Troubleshooting High Bacteria Counts in Bulk Milk: What needs cleaning, the machine or the cows?

Douglas J. Reinemann
Paper Presented at the June 2011 Countdown Meeting, Melbourne, Australia

Introduction and Overview

I have had a professional fascination with bulk tank cultures for the past 20 years. This odd obsession developed from my interest in the mechanics of milking machine cleaning and sanitation. Over the course of the past two decades I have been involved in numerous situations in which bulk tank bacteria counts were higher than desired, most often approaching or exceeding the legal limit. A remarkable number of these crisis situations share a common thread: an argument between the milking machine service technician (or cleaning chemical supplier) and the farmer over whether the cause of the high bacteria counts was a failure in the cleaning system or the excessive bacteria load from milking wet/dirty cows. A very crude estimate based on my memory of interesting cases is that there are not overwhelming odds for either of these 2 primary sources, but that when the legal limit is being exceeded, it is common that both sources contribute to the crisis situation. In this paper I will provide the tools that I have found most useful and effective in diagnosing the source of bacteria in bulk tanks, which in turn, will allow the investigator to focus intervention in the right place to solve the problem.

Bulk tank Cultures: Know Your Enemy

A simple, yet powerful, method for diagnosing high bacteria counts in bulk tank milk using the relative relationships between bulk tank standard plate count (SPC), somatic cell count (SCC), laboratory pasteurized count (LPC), and coliform count was presented by Guterbok and Blackmer (1984) and is used as the basis for the NMC Guide, Troubleshooting Cleaning Problems in Milking Systems (Figure 1).

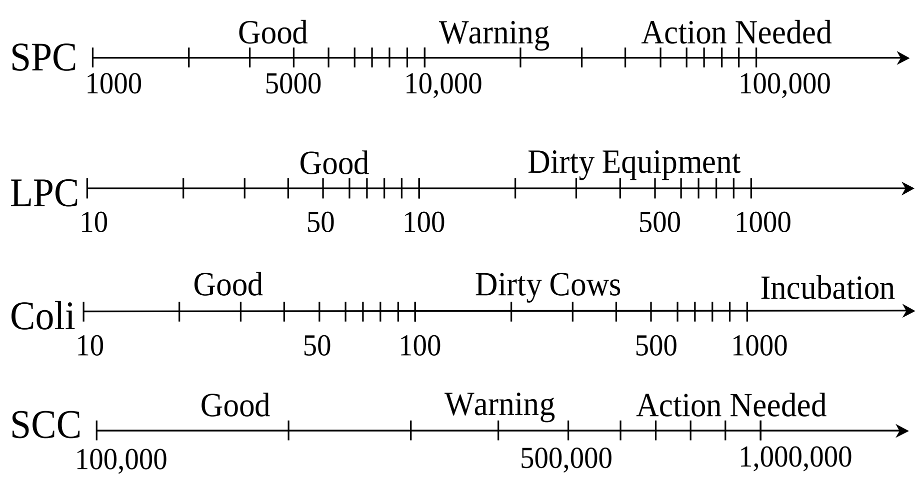


Figure 1. Diagnostic Chart for Bulk Tank Bacteria Counts.

This method uses coliform bacteria as an indicator of the level of environmental contamination (organisms drawn into the milk from the environment – mainly from the skin of teats and udders) in bulk tank milk. There are, however, many other types of environmental bacteria and elevated coliform counts can occur for other reasons. The method also uses the LPC (or thermoduric count) as the primary indicator of a cleaning failure in milking and milk storage equipment. Again, there are many different types of thermoduric bacteria and elevated LPC can occur for reasons other than cleaning failures.

This method relies on the RELATIVE COMPARISON between numbers to formulate a diagnosis. The most common misapplication of the method is the formulation of a diagnosis based on only one of these numbers without considering the relative values of the others. The simple chart in figure 1 is intended to highlight the diagnostic measure that is the most elevated compared to the others. Note that the different types of counts have different 'target' values based on the performance of typical dairy herd.

LPC's are correlated with coliform counts because there are thermoduric bacteria that are present in the environment. Coliform (and other environmental organisms) make up a larger percentage of the population than thermoduric organisms so that the increase in coliform count is larger than the increase in LPC. Following are some examples of the 3-part decision tree to properly implement this diagnostic technique. Following are some examples and the decision rules for the diagnosis:

Milking wet and/or dirty cows:

- Coliform count is between 100 and 1000 cfu/ml
- AND LPC is less than Coli
- AND SPC is moderately elevated (5000 – 20,000) cfu/ml

Persistent milking machine cleaning problem

- LPC is between 100 and 1000 cfu/ml
- AND Coli less than LPC (probably because of the use of an effective sanitize cycle)
- AND SPC is moderately elevated (5000 – 20,000 cfu/ml)

Incubation in the milk handling system

- Coli is greater than 1000 (or to numerous to count TNTC)
- AND LPC is greater than 100 but less than Coli (Or TNTC)
- AND SPC is extremely elevated (greater than 50,000 to 100,000 or TNTC)

Multiple sanitation problems are likely contributing to these elevated counts and further investigation is recommended (strategic sampling from various points in the milk handling system both early and late in the milking process).

Acquiring more information about the specific bacterial species represented in bulk tank milk will improve the power of a diagnosis. Quantitative bulk tank cultures (QBTC) enumerate a range of specific organisms. QBTC is typically focused on types of bacteria related to the level of mastitis in the herd (environmental and contagious). Some of these organisms are also useful in diagnosing sources of environmental contamination of milk, cleaning failures and incubation in milk handling equipment. Following is a summary of sources and growth characteristics of specific bacteria types commonly found in bulk tanks from the excellent review by Murphy and Boor (2008) *(additional comments by the author in italics).* This continually updated document on the E-extension website is required reading for anyone interested in the diagnosis of bulk tank bacteria counts. Applying this research based information for a broader range of bacteria types will greatly improve your diagnostic abilities.

1) Mastitis organisms

   a) Mastitis organisms that most often influence bulk milk count are *Streptococcus spp*., most notably *S. agalactiae* and *S. uberis.*

   b) *Staphylococcus aureus* is not a frequent contributor to total bulk tank bacteria count.

   c) Detection of *(environmental)* mastitis pathogens does not necessarily indicate that they originated from cows with mastitis as environmental mastitis pathogens occur in milk as a result of factors other than mastitis infection.

   d) Correlation of somatic cell responses and bulk tank environmental mastitis organisms is poor.

2) Environmental Contamination

   a) Organisms associated with bedding materials that contaminate the surface of teats and udders include streptococci, staphylococci, spore-formers *(or thermodurics)* coliform, and other Gram-negative bacteria.

   b) Both thermoduric (bacteria that survive pasteurization) and psychrotrophic (bacteria that grow under refrigeration) strains of bacteria are commonly found on teat surfaces. Contamination from the exterior of the udder can influence Lab Pasteurization Counts (LPC) and Preliminary Incubation Counts (PIC).

   c) Milking heavily soiled cows could potentially result in bulk milk bacteria counts exceeding 10^4 (or 10,000) cfu/ml, although higher coliform *(or other environmental bacteria)* counts are more likely to occur due to incubation in milk handling equipment. Elevated bulk tank coliform counts can also result from coliform mastitis in the herd.

3) Cleaning and Sanitation

   a) Significant buildup of (thermoduric) organisms in milk residue to a point where they influence the total bulk tank count may take several days to weeks *(and are therefore an indication of a persistent cleaning failure).* Old cracked rubber parts are also associated with higher levels of thermoduric bacteria.

   b) Some types of cleaning failures can also select for faster growing, less resistant organisms, principally Gram-negative rods (coliforms and Pseudomonads) and lactic streptococci and can result in high PIC.

   c) Effective use of chlorine or iodine sanitizers has been associated with reduced levels of psychrotrophic bacteria that cause high PIC.

4) Refrigeration

   a) Elevated psychrotrophic bacteria counts are often associated with poorly cleaned refrigerated bulk tanks.

   b) In milk produced with low initial psychrotrophic populations, psychrotrophic bacteria can quickly become dominant after incubation at 4.4°C (40°F) resulting high PIC.

Statistics: Transforming data into information

Bacteria of all types grow at an exponential rate and therefore produce highly skewed distributions. The same is true for the increase in somatic cell counts in cows infected with mastitis. The linear mastitis score was developed to adjust these highly skewed SCC indicators into a linear effect on milk production. A log transformation will convert bacteria count data into a more normally distributed population and give a better estimate of the resulting milk quality effects of increased bacteria counts. Log transformations therefore offer a better yardstick for true deviations in bacteria count data and provide a more accurate assessment of deviations over time.

Statistical process control uses a combination of a moving average (an average of the last x data points, where x may be from 3 days to many months) and the deviation from this moving average based on the standard deviation of the data. A time series plot (one data point per day) of log10 SPC is shown in Figure 2. A 5 day moving average trend line has been added to figure 2. A moving average trend line in an Excel graph is an excellent way to visually assess real responses from the ‘noise’ in the data and also helps to visualize longer term trends in data. Always use log transformed bacteria count data for statistical process control or when averaging or exploring trends.

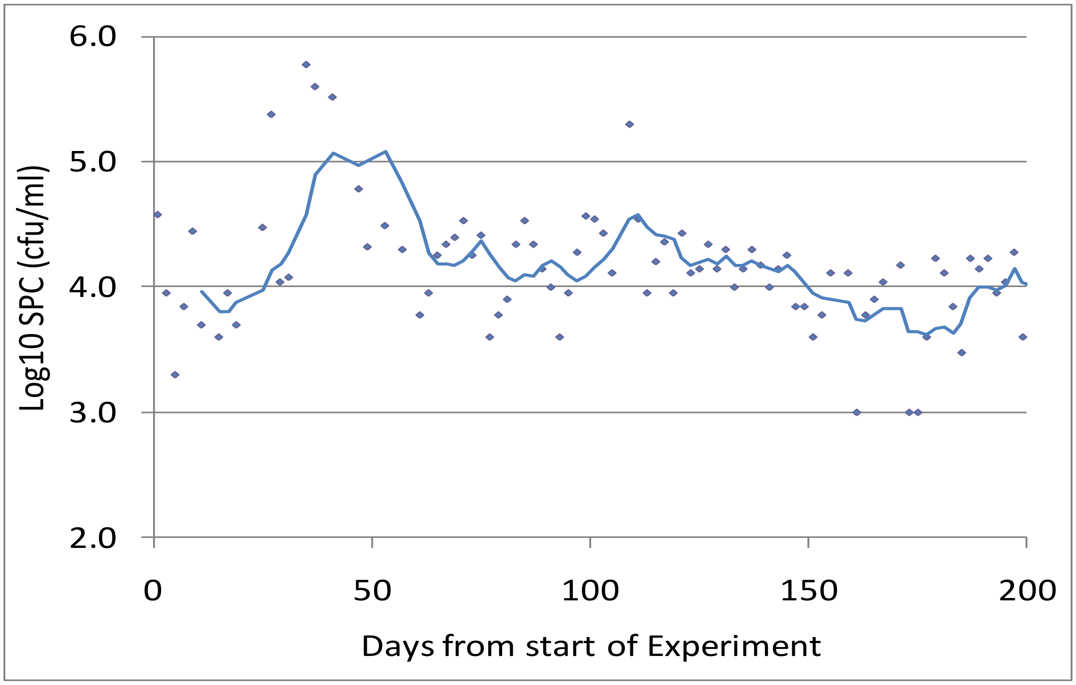


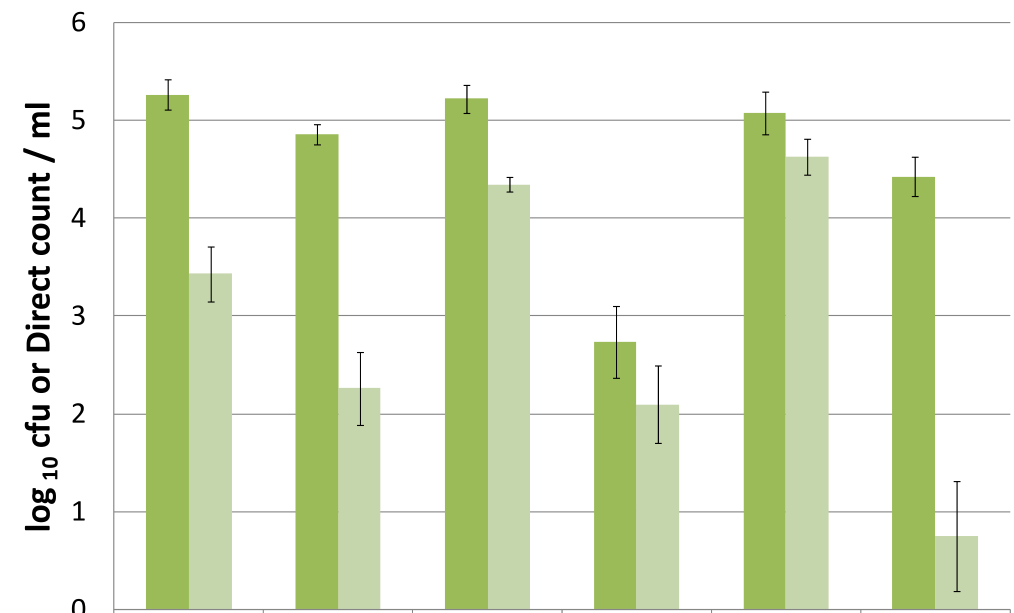
Figure 2. Time series plot of log10 SPC data with a 5 day moving average trend line added.

Correlation between different data sets tells us which data deviate from their average values in the same manner. Data that is collected over time are positively correlated when both increase at the same time. For data that is negatively correlated, one data point tends to deviate in a positive direction while the other deviates in a negative direction. If two data sets have no significant correlation the deviations have no relationship to each other over time (sometimes one is up and the other down and sometimes the other way). Correlation is a powerful tool to help diagnose which bacteria types are the major contributor to short term elevations in SPC and therefore the likely sources to be investigated.

Direct bacteria counting (DBC) methods are becoming more widely used in the dairy industry (e.g. Bactoscan). DBC methods have much better repeatability than plate culture methods and virtually eliminate false-negative results that are common in plate cultures. A combination of DBC to enumerate total bacteria populations combined with plate count methods to estimate the relative magnitude of different bacteria species (if needed) maximizes the strengths and minimizes the weakness each method.

Investigating the Influence of Udder Hygiene and Pre-Milking Sanitation

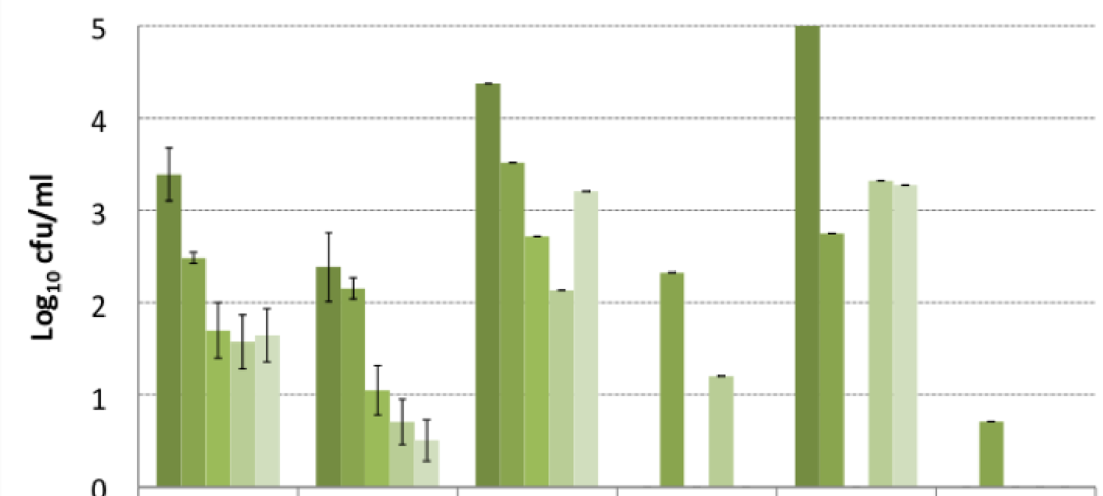
A wide variety of bacteria species can be harvested from the skin of teats and udders during milking. The ’wipe test’ method described in Reinemann et al. (2008) has been used to assess the bacteria population on teat skin both before and after pre milking sanitation. The results of this method used on 3 farms using both SPC and DBC methods pre and post teat sanitation are shown in figure 3. These 2 tests allow a comparison across farms or within a farm over time of the general bacterial condition of teat skin as influenced by farm management and weather conditions. Comparison of the pre and post teat sanitation practice can be used to assess the effectiveness of that practice.



	A. Pre	A. Post	B. Pre	B. Post	C. Pre	C. Post
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 3. DBC and SPC data for Farms A, B, and C. pre and post teat sanitation. Error bars indicate 95% confidence intervals of the mean values

DBC technology 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. In this study the reduction in DBC were used as an estimate of the effectiveness of 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 + unviable bacteria reductions also allowed for an estimate of the killing action of pre-milking teat disinfectants. Further insight is gained by measuring the prevalence of specific bacteria types, as illustrated in figure 4. It is interesting to note large differences in the LPC counts across farms as well as differences in the relative populations of LPC vs. Coli and other environmental bacteria.   Experience with this method on a number of farms has indicated that contamination of bulk milk by thermoduric bacteria can occur because of the harvest of these bacteria from teat skin. It is also common for the environmental bacteria to also be present in large numbers in these situations. It is also interesting to note that pre-milking disinfectants are less effective at reducing thermoduric bacteria counts than common environmental such as coliform, streptococcal and staphylococcal organisms.



	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 4. 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

Cleaning the Milk Handling Equipment

A procedure for troubleshooting cleaning problems was developed by the machine milking committee of the NMC and last updated in 2004. This method begins with an analysis of bulk tank bacteria cultures. The methods presented above provide further clarification and new developments in this method. The purpose of this exercise is to determine if cleaning problems are the most likely cause of elevated bacteria counts. There have been innumerable attempts to fix a high bacteria count by focusing attention on the cleaning system when the likely cause of an elevated count (especially a 'spike' or short term or singe event of a rise in SPC or LPC) was actually caused by environmental contamination. A common response in such a situation is to apply a 'shock' treatment to the milking machine in which chemical concentrations and wash water temperatures are greatly increased. This practice usually results in a substantial degradation of the rubber components in the milk handling system resulting in a milking machine that is much more difficult, or impossible to clean effectively.

If bulk tank cultures indicate that a cleaning failure is the likely cause of elevated bacteria counts, there are a few basic concepts to further determine the causal mechanism.

1. Are the cleaning procedures being implemented properly and with the appropriate frequency?
    a. Proper implementation of the cleaning procedures includes using the appropriate concentration of cleaning chemicals and water temperature.
        i. Check the product labels to ensure that the cleaning chemicals are being used correctly. Increasing water hardness requires increasing detergent concentration for the same cleaning efficacy. Recommendations for the proper detergent concentration

should include a test of water hardness in addition to a test of the alkalinity of the cleaning solution as mixed on the farm.

   ii. Water temperatures can be checked with a pocket thermometer.

b. It is often difficult to determine if the cleaning cycles are being executed with the proper frequency. A temperature recorder in the bulk tank can be used to determine if the expected temperature rise occurs at the frequency that cleaning should be performed. Many modern milking machine wash controllers also record this information for the cleaning cycles in the milking machine. These are very useful sources of information and should be used whenever available.

2. Are the cleaning solutions being circulated throughout the entire milking machine?
   a. There are 2 fundamentally different flow patterns in a milking machine cleaning system.
      i. The milking units and milk meters if present: Cleaning of these components requires that cleaning solutions have long contact times as the agitation and turbulence developed in them is very low. This can be achieved by ensuring that these components are 'flooded' or full of cleaning solution, during a majority of the cleaning cycle. This can be assessed by visual assessment, or more accurately by measuring the water flow through each unit during the cleaning cycle with a simple flow meter
      ii. Flow dynamics in the milk line: In smaller milking machines the milk line may also be cleaned by flooding the milk line. As the diameter and length of the milk line increase this becomes impractical due to the large volume of water required. Air injection provides increased mechanical cleaning action in the milking and reduced the amount of water and cleaning chemical required to achieve effective cleaning. A vacuum recorder can be used to determine if air injected slug flow results in complete coverage and agitation in the entire milk line. A vacuum drop of at least 15 kPa at each milk inlet on the milk line during each air injection cycle is a good indication that milk line flow dynamics are effective

Further details of test methods and diagnostics are available in references at the end of this paper.

Summary Points

- Know your enemy: Acquiring information about the specific bacterial species represented in bulk tank milk will improve the power of a diagnosis. Use bulk tank cultures to determine that a cleaning failure is the most likely cause of elevated bacteria counts before trying to 'fix' the cleaning system.
- Bacteria count data should be converted into log values so that statistical analysis and process control algorithms are valid and more sensitive.
- The moving average is a useful tool to assess long term trends in bacteria populations in bulk milk.
- Correlation is a useful tool to aid in the diagnosis of the types of bacteria contributing to short term changes in bulk tank bacteria counts.
- Incubation causes dramatic increases in some bacteria types but not in others. Knowledge of the growth rates of different bacteria types under different conditions will improve diagnostic methods.
- Premilking sanitation will reduce bacteria population on teat skin but not eliminate bacteria. Cows entering the milking with higher degree of teat contamination will still have a higher degree of teat contamination for the same method of pre-milking teat sanitation than cows with lower initial teat contamination.
- Pre-milking sanitizers are more effective at reducing the population of some bacteria types (environmental) than others (thermoduric).
- If bulk tank cultures indicate that a milking machine cleaning failure is likely, begin your troubleshooting by checking the simple things first.
    - Is something broken? (Water heater, chemical mixer, valves, switches, etc.)
    - Are chemicals being used properly (according to label directions for concentration adjusted for water hardness, temperature, and circulation time?
- Move to an analysis of flow dynamics after these have been confirmed.

References

Guterbok, W.M., and P.E. Blackmer, 1984. Veterinary Interpretation of Bulk Tank Milk. Veterinary Clinics of North America: Large Animal Practice, Vol. 6, No. 2, July 1984. pp. 257-268.

Murphy S.C., and K.J. Boor, 2008. Sources and Causes of High Bacteria Counts in Raw Milk: www.extension.org/pages/Sources_and_Causes_of_High_Bacteria_Counts_in_Raw_Milk:_An_Abbreviated_Review

Reinemann, D.J., 2009. Methods for Interpretation of Bulk Tank Cultures and Udder Hygiene Tests for Diagnosing High Bacteria Counts: or, A High LPC count is not always a Cleaning Failure. Proc. NMC 47th Annual Meeting, Charlotte, NC, 2009. Available at www.uwex.edu/uwmril

Reinemann, D.J., R.D. Bade and P.D. Thompson, 2008. Method for Assessing Teat and Udder Hygiene. Paper No. 083796, Written for presentation at the 2008 ASABE Annual International Meeting. Available at www.uwex.edu/uwmril

Reinemann, DJ, GA Mein, J. Britt, D. Reid, 1998, Troubleshooting High Bacteria Counts in Farm Milk. University of Wisconsin Extension, Bulletin A3705. Available at www.uwex.edu/uwmril

Reinemann, DJ, 1996, Testing Cleaning Performance of Milking systems with a Vacuum Recorder. University of Wisconsin Extension, Bulletin A3649. Available at www.uwex.edu/uwmril