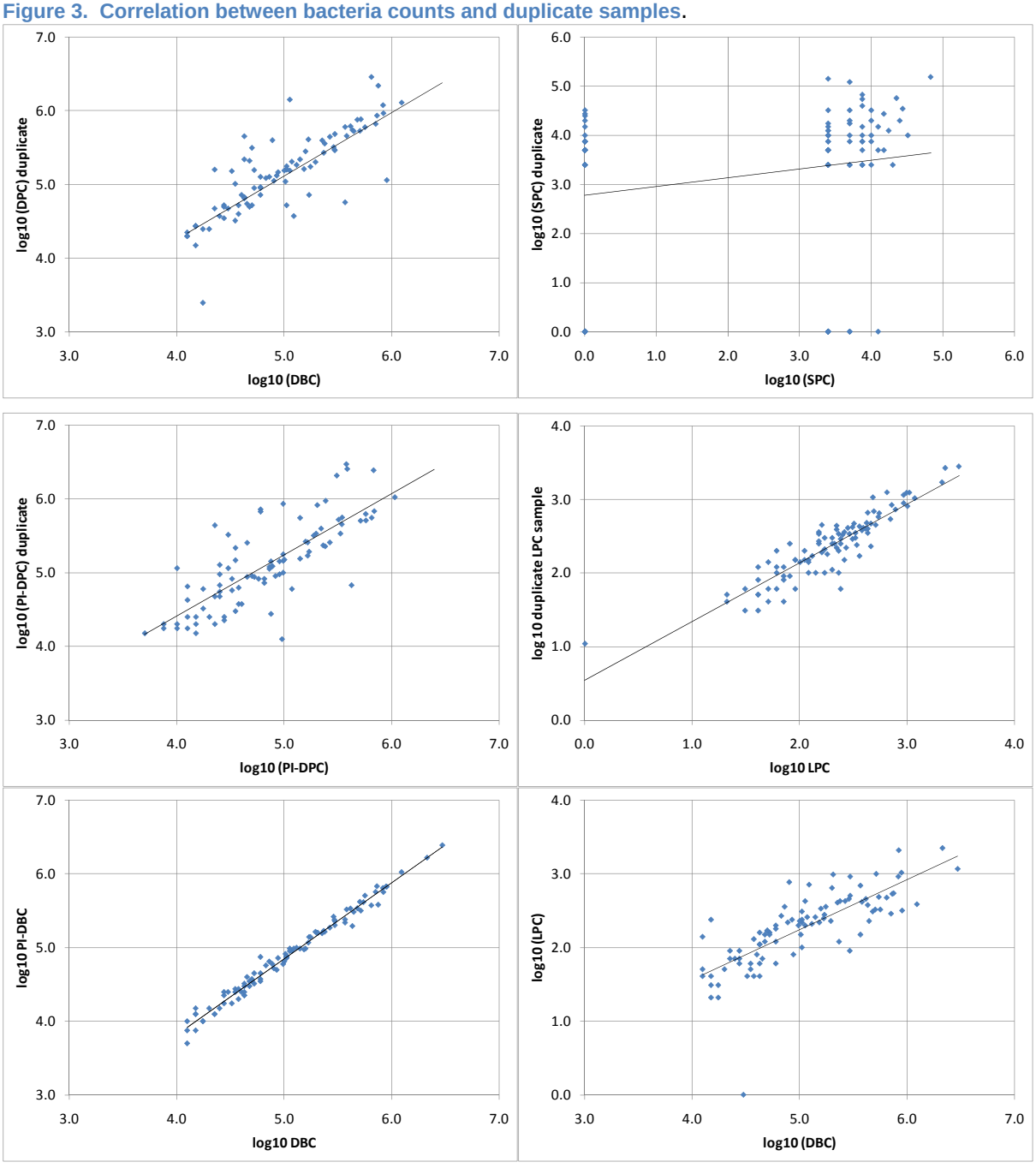
Figure 2. Results of bacteria speciation for farms A, B, and C. pre and post teat sanitation. Error bars indicate 95% confidence intervals of the mean values. Columns with no error bars are the geometric mean of 2 or 3 composite samples.

Table 1. Cross-correlation table for bacteria counts from farm A.

	DBC	*PI-DBC*	*SPC*	*PI*	*LPC*	*staph*	*strep*	*Coli.*	*mold*
PI-DBC	0.99								
SPC	0.39	0.40							
PI	0.44	0.44	0.45						
LPC	0.76	0.75	0.46	0.44					
staph	0.34	0.36	0.30	0.29	0.27				
strep	0.43	0.43	0.36	0.41	0.35	0.40			
coliform	0.19	0.19	0.19	0.23	0.06	0.13	0.28		
mold	0.54	0.55	0.26	0.33	0.55	0.23	0.27	-0.01	
Duplicate	0.59	0.80	0.20	0.19	0.90	0.19	0.41	0.21	0.24

Correlations between bacteria counts and duplicate samples are presented in Table 1 and selected correlations in Figure 3. Reasonable correlations (>0.75) were observed between DBC and PI-DBC, LPC and its duplicate, PI-DBC and its duplicate, and DBC and LPC. The correlation between the SPC, PI, staph, coli and mold bacteria and their duplicates was quite low (<0.25). In general, the direct bacteria counts had better repeatability than plate culture methods, for which many (presumably) false negatives occurred. It is interesting that the correlation between DBC and LPC and between LPC and its duplicate was quite high. The recovery of viable thermoduric bacteria colonies thus appears to be more repeatable than for other bacteria types and thermoduric bacteria may be disproportionately represented in direct bacteria count methods.

Figure 3. Correlation between bacteria counts and duplicate samples.



Conclusion

The method developed for quantifying bacteria populations on cow teats appears to have promise. Statistically significant differences were detected between pre and post teat sanitation for all bacteria types tested on 3 different farms with practical sample sizes. The method is simple enough to be performed by farm operators using reasonable care and consistency of bacteria harvest and suspension techniques. Enumeration of bacteria can be performed in conventional milk quality labs. The order of magnitude of bacteria populations was comparable across farms and the differences between bacteria types and across teat sanitation practices were plausible.

The DBC and LPC had much better repeatability across duplicate samples than did the other plate culture methods (SPC, coliform, strep, staph, mold bacteria). The recovery of viable thermoduric bacteria colonies thus appears to be more repeatable than for other bacteria types and thermoduric bacteria may be disproportionately represented in direct bacteria count methods. Although DBC has much better repeatability and requires a substantially smaller sample size to detect biologically important differences, it does not indicate the type of bacteria present in the sample. A combination of DBC of individual samples and bacteria speciation using fewer composite samples as well as visual assessment methods is recommended to provide the best benefit-cost ratio for assessing teat and udder hygiene as a means of assessing the hygienic quality of cow bedding materials and management as well as methods of pre-milking teat sanitation. The authors are exploring techniques to make this test more practical for field application.

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