

APPLICATION OF CLEANING AND COOLING PRINCIPLES TO ROBOTIC MILKING

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Some basic principles of cleaning and cooling are presented in this paper with ideas for application to robotic milking:

1. Monitoring methods for milk quality control, failure diagnosis, and as a basis for recommending practices such as cleaning intervals,
2. The influence of soil/bacterial contamination drawn into the milking machine and milk from cow's udders and the environment,
3. The removal of soils and bacteria from milk contact surfaces effectively and at appropriate intervals,
4. Milk cooling to inhibit bacterial growth, and
5. Design of milk handling systems for automated milking.

Monitoring Methods

The key to properly diagnosing cleaning and sanitation problems is identifying the types of bacteria present in bulk tank milk. Periodic culturing of bulk tank milk is also an essential tool to diagnose milk quality issues related to mastitis. These methods are described in greater detail in Reinemann et al. (2002) and Ruegg and Reinemann (2002). Collection of the type of bulk tank culture data presented here should be a routine practice on any farm concerned with product quality, especially those learning to use new technology such as automatic milking. If the dairy producer is not sufficiently motivated to acquire this type of data, it would behoove milk quality consultants to use these methods to correctly diagnose the source of elevated bacteria counts and solve these problems in an expeditious manner, before they become a crisis.

Some form of testing for bacterial contamination of bulk milk is done periodically on all farms to assure compliance with national, state, and local requirements (usually standard plate count, SPC). The two main sources of bacteria in raw milk are organisms transported from the environment into the milking machine and mastitis organisms from within the udder. Bacteria deposited in milk handling equipment will multiply and become a major source of contamination if this equipment is not cleaned and sanitized properly. Differential diagnosis can be performed using several bacterial culture methods to determine the likely source of elevated bacterial counts in bulk milk.

Quantitative bulk tank cultures (QBTC), which enumerate the various types of organisms, are commonly used in mastitis control programs to identify the type of mastitis organisms in bulk milk.

Organism types identified in the QBTC are typically:

<i>staph. aureus</i>	<i>strep. agalactiae</i>	<i>coliforms</i>
<i>staph. non-aureus.</i>	<i>strep. non agalactiae.</i>	<i>other</i>

The “other” category can include a variety of organisms including:

<i>pseudomonas</i>	<i>bacillus cereus</i>	<i>klebsiella sp.</i>
<i>serratia sp.</i>	<i>c. pyogenes</i>	<i>yeasts</i>

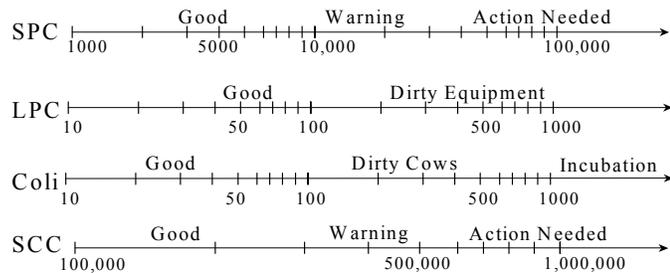
The quantitative bulk tank culture yields a Total Bacteria Count (TBC) that should correspond closely to the SPC. All of these bacterial tests rely on culture media and incubation from two to three days. The SPC and plate loop count are direct methods based on recovering and growing viable bacteria into colonies. The Bactoscan™ method is a recent technological advance that uses continuous epifluorescent microscopy to count bacterial cells stained with acridine orange. Bactoscan™ has compared favorably to traditional bacteriologic methods and is considered to be less variable and more reproducible.

Bacteria in raw milk can come directly from the environment or originate as mastitis organisms. Bacterial “spikes” (defined as “transient sporadic increases in SPC values that exceeded a 95% confidence interval for mean SPC and were >10,000 cfu/ml”) have been associated with streptococci (primarily *strep. uberis*) and gram-negative organisms. Very high shedding has been documented for cows infected with *strep. uberis* mastitis and *strep. agalactiae*. Sub-clinical mastitis problems should be considered when both the somatic cell count (SCC) and SPC are high. This situation is most common on small herds in which one or two shedding cows can have a significant influence on the bulk tank totals. In these instances the SCC and the SPC are generally both high, and the causative organism should be apparent from QBTC.

The coliform count provides an indication of both the effectiveness of cow preparation procedures during milking and the cleanliness of the cows’ environment as a major source of coliform bacteria in bulk tank milk is transportation of soil from the teats and udders into the milking machine. Coliforms can also incubate on residual films of milking equipment, however. Coliform counts less than 10/ml indicate excellence in both pre-milking hygiene and equipment sanitation. Coliform counts must be less than 10/ml where raw milk may be sold to consumers. Poor milking hygiene usually results in coliform counts between 100 and 1000 with a commensurate rise in SPC and near normal LPC if the milking machine is clean. Coliform counts in excess of about 1000 suggest that bacterial growth is occurring on milk handling equipment. In cases of prolonged cleaning failure Coliform, SPC and LPC will be elevated due to coliforms and other bacteria growing in soil films in the milking machine.

Another bulk milk test that provides diagnostic value is the thermoduric count or Laboratory Pasteurized Count (LPC). Thermoduric organisms are often related to spoilage of pasteurized milk. The LPC is a SPC performed on milk that has been heated to 145F (62.8C) and held for 30 minutes (low temperature-long time pasteurization). Typical mastitis causing organisms (including coliforms) do not survive pasteurization. Thermoduric bacteria may include *Micrococcus*, *Microbacterium*, *Lactobacillus*, *Bacillus*, *Clostridium* and occasional *Streptococci*. These organisms will grow and multiply in the milk handling equipment if cleaning and sanitation procedures are inadequate. The LPC should be below 200 cfu/ml, and a LPC below 10 cfu/ml indicates excellent equipment hygiene.

A reasonable minimum testing schedule for users of AMS is weekly QBTC and LPC. The QBTC will provide information for management of mastitis as well as environmental sanitation (coliform count). The PLC will provide additional diagnostic capability for equipment cleaning and sanitation. A summary of the interpretation of these tests is given in the chart below. (Note that TBC from the QBTC can be substituted for SPC.) An outstanding example of the application of these methods for diagnosing sanitation problems in an automatic milking machine was presented by Knapstein et al. (2002). In this study the elevated LPC detected an equipment cleaning failure long before the problem became apparent with an elevated SPC.



When routine bulk tank testing indicates that a problem exists, more detailed tests can be performed to further isolate the source of the problem and to recommend the most effective methods to solve it. Strategic sampling of milk in different locations will determine if the location of a cleaning failure and/or incubation problem is in the milking units, milkline and receiver, in the milk transfer line (including filters and pre-coolers), or in the bulk tank. Strategic sampling of milk at different times during the milking process will determine if incubation in the milk handling system during milking is a major source of contamination.

Recent developments of ATP detection methods using a bioluminescence have been proposed as a rapid method for assessing the effectiveness of sanitation in the dairy industry. ATP bioluminescence is a rapid detection method suited for on-site sampling and takes less than five minutes to perform. The ATP method appears to be a more sensitive method to detect differences in cleaning effectiveness than bulk tank culture methods (Reinemann and Ruegg, 2000). Plate count methods also detect the presence of bacterial contamination on equipment surfaces, whereas ATP bioluminescence can detect both bacterial contamination and non-microbial contamination such as milk soil. ATP bioluminescence has the potential to be a useful tool to evaluate the effectiveness of cleaning procedures used on the milking machines.

There is considerable variation in the ATP data, and the method must be used carefully and with sufficient number of tests to obtain meaningful results. The required sample size will depend on the skill of the user and the stability of the system being monitored. Care must be taken to avoid contaminating the inner surfaces of components as they are opened for swabbing. The variability in the ATP data can be reduced significantly by using the same measurement locations over time. Bioluminescence technology may be of use to cleaning and sanitation troubleshooters in the field, and is most useful in determining relative differences between different locations in the milk flow path (dirty spots) and to detect differences over time in the same location (does cleaning regime A work better than cleaning regime B). Bioluminescence has limited value as a one-time, absolute measure of whether an AMS is 'clean' or 'dirty'.

Case Studies

These two case studies illustrate the use of culture methods and typical situations expected for automatic milking. Both farms and underwent a herd expansion and changed from cleaning

three times per day (8-hr interval) to twice per day (12-hr interval). Both of these example farms were investigated to determine the cause of elevated SPC.

Farm A was investigated because the SPC was elevated ten-fold from about 2000 to slightly above 20,000 cfu/ml. When this elevated count occurred, milk samples were taken from the receiver at the start of milking and after 4 hours and 11 hours of milking. A bulk tank sample was taken at the end of the milking period, just before the cleaning cycle started. These milk samples were tested for SPC, coliform and LPC. Farm A has a well established history of outstanding cow hygiene, both in the housing area and pre-milking hygiene in the milking parlor. The quantitative confirmation of this observation is that, over a 3 month period the majority of the coliform counts in the bulk tank were under 10/ml. (Note the diagnostic benefits of a well established and well quantified history for the farm.) One of the actions taken on this farm to resolve the elevated SPC concern was to change from 2x cleaning to 3x cleaning. This did not resolve the elevated SPC problem. Data from Farm A are presented for both the 2x and 3x cleaning scenarios. The investigation on Farm A eventually revealed a flow problem in the CIP circuits, which resulted in reduced SPC when resolved.

Farm B was investigated because the SPC was also elevated by about 10 fold, from about 20,000 to in excess of 200,000. Milk samples were taken at the receivers and at the outlet of the milk transfer lines at the start of milking (after only 10 cows milked on each side of the parlor), after 7 hours of milking. A sample was also taken at the bulk tank at the end of the 11-hr milking shift. A QBTC was done on all samples and LPC on some samples. The cow hygiene in the barn and in the parlor on Farm B was adequate, but not outstanding, by most observers' accounts. The coliform counts were typically in the 100/ml to 200/ml range, or 10 to 20 times higher than the first farm. Coliform counts in the 100/ml to 200/ml range are considered in the "warning" zone, not the "critical" zone, and commonly indicate that cow hygiene is marginal. There was also a large population of other environmental organisms in the bulk tank (*non ag. strep.*). No major deficiencies were noted in the milking machine cleaning and sanitation procedures on Farm B. The cleaning chemical concentrations and temperature were verified to be in the recommended ranges. The flow dynamics of the CIP circuits were also verified to be in the recommended range. It was noted on this farm, however, that several of the rubber components of the milking machine were quite aged and have cracked and rough surfaces. It was also noted on Farm B that at the end of an 11-hr milking shift there was a considerable build up of manure on the inner surfaces of the liner mouthpieces and that the filter sock was not changed during these 11 hours and appeared quite soiled.

The milking and cleaning schedules on these two farms were thus similar to those expected for automatic milking machines with one of the major differences between these two farms being the difference between outstanding hygiene (low soil and bacterial load) and marginal hygiene (moderate soil/bacterial load). There was also a confirmed deficiency in the flow dynamics of the cleaning system for the farm with excellent hygiene. The data from these various scenarios illustrate all three of the major points outlined above.

Farm A: 15 hr/day milking duration, 8-hr cleaning interval, no detectable milking machine cleaning problem (previous history, well established by weekly bulk tank cultures).

Location	Time of Sample	SPC (cfu/ml)	Coli (cfu/ml)	LPC (cfu/ml)
Bulk Tank	5 hrs of milking	2,000	10	100-200

Farm A: 21 hr/day milking duration, 12-hr cleaning interval, milking machine cleaning flow problem.

Location	Time of Sample	SPC (cfu/ml)	Coli (cfu/ml)	LPC (cfu/ml)
Receiver	Start of Milking	25,000	8	88,000
Receiver	4 hrs of milking	35,000	13	22,000
Receiver	11 hrs of milking	38,000	2	12,000
Bulk Tank	11 hrs of milking	26,000	15	19,000

Farm A: 21 hr/day milking duration, 8-hr cleaning interval, milking machine cleaning flow problem still existing.

Location	Time of Sample	SPC (cfu/ml)	Coli (cfu/ml)	LPC (cfu/ml)
Receiver	Start of Milking	28,000	51	14,000
Receiver	4 hrs of milking	22,000	17	17,000
Receiver	7 hrs of milking	51,000	(?) 130	8,600
Bulk Tank	7 hrs of milking	22,000	13	12,000

Farm B: 22 hr/day milking duration, 12-hr cleaning interval, no detectable failures in milking machine cleaning and sanitation processes (time, temperature, chemical concentration, flow dynamics), however, rubber goods noted to be aged with rough surfaces.

Location	Time of Sample	SPC (cfu/ml)	Coli (cfu/ml)	Non ag strep (cfu/ml)	LPC (cfu/ml)
Receivers	Start of Milking	3200	100	800	300
Transfer Line	Start of Milking	12,000	200	5,400	na
Receivers	7 hrs of milking	66,000	2000	45,000	na
Transfer Line	7 hrs of milking	200,000	1500	185,000	na
Bulk Tank	11 hrs of milking	280,000	(?) 200	200,000	na

Effects of Soil Load

The effect of soil load on the bacterial quality of milk is illustrated by data collected from two case study farms in Wisconsin. A large portion of the rise in the Bulk Tank SPC (+ 22,000 to 24,000 cfu/ml) on Farm A was explained by the increase in LPC (+ 19,000 to +12,000). The cleaning flow problem that was eventually identified on this farm was severe enough and had persisted long enough so that a visible film had built up in one or two milking unit flow sensors. The LPC is specific to the types of bacteria that thrive in these wet, nutrient rich environments (e.g., *pseudomonas* and *bacillus*). The film had developed to the point that bacteria were shed into the milk. (Note that there is no trend in the LPC counts over time, an indication of sporadic shedding as would be expected for an organic film.) It is also interesting to note that this rather major cleaning failure resulted in moderately high SPC. These counts were high enough that this quality-conscious producer took action, but not high enough to be illegal. The soil and bacteria load from the environment was low (as indicated by the low coliform counts) and the milk was cooled quickly in a plate cooler before being deposited in the bulk storage tank. It appears that there was very little incubation occurring in other parts of the system tank. The main source of the bacteria in the bulk tank can be deduced to have been bacteria incubating in the organic films found on a small portion of the machine.

The situation on Farm B is quite different. The soil load from the environment is moderately high in the receiver at the beginning of milking (coliform counts 100-200 cfu/ml). No major defects were found in the cleaning procedures in the milking machine. However there is some evidence that cleaning was not entirely effective as the SPC and LPC at the receiver are higher than expected for a clean system at the start of milking. This is likely due to the difficulty of removing attached bacteria from surfaces such as worn rubber components. The rise in SPC at transfer line outlet at the beginning of milking suggests a cleaning failure somewhere between the receiver and the bulk tank. The rise in coliform count, as well as in other environmental bacteria, at the receiver after 7 hours of milking indicates that incubation is occurring in milking machine components. The major contribution (75%) to the SPC in these milk samples taken from the receiver after 7 hours of milking is from environmental bacteria (non *ag. strep.*).

These examples suggest that incubation during the milking process can be a considerable source of bacteria in milk tank milk for systems with high soil and bacteria loading. Minimizing the soil load of an automatic milking system can be accomplished by a combination of clean resting areas in the barn along with effective pre-milking sanitation of teats and udders. Systems with low soil loads will require less frequent cleaning while systems that are heavily loaded will require more frequent cleaning. Enumeration of the population of coli forms and other environmental organisms in bulk tank milk is a useful tool to quantify soil/bacteria loading in automatic milking systems and can be used as to evaluate barn and pre-milking hygiene and as a basis for recommending cleaning frequencies.

Milk Soil Removal

Laboratory tests have confirmed field experience that the most difficult milk soil to remove is a combination of an organic deposit in which bacteria have incubated and formed attachment matrices to surfaces (Muljadi et al., 1996). These biofilms are most likely to form on surfaces that are protected because of rough or cracked finish and irregularities created by gaskets and

joints. These locations are the most difficult to remove biofilms from as the mechanical action is least effective in these protected zones. These biofilms also protect bacteria from the action of sanitizers.

There are two strategies to minimize this problem: 1. avoid situations in which bacteria can incubate and form attachment mechanisms, and 2. remove attached bacteria in an effective manner and at a frequent enough interval so that films will not build up.

Water rinses are effective at removing gross soils. Rinsing components such as teatcup liners frequently can help to reduce the buildup of manure and other soils in the liners and eliminate incubation sites. The primary function of an acid rinse is to remove mineral deposits from water and milk. The recommended frequency of acid treatment will depend primarily on the hardness of the water used for cleaning the milking system.

The removal of biofilms is most effectively accomplished using a chlorinated alkaline detergent that is capable of dissolving the extra-cellular polymeric bonds created when bacteria grow and attach to surfaces. Detergents reduce the surface tension of water so the solution can more effectively wet and penetrate soils that have adhered to surfaces. Chlorine is often added to alkaline detergents as a peptizing agent to aid in protein removal and to improve the rinse-ability of the detergent. A chlorinated alkaline detergent may not be required at each cleaning of the automatic milking system but should be done periodically to remove protein and biofilm deposits.

The acidified boiling water method is used in some parts of the world. An acid detergent is used at a temperature of nearly 100°C. The wash solution makes a single pass through the system and is not circulated. The objective is to maintain all surfaces at a temperature above 77°C for at least two minutes. This method replaces the chemical action of alkaline detergents with intensified thermal action. The flow dynamics and heat transfer characteristics of the many components are an extremely important element to achieve the design objective of this wash strategy. The success of this system rests on careful system design and control, special equipment to achieve elevated water temperatures, and milking system components that can withstand these high temperatures. If acidified boiling water washes are used in automatic milking systems, special attention should be given to inspection (perhaps with ATP bioluminescence techniques) to ensure that protein and/or biofilm development is not occurring in critical locations.

Maintaining the temperature of wash solutions has always been a challenge in milking machine sanitation. Thermal energy is typically stored in a small volume of wash solution with no supplemental heat added during the process. It is common to inject steam into wash solutions in dairy plants. This technology should be considered for cleaning processes of automatic milking systems that depend on high temperature to be effective.

Milk Cooling

The dramatic effect of incubation on bacteria counts is illustrated in the case studies cited above. Bacterial incubation rates are suppressed at low temperatures and reduce the risk of elevated bacteria populations in the bulk tank (de Konig et al., 2002). Even though there was a consistent and considerable bacteria load on Farm A, there was no increasing trend in SPC, coliforms or LPC over the 11 hours of operation. This is due in part to the use of a pre-cooler immediately

after the receiver. Milk was cooled to storage temperature within minutes of extraction. Cooling milk as quickly as possible will reduce the problems associated with incubation and avoid exponential growth rates of bacterial populations.

System Design

A cardinal rule for efficient and effective CIP system design is to keep pipe lengths and number of fittings to a minimum. Irregularities on inner surfaces produced by fittings and gaskets should be kept to a minimum to reduce the number of preferred incubation sites. The use of components that must be disassembled and manually cleaned should be kept to a minimum as it is unlikely that these components will be cleaned as frequently and as regularly as components that are automatically cleaned.

Bulk tanks and large vessels, such as the milk collection vessels and milk meters commonly used for automatic milking systems, are cleaned by covering their inner surfaces with a sprayed sheet of water. It is generally more difficult to maintain surface temperatures in spraying operations than in pipe flow conditions. Mechanical action is also significantly reduced compared to circulation cleaning. The chemical concentrations and wash water temperatures are therefore of critical importance for successful spray cleaning. It is best to avoid the use of large volume vessels that are part of the milk flow path wherever possible.

Most of the tubes and hoses that make up the milk flow path in automatic milking systems rely on a flooded condition to provide contact of cleaning chemicals and develop mechanical cleaning action. These flow circuits must be designed so that it is possible to maintain flooded conditions in all components with a flow velocity above 3 m/s.

Cycled air admission is commonly used on milking machines pipelines with diameter 48 mm or greater to reduce the volume of cleaning solution required to clean long pipelines. The objective in air-injected flow is to form a 'slug' of cleaning solution and move this slug around the entire pipeline. Slug velocities of 7 to 10 m/s maximize the wall shear stress developed while minimizing the variation of shear stress along the pipe. The slug velocities developed with air injected two-phase flow can be 3 to 5 times higher, and the wall shear stress developed 10 to 20 times higher than those in flooded CIP circuits. The contact time between the slug and pipe wall is significantly reduced, however. Cycled air admission has clear advantages to flooded flow in large diameter pipelines. In small diameter lines, such as those typically found in automatic milking systems, its value is questionable.

A careful evaluation of all system components is required to assess the most practical and effective low regime for cleaning (flooded, sprayed, or slugged) and to design the circulation systems to achieve these effectively. The motive force can be produced by pumps, air injection or steam injection. Steam injection is a technology worthy of serious consideration for automatic milking systems. The resulting effectiveness of the cleaning process is a balance of contact time (the true contact time between cleaning chemicals and all milk contact surfaces), the mechanical cleaning action produced by the velocity of the solutions near the surface, the surface temperature of the components being cleaned, and the effectiveness of the chemicals in solution at dissolving deposits that are not easily dislodged.

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