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Dairy Foods Science Notes

- RAW MILK BACTERIA TESTS -
*Standard Plate Count, Preliminary Incubation Count,
Lab Pasteurization Count and Coliform Bacteria Counts*
&
SOURCES AND CAUSES OF HIGH BACTERIA COUNTS
- AN ABBREVIATED REVIEW -

Dairy product quality assurance begins at the farm and ends in the hands of the consumer. In this regard, raw milk quality is essential and is closely monitored. Regulations require that bacteria and somatic cell counts of Grade "A" raw milk not exceed 100,000 Standard Plate Count (SPC) and 750,000 Somatic Cell Count (SCC), respectively. Raw milk must also meet other quality standards; it should be free of drug residues, free of added water and free of sediment, contaminants and other abnormalities. The overall condition and cleanliness of the dairy farm, as determined by routine inspections, are also considered. Although regulatory requirements have been instrumental in ensuring the quality of raw milk, most segments of the dairy industry feel that more stringent standards (e.g. $SPC \leq 10,000$, $SCC \leq 200,000$) would result in an even higher quality milk supply. Depending on the purchaser of the milk, dairy farmers can also receive substantial monetary premiums by producing quality milk that meets standards far more demanding than regulatory requirements.

The SPC, which measures the overall bacterial quality of a sample, is used extensively in both regulatory and premium testing programs. In addition to the SPC, producer raw milk is often subjected to a number of other bacteriological tests used as indicators of how that milk was produced. These tests may be included in determining eligibility for premium payments or they may be used simply as an added quality assurance tool. The procedures most often used in addition to the SPC are the *Preliminary Incubation Count* (PIC), the *Lab Pasteurization Count* (LPC) and/or the *Coliform Bacteria Count* (Richardson, 1985). While the SPC gives an estimated count of the total bacteria in a sample that can be detected with the method, the PIC, LPC and Coliform Count select for specific groups of bacteria that are often associated with poor dairy practices. Results of these testing procedures are used to help identify potential problems that may not be evident or detected with the SPC alone. This document provides an overview of these bacteriological procedures and provides a general discussion on the causes of high bacteria counts in raw milk.

Standard Plate Count

The Standard Plate Count (SPC) of a producer raw milk samples gives an indication of the total number of aerobic bacteria present in the milk at the time of pickup. Milk samples are plated in a semi-solid nutrient media and then incubated for 48 hours at 32°C (90°F) to encourage bacterial growth. Single bacteria or tight clusters (e.g. chains or clumps) grow to become visible colonies that are then counted. All bacterial plate counts are expressed as the number of colony forming units (cfu) per milliliter (ml).

Aseptically collected milk from clean, healthy cows generally has SPC values of less than 1,000. Higher counts suggest that contaminating bacteria are entering the milk from a variety of possible sources. Although it's impossible to eliminate all sources of contamination, counts of less than 5,000 or even 1000 are possible; counts of 10,000 or less should be achievable by most farms. One of the most frequent causes of high SPCs is poor cleaning of the milking system. Milk residues on equipment surfaces provide nutrients for growth and multiplication of bacteria that can then contaminate the milk of subsequent

milking. Other practices that might contribute to increased bulk-tank SPCs are milking soiled cows, maintaining an unclean milking and housing environment, and failing to rapidly cool the milk to or maintain it at less than 4.4°C (40°F). On rare occasions, mastitic cows that shed infectious bacteria can also contribute to or cause high SPCs.

Preliminary Incubation Count

The Preliminary Incubation Count (PIC) reflects milk production practices. This procedure involves holding the milk at 12.8°C (55°F) for 18 hours prior to plating. This step encourages the growth of groups of bacteria that grow well at cool temperatures. Bacteria in the incubated sample are counted with the SPC procedure and compared to the SPC of the un-incubated sample to determine if a significant increase has occurred. PICs are generally higher than SPCs, although in some cases no growth occurs or counts may even be lower. Counts 3-4 fold higher are often considered significant, but this depends on the initial SPC. Some consider counts of > 50,000 to be of concern regardless of the SPC, though in some cases the counts may be equal and in rare cases the PIC may be lower. It should be noted that contaminating bacteria from the same source can vary in their growth rates in this procedure, which can result in very different PICs at the same level of contamination.

High PICs are most often associated with a failure to thoroughly clean and sanitize either the milking system or, in some cases, the cows. Bacteria considered to be natural flora of the cow, including those that cause mastitis, are not thought to grow significantly at the PI temperature, although there may be a few exceptions. If a PIC is approximately equal to or slightly higher, or even lower, than a high SPC (e.g., > 50,000), it may suggest that the high SPC is possibly due to mastitis. Marginal cooling (e.g., milk is held over 40°F) or prolonged storage times may also result in unacceptable PIC levels by allowing organisms that grow at refrigeration temperatures to multiply. Bacteria that grow well at refrigeration temperatures (*psychrotrophic bacteria*), are most frequently associated with high PICs. Although the same types of bacteria that cause high PICs can also cause defects in pasteurized milk products, *the PIC of a raw milk supply does not indicate the potential quality or shelf-life of a pasteurized product made from that milk*. These types of bacteria are mostly destroyed by pasteurization, but can occur as post-pasteurization contaminants in pasteurized milk.

Lab Pasteurized Count

Although most bacteria are destroyed by pasteurization, there are certain types that are not. The Lab Pasteurized Count (LPC) estimates the number of bacteria in a sample that can survive the pasteurization process. Milk samples are heated to 62.8°C (145°F) for 30 minutes, which simulates batch pasteurization. Bacteria that survive the heat treatment (*thermoduric bacteria*) are enumerated using the SPC procedure. LPCs are generally much lower than SPCs, with counts > 200-300 deemed high. The natural bacterial flora of the cow, as well as those associated with mastitis, are generally not thermoduric, although there may be a few exceptions. High LPCs are most often associated with a chronic or persistent cleaning failure in some area of the system or significant levels of contamination from soiled cows. Other common causes of high LPCs are leaky pumps, old pipe-line gaskets, inflations and other rubber parts, and milkstone deposits.

Coliform Count

The Coliform Count procedure selects for bacteria that are most commonly associated with manure or environmental contamination. Milk samples are plated on a selective bacterial media that encourages the growth of coliform bacteria, while preventing the growth of others. Though coliforms are often used as indicators of fecal contamination, there are strains that commonly exist in the environment. Coliforms may enter the milk supply as a consequence of milking soiled cows or dropping the milking claw into manure during milking. Generally, counts >50 would indicate poor milking hygiene or other sources of contamination. Higher coliform counts more often result from dirty equipment and in rare cases result from milking cows with environmental coliform mastitis.

Quality Standards - SPC, LPC, PIC and Coliform Count

Suggested standards used for quality premiums or for trouble-shooting purposes are listed in table 1. The tests included in a quality program, as well as the limits used, will vary depending on the philosophy and requirements of the processor or cooperative. Generally, standards used to determine premium eligibility are based on values established for well managed farms. Although some of the test methods are not considered “official,” it is important that they be carried out in a professional manner using standardized procedures. It is not recommended that these tests be used to penalize dairy farms.

Table 1. Suggested Bacterial Test Standards for Quality Premiums

<u>Testing Procedure</u>	<u>Suggested Standard¹</u>	<u>Regulatory Standard¹</u>
Standard Plate Count	< 10,000	≤ 100,000
Laboratory Pasteurization Count	< 200 - 300	none
Preliminary Incubation Count	50,000 – 100,000 and/or Significantly > SPC	none
Coliform Bacteria Count	< 50	California (< 750)

¹ All counts expressed as colony forming units per ml.

Sources and Causes of High Bacteria Counts in Raw Milk: An Abbreviated Review

Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (Tolle, 1980). Beyond this stage of milk production, microbial contamination can generally occur from three main sources (Bramley & McKinnon, 1990); from within the udder, from the exterior of the udder and from the surface of milk handling and storage equipment. The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are all key factors in influencing the level of microbial contamination of raw milk. Equally important are the temperature and length of time of storage, which allow microbial contaminants to multiply and increase in numbers. All these factors will influence the total bacteria count (SPC) and the types of bacteria present in bulk raw milk.

Microbial Contamination from within the Udder: Raw milk as it leaves the udder of healthy cows normally contains very low numbers of microorganisms and generally will contain less than 1000 total bacteria per ml (Kurweil, 1973). In healthy cows, the teat cistern, teat canal, and the teat apex may be colonized by a variety of microorganisms though microbial contamination from within the udder of healthy animals is not considered to contribute significantly to the total numbers of microorganisms in the bulk milk, nor to the potential increase in bacterial numbers during refrigerated storage. Natural flora of the cow generally will not influence LPCs, PICs or Coliform Counts.

While the healthy udder should contribute very little to the total bacteria count of bulk milk, a cow with mastitis has the potential to shed large numbers of microorganisms into the milk supply. The influence of mastitis on the total bacteria count of bulk milk depends on the strain of infecting microorganism(s), the stage of infection and the percentage of the herd infected. Infected cows have the potential to shed in excess of 10^7 bacteria per ml. If the milk from one cow with 10^7 bacteria per ml comprises 1% of the bulk tank milk, the total bulk tank count, disregarding other sources, would be 10^5 per ml (Bramley & McKinnon, 1990).

Mastitis organisms found to most often influence the total bulk milk count are *Streptococcus* spp., most notably *S. agalactiae* and *S. uberis* (Bramley & McKinnon, 1990; Bramley et al., 1984; Gonzalez et al., 1986; Jeffrey and Wilson, 1987) though other mastitis pathogens have the potential to influence the bulk

tank count as well. *Staphylococcus aureus* is not thought to be a frequent contributor to total bulk tank counts though counts as high as 60,000/ml have been documented (Gonzalez et al., 1986). Detection of implied pathogens does not necessarily indicate that they originated from cows with mastitis. Potential environmental mastitis pathogens and/or similar organisms can occur in milk as a result of other contributing factors such as dirty cows, poor equipment cleaning and/or poor cooling. An increase in SCC can sometimes serve as supportive evidence that a mastitis bacterium may have caused an increase in the bulk milk bacteria count. This seems to hold true more for *Streptococcus* spp. than for *S. aureus*, which appears to be shed into the milk in lower numbers (Fenlon et al., 1995). Correlations of somatic cell responses and environmental mastitis organisms, including coliform bacteria, streptococci, and certain coagulase-negative *Staphylococcus* spp., were found to be poor as well. These organisms are by nature associated with the cow's environment and may influence bulk milk bacteria counts through other means (Bramley, 1982; Zehner et al., 1986). *S. agalactiae* and *S. aureus* are not thought to grow significantly on soiled milking equipment or under conditions of marginal or poor cooling. Their presence in bulk tank milks is considered strong evidence that they originated from infected cows (Gonzalez et al., 1986; Bramley and McKinnon, 1990). In general, mastitis organisms will not influence LPCs or PICs though in some cases of coliform mastitis, coliform counts may be elevated.

Microbial Contamination from the Exterior of the Udder: The exterior of the cows' udder and teats can contribute microorganisms that are naturally associated with the skin of the animal as well as microorganisms that are derived from the environment in which the cow is housed and milked. In general, the direct influence of natural inhabitants as contaminants in the total bulk milk count is considered to be small and most of these organisms do not grow competitively in milk. Of more importance is the contribution of microorganisms from teats soiled with manure, mud, feeds or bedding.

Teats and udders of cows inevitably become soiled while they are lying in stalls or when allowed in muddy barnyards. Used bedding has been shown to harbor large numbers of microorganisms. Total counts often exceed 10^8 - 10^{10} per gram (Bramley, 1982; Bramley & McKinnon, 1990; Hogan et al., 1989; Zehner et al., 1986). Organisms associated with bedding materials that contaminate the surface of teats and udders include streptococci, staphylococci, spore-formers, coliforms and other Gram-negative bacteria. Both thermophilic and psychrotrophic strains of bacteria are commonly found on teat surfaces (Bramley & McKinnon, 1990) indicating that contamination from the exterior of the udder can influence LPCs, PICs and coliform counts.

The influence of dirty cows on total bacteria counts depends on the extent of soiling of the teat surface and the wash procedures used immediately before milking. For example, if one gram of teat soil containing 10^8 bacteria is allowed into the milk of one cow giving approximately 30 lb. (~13,400 gm) of milk, the total bacteria count for that cow's milk, excluding other sources, would be in excess of 7,000 cfu/ml. Milking heavily soiled cows could potentially result in bulk milk counts exceeding 10^4 cfu/ml. Several studies have investigated pre-milking udder hygiene techniques in relation to the bacteria count of milk (Bramley & McKinnon, 1990; Galton et al 1984; McKinnon et al 1990, Pankey, 1989). Generally, thorough cleaning of the teat with a sanitizing solution (spray, wet towel or dip) followed by thorough drying with a clean towel is effective in reducing the numbers of microorganisms in milk contributed from soiled teats. Counts of coliform bacteria, though highly associated with manure, barnyard mud, and used bedding, were relatively low in these studies, even for the untreated cows suggesting that higher coliform counts in bulk milk are more likely to occur due to other factors (e.g., equipment, mastitis).

Influence of Equipment Cleaning and Sanitizing Procedures: The degree of cleanliness of the milking system probably influences the total bulk milk bacteria count as much, if not more than any other factor (Olson and Mocquat, 1980). Milk residues left on equipment contact surfaces support the growth of a variety of microorganisms. Organisms considered as natural inhabitants of the teat canal, apex and skin are not thought to grow significantly on soiled milk contact surfaces or during refrigerated storage of milk. This generally holds true for a number of organisms associated with contagious mastitis (e.g., *S.*

agalactiae), although there may be exceptions. Certain strains associated with environmental mastitis (e.g., coliforms) may be able to grow to significant numbers on milk contact surfaces. In general, environmental contaminants (e.g., from bedding, manure, feeds) are more likely to grow on soiled equipment surfaces. Water used on the farm might also be a source of microorganisms, especially psychrotrophs that might seed soiled equipment and/or the milk (Bramley & McKinnon, 1990).

Cleaning and sanitizing procedures can influence the degree and type of microbial growth on milk contact surfaces by leaving behind milk residues that support growth, as well as by setting up conditions that might select for specific microbial groups. More resistant and/or thermophilic bacteria may endure in low numbers on equipment surfaces that are considered to be efficiently cleaned with hot water. If milk residue is left behind (e.g., milk stone) growth of these types of organisms, though slow, may persist. Old cracked rubber parts are also associated with higher levels of thermophilic bacteria. Significant build-up of these organisms to a point where they influence the total bulk tank count may take several days to weeks (Thomas et al., 1966), although increases could be detected in the LPC procedure.

Less efficient cleaning, using lower temperatures and/or the absence of sanitizers tends to select for the faster growing, less resistant organisms, principally Gram-negative rods (coliforms and Pseudomonads) and lactic streptococci. This will result in potential high PICs and in some case elevated LPCs. Effective use of chlorine or iodine sanitizers has been associated with reduced levels of psychrotrophic bacteria that cause high PICs (Jackson & Clegg, 1965). Psychrotrophic bacteria tend to be present in higher count milk and are often associated with occasional neglect of proper cleaning or sanitizing procedures (Olson & Mocquat, 1980; Thomas et al., 1966) and/or poorly cleaned refrigerated bulk tanks (MacKenzie, 1973; Thomas, 1974).

Milk Storage Temperature and Time: Refrigeration storage, while preventing the growth of non-psychrotrophic bacteria, will select for psychrotrophic microorganisms that enter the milk from soiled cows, dirty equipment and the environment. Minimizing the level of milk contamination from these sources will help prevent psychrotrophs from growing to significant levels in the bulk tank during the storage period on the farm or at the dairy plant. In general these organisms are mostly not thermophilic and will not survive pasteurization. The longer raw milk is held before processing (potential up to 5 days; 2 days on the farm, 3 at the plant), the greater the chance that psychrotrophs will increase in numbers. Holding milk near the legal limit of 7.2°C (45°F) allows much quicker growth than milk held below 4.4°C (40°F). Although milk produced under ideal conditions may have an initial psychrotroph population of less than 10% of the total bulk tank count, psychrotrophic bacteria can become the dominant microflora after 2-3 days at 4.4°C (40°F) (Gehring, 1980). This could result in a significant influence on PICs. Colder temperatures of 1-2°C (34-36°F) will delay this shift, although not indefinitely.

Under conditions of poor cooling with temperatures greater than 7.2°C (45°F), bacteria other than psychrotrophs are able to grow rapidly and can become predominant in raw milk. Though incidents of poor cooling still occur, this defect is not as common as when milk was held and transported in cans. Streptococci have historically been associated with poor cooling of milk, appearing as pairs or chains of cocci (spherical bacteria) on microscopic examination of milk smears (Atherton & Dodge, 1970). These bacteria will potentially increase the acidity of milk. Certain strains are also responsible for a “malty defect” that is easily detected by its distinct odor. Storage temperatures greater than 15°C (60°F) tend to select for these types of contaminants (Gehring, 1980). Although poor cooling conditions allow growth of bacteria that normally will not grow in properly refrigerated milk, it will not prevent typical psychrotrophic strains from growing. The types of bacteria that grow and become significant will depend on the initial microflora of the milk (Bramley & McKinnon, 1990).

Summary

Microbial contamination of raw milk can occur from a variety of microorganisms from a variety of sources. Because of this, determining the cause of bacterial defects is not always straight-forward. High bacteria counts can result from a combination of factors (e.g., dirty equipment coupled with marginal cooling). Other than the SPC, a number of testing procedures may be used to evaluate the quality of raw milk, including the LPC, PIC and the coliform count. These tests generally select for bacteria that occur as contaminants that are not considered to be the natural flora of the cow. In some cases, though not covered in this paper, more extensive procedures (e.g., bacterial culturing for mastitis bacteria) may be useful. The likelihood of bacteria from different sources being responsible for high counts in the discussed procedures is summarized in table 2.

Table 2. Sources Of Microbial Contamination As Detected By Bacteriological Procedures.

<u>Procedure</u>	<u>Natural Flora</u>	<u>Mastitis</u>	<u>Dirty Cows</u>	<u>Dirty Equip.</u>	<u>Poor Cooling</u>
SPC >10,000	Not likely	Possible	Possible	Possible	Possible
SPC >100,000	Not likely	Possible (rare)	Not likely	Possible *	Possible *
LPC >200-300	Not likely	Not likely	Possible	Possible *	Not likely
PIC High vs. SPC	Not likely	Not likely	Possible	Possible *	Possible *
SPC High/ no increase in PIC	Not likely	Possible	Possible but not likely	Possible but not likely	Not likely but possible
Coliform Count High	Not likely	Possible (rare)	Possible	Possible	Not likely But Possible

* A more likely possible source.

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