# Technews

**National Dairy Development Board**

**For Efficient Dairy Plant Operation**

**March-April 2005** **No.55**


# MICROBIOLOGICAL TROUBLESHOOTING OF LIQUID MILK

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue is **Microbiological Troubleshooting of Liquid Milk**. It may be understood that the information given here is by no means complete.

***In this issue:***

# 1. INTRODUCTION

Microbial contamination of milk, leading to various quality defects, can occur with a variety of microorganisms from a variety of sources. Because of this, determining the cause of bacterial defects is not always straightforward. Though there is often one source of bacteria that causes high counts, such high bacterial counts can also result from a combination of factors (i.e. animal, dirty equipment and inadequate cooling). Further, specific type of microbial contamination at any point before processing may manifest itself, in terms of product quality defects, at a subsequent stage even after appropriate and effective heat processing due to prevalence of conditions that favour the contaminant's growth. Therefore, it becomes important for a dairy processor to diagnose the defect in the product, pinpoint the type of contamination through selective plating procedures, identify sources of contamination, and take appropriate preventive and corrective measures.

This issue of Technews details the sources and type of microbial contamination and control measures to minimize such microbial contamination and resultant growth in raw and pasteurized milk.

# 2. HIGH COUNTS IN RAW MILK

Milk is synthesized in mammary glands and is virtually sterile when secreted into the udder. But even before it leaves the udder it is infected by bacteria which enter through the teat canal. These bacteria are usually harmless and few in number, only a few tens or hundreds per ml of milk[1, 2, 3]. Beyond the stage of secretion, microbial contamination of milk can generally occur from three main sources - 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 milk animal, the environment in which the milk animal is housed and milked, the procedures used in cleaning and sanitizing the milking and milk storage and handling equipment are all key factors influencing the type and level of microbial contamination of raw milk. Equally important are the temperature and length of time of storage which allow specific microbial contaminants to multiply and increase in numbers. All these factors influence the total bacteria count or Standard Plate Count (SPC) in bulk raw milk. Raw milk may contain anything from a few thousand bacteria per ml, if it is hygienically produced, up to tens of lakhs if the standard of cleaning, disinfection and chilling is poor[(2)]. Table 1 presents possible causes of high bacterial content in raw milk, symptoms of such highly contaminated milk and suggested remedial measures to prevent contamination of raw milk.

**Table 1: Sources of microbial contamination of raw milk and measures to control the contamination**

| Possible causes | Suggested remedial measures |
|---|---|
| **a) Animal**<br>Soiled teats and udders of cows: Milking cows with heavily soiled and unwashed teats may result in bacterial count in milk approaching 1 lakh per ml.[(1)] Both thermoduric and psychrotrophic strains of bacteria are commonly found on teat surfaces.<br><br>For milk to be classed as top quality, the bacterial count should be less than 1 lakh per ml.[(2)] | ►Thorough cleaning of the teat with iodophore solution (iodine content 0.71% w/v) spray or dip followed by drying is effective in reducing the numbers of microorganisms in milk contributed from soiled teats.<br>►Avoiding animal access to unclean areas. |

| Possible causes | Suggested remedial measures |
|---|---|
| Surface of teats and udders are usually contaminated with organisms associated with animals' bedding materials. The contaminating organisms include micrococci (including coagulase-negative staphylococci), streptococci (mainly faecal type), Gram-negative (psychrotrophs) and Gram-positive (both spore formers and non-spore formers) rods.[1,3] Bedding material in winter may have very high bacterial counts, $10^8$-$10^{10}$ per g, although the bedding may appear relatively clean and dry.[1] | ►Adequate and frequent replenishment of clean straw for animal bedding. |
| • Mastitic cows: Potential to shed in excess of $10^7$ bacteria per ml of milk, depending upon the strain of infecting microorganism(s), the stage of infection etc.[3]<br><br>Prominent mastitis bacteria influencing total bulk milk counts are *Streptococcus* spp., most notably *S. agalactiae* and *S. uberis*. *Staphylococcus aureus* infrequenly influences total bulk milk counts. Potential mastitis environmental pathogens, including coliforms, are by nature associated with milk animal's environment and | ►Preventing mix-up of mastitic milk with normal milk supplies<br>►Milking hygiene and post milking teat disinfection to reduce the transfer of bacteria during milking, and to destroy pathogens left on the teat skin at the end of milking.<br>►Timely and adequate treatment of animals suffering from mastitis. Preventing new cases of infection or eliminating existing sub-clinical mastitis through antibiotic treatment at dry-off (end |

| Possible causes | Suggested remedial measures |
|---|---|
| may influence bulk milk bacteria counts through other means also.[3] | of lactation period) and possible vaccination.<br>►Drying-off of chronically infected quarter. |
| **b) Contact surfaces: Improperly cleaned equipments and equipment surfaces**<br>More resistant and/or thermoduric bacteria (such as *Micrococci* and *Microbacterium*) surviving hot water cleaning of equipment and growing in residual soils on the surfaces of milk handling and storage equipment.[1, 3] Old cracked rubber parts are also associated with higher levels of thermoduric bacteria. Significant build-up of these organisms in the residual soils on equipment surfaces to a point where they influence the total bulk tank count may take several days to weeks though increase would be detected by Laboratory Pasteurization Counts (LPC). | ►Preventing build-up of milk soils by regular and effective cleaning of the cold milk equipment **(please see *Technews* Issue Nos. 48 (Jan.-Feb. 2004) and 49 (Mar.-Apr. 2004)).**<br>►Replacing worn-out rubber parts such as gaskets, seals etc. in the raw milk handling and storage equipment. A preventive maintenance programme for equipments is desirable**.** |
| • Less resistant organisms, principally Gram-negative rods (coliforms and pseudomonads) and lactic streptococci surviving less efficient cleaning, using lower temperatures and/or the absence of sanitizers. Psychrotrophic bacteria tend to be present in higher count milk | ►Effective use of chlorine or iodine sanitizers for cold milk equipment cleaning helps reducing levels of psychrotrophic bacteria **(please see also *Technews* issues 48 (Jan.-Feb. 2004) and 49 (Mar.-Apr. 2004)).** |

| Possible causes | Suggested remedial measures |
|---|---|
| and are often associated with neglect of proper cleaning or sanitizing procedures and/or poorly cleaned refrigerated bulk tanks procedures.[3] | |
| **c) Milk storage temperature and time**<br>• High levels of psychrotrophic microorganisms in milk after 2-3 days storage at refrigeration temperatures (4°C)**.**<br>Refrigeration temperatures favour growth of psychrotrophic microorganisms that enter milk from soiled cows, dirty equipment and environment.[3] Some of these psychrotrophs, when growing in refrigerated milk, produce heat resistent lipases and proteinases, responsible for off-flavours in raw / pasteurized milks.[1] | ►Minimizing the level of milk contamination from soiled milk animals, dirty equipment and the environment sources (as specified in measures against (a) and (b) above) helps preventing psychrotrophs from growing to significant levels in the bulk tank during the storage period on the farm or at the dairy plant. **For detailed guidance on reducing contamination of raw milk please refer to *Codex Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004).*** |
| • High levels of streptococci in milk that is poorly cooled. Storage temperatures greater than 15°C tend to favour growth of these types of contaminants[3]. These bacteria will increase the acidity of milk. | ►Storage of milk at appropriate refrigeration temperatures prevents growth of non-psychrotropic organisms. |

## 3. SPOILAGE OF PASTEURIZED MILK

Quality defects, such as abnormal flavours, developed acidity and reduced shelf life, in pasteurized milk products are most often the result of microbial contamination, and their metabolic activities and growth. Though poor quality raw milk can result in defective products, post-pasteurization contamination with psychrotrophic spoilage bacteria is most detrimental.

In most cases, product contamination and spoilage is the result of insufficient cleaning and sanitation of the processing equipment and plant environment. Product contamination may occur even when it appears that a well-designed sanitation and quality control programme is in place.

In the absence of post-pasteurization contamination, certain strains of microorganisms (i.e. *Bacillus* spp) that are capable of surviving pasteurization and growing under refrigeration (thermoduric psychrotrophs) can eventually grow and cause spoilage, generally later in shelf-life.

Generally the SPC of freshly pasteurized milk is less than 500/ml. Most often this initial SPC represents those bacteria that survive pasteurization (thermoduric bacteria). When pasteurized milk is prepared from raw milk with a low level of thermoduric organisms, initial counts in pasteurized milk, prior to storage and distribution, higher than 1000 SPC/ml suggest a potential post pasteurization contamination.[4]

Table 2 presents possible causes of high bacterial content in pasteurized milk and resultant spoilages, and remedial measures to prevent such defects.

**Table 2: High bacterial counts in pasteurized milk and remedial measures**

| Possible causes | Suggested remedial measures |
|---|---|
| **a) Raw milk contaminants surviving pasteurization** | |
| • Contamination of raw milk supply with thermoduric microorganisms - primarily psyc**h**rotrophic spore formers (*Bacillus cereus, B. circulans and B. mycoides etc.)* - their survival during pasteurization and subsequent growth at refrigeration temperatures. Thermoduric psychrotrophs occasionally spoil milk in the absence of Gram-negative post-pasteurization contamination. These types of organisms generally grow slower and/or begin growth later, causing problems later in shelf-life.[4] | ► The likely occurrence of these organisms that survive pasteurization and eventually cause spoilage can be minimized through hygienic raw milk production and handling procedures from the farm to the plant. |
| • Contamination of raw milk supply with thermoduric microorganisms- *Streptococcus* spp (*Str. thermophilus, Ent. faecalis & Str. brevis*), *Bacillus* spp. (*B, subtilis*, *B. licheniformis etc.)* - their survival during pasteurization and subsequent growth in inadequately refrigerated product. | ► Maintenance of appropriate cold-chain following pasteurization process throughout the shelf life of the pasteurized product. |

| Possible causes | Suggested remedial measures |
|---|---|
| **b) Post pasteurization contamination**<br>• Post pasteurization contaminants (PPC), mostly gram-negative rods, generally arising from improperly cleaned equipment (balance tank, filling machines etc.) downstream of the pasteurization process. Of these, psychrotrophic bacteria are generally the cause of most shelf-life problems in pasteurized milk.<br>The most common psychrotrophic organisms implicated in the spoilage of fluid milk are Gram-negative rods, primarily belonging to the genus *Pseudomonas*. As a rule, Gram-negative bacteria in general do not survive pasteurization. However, Gram-negative psychrotrophic spoilage organisms re-contaminating product even at very low levels, often less than 1 per ml, still present a major concern because one psychrotrophic bacterium with a doubling time of 6 hours can spoil even refrigerated milk in less than ten days. Generally, when the SPC exceeds 1 crore per ml the product will become unacceptable due to flavor defects related to bacterial | ►Preventing post pasteurization contamination of milk **(for detailed guidance please see *Technews* Issue No.46, (Sep.-Oct. 2000)).**<br>►Proper cleaning and sanitization of post process equipment such as pipelines, pumps, storage tanks and especially filling machines.<br>►Effective use of chlorine or iodine sanitizers for equipment cleaning helps in reducing levels of psychrotrophic bacteria **(see also *Technews* issues 48 (Jan.-Feb. 2004) and 49 (Mar.-Apr. 2004)).** |

| Possible causes | Suggested remedial measures |
|---|---|
| growth and metabolism. The extent and type of spoilage will depend on the strain(s) of psychrotrophic bacteria present.[4] | |

# 4. FLAVOUR DEFECTS ASSOCIATED WITH BACTERIAL ACTIVITY IN RAW / PASTEURIZED MILK

Milk is an excellent growth medium for bacteria. It provides the nutrients and moisture and has a near neutral pH. Off-flavors are the results of bacterial growth (mainly psychrotrophs). The type of bacteria is more important than the number. Table 3 presents most common flavour defects encountered in raw / pasteurized milk as a result of bacterial activity, and possible measures to control occurrence of such defects.

**Table 3: Bacteria induced flavour defects in raw / pasteurized milk and measures to prevent such defects**

| Possible causes / symptoms | Suggested remedial measures |
|---|---|
| **Malty flavour**<br>• Causative agent *Streptococcus lactis*.<br>• Develops in poorly cooled raw milk.<br>• Rarely develops in pasteurized milk. However, the characteristic flavor developed in raw milk persists after processing. | ►Adequate sanitation of equipments used for storage and handling of raw milk **(see *Technews* Issues Nos. 48 (Jan.-Feb. 2004) and 49 (Mar.-Apr. 2004)).**<br>►Replacement of old rubber parts before they become worn-out. A preventive |

| Possible causes / symptoms | Suggested remedial measures |
|---|---|
| • Problem can be exacerbated due to commingling of defective milk with fresh milk from different bulk tanks etc.<br>• Malty flavor is generally a forerunner of a high acid flavor. | maintenance programme is desirable.<br>►Raw milk storage at less than 7°C, preferably 1-4°C. While mixing and storing raw milk from more than one milking, adequate cooling of first milking is critical.(5)<br>►Timely pasteurization of raw milk to arrest the defect, which otherwise might lead to high acid flavour. |
| **High acid flavour**<br>• Prolonged storage of raw milk in a bulk tank for more than three days has the potential of high bacteria counts and off-flavors. Longer time periods between pickup and partial emptying of a milk storage tank causes problems.<br>• Bacterial degradation of lactose (milk sugar), during prolonged storage of milk, resulting in acid production. Acidity development usually above 0.20 percent. | ►Adequate sanitation of equipments used for storage and handling of raw milk All milk handling equipment surfaces should be washed after each use and sanitized just prior to reuse. This includes washing all milking units, pails, and pipelines on a twice per day basis. Bulk tanks must be washed each time they are emptied **(see *Technews* Issues Nos.48 (Jan.-Feb. 2004) and 49 (Mar.-Apr. 2004)).**<br>►Raw milk from the bulk tank should be picked up at least every other day. |
| **Putrid flavour**<br>• Causative agents are psychrotrophs.<br>• The most common source of the problem is contamination of | ►Psychrotrophs do not generally survive pasteurization unless large numbers of the bacteria are present in the raw milk. Proper pasteurization and |

| Possible causes / symptoms | Suggested remedial measures |
|---|---|
| equipment used in milk handling following pasteurization.<br><br>• Putrid flavours may also originate in raw milk caused by psychrotrophic contamination and holding raw milk for three or four days after collection from farms. Defect facilitated when storage temperature is above 4°C for longer periods.<br>• Spoilage of the milk is by bacterial action on the protein rather than on the lactose. Frequently the titratable acidity may be near normal.<br>• Putrid milk will curdle, separate and may smell rotten if left for a few days. Heating, vacuum treatment or other processing of the defective does not lessen the flavour defect. | packaging without contamination eliminates most of the psychrotrophs.<br>► Proper cleaning and sanitization of all the parts of the pumping, holding, and filling systems. |

## 5. PROCEDURES FOR SELECTIVE ENUMERATION OF VARIOUS TYPES OF BACTERIA[4]

Milk product quality is generally determined by sensory, chemical and microbiological analysis. While sensory evaluation is somewhat subjective, the microbiological analysis lends more insight into potential quality defects in dairy products. In practice, selective plating procedures or bacterial culturing is generally useful in identifying the source of high bacterial counts in fluid milk. Some selective procedures for enumeration of different types of spoilage bacteria in milk are given below:

**Psychrotroph Count:** Psychrotrophic bacteria are generally the cause of most shelf-life problems in fluid milk. The standard psychrotroph count is determined by plating the milk using the SPC procedure with incubation of the plates for 10 days at 7°C. The psychrotroph count will detect Gram-negative and Gram-positive psychrotrophs, though Gram-positive organisms rarely cause problems in pasteurized fluid milk.

**Coliform Bacteria Count:** Coliforms are killed by pasteurization, thus their detection in pasteurized milk suggests that some point in post-pasteurization handling has been neglected in regard to effective cleaning/sanitation procedures**.**

The coliform count is performed by plating milk samples on Violet Red Bile Agar (VRBA), which is selective for these types of Gram-negative bacteria. The plates are incubated at 32°C for 24 hours after which dark red colonies are counted. Since VRBA may allow growth of non-coliform Gram-negative bacteria (generally smaller, light colored colonies) also, any growth would indicate a PPC concern. This procedure is routinely used for fresh pasteurized milk.

**Gram-Negative Bacteria Count:** Gram-negative bacteria, including coliforms, in general do not survive pasteurization. This is why coliforms are used as indicators of poor hygiene and possible concurrent contamination of milk products with Gram-negative psychrotrophs and/or potential pathogens during post pasteurization handling. The Gram-negative bacteria count is determined by plating the sample on Crystal Violet Tetrazolium Agar (CVTA) which is selective for Gram-negative bacteria.

**Thermoduric Psychrotrophs:** Thermoduric bacteria are those that survive pasteurization. Most thermoduric bacteria are not psychrotrophic, though certain bacteria that survive pasteurization are capable of growth at refrigeration temperatures. The Laboratory Pasteurization Count (LPC) is often used to estimate

the number of thermoduric bacteria in a raw milk supply that will survive pasteurization. The LPC is performed by heating raw milk to 62.8°C for 30 minutes before plating for the SPC. To detect thermoduric psychrotrophs, milk that is laboratory pasteurized is plated for psychrotrophic organisms (SPC procedure incubated at 7°C for 10 days) as well as for the standard SPC. Alternatively, the heated milk can be stored at 7°C for 10 days or more and then plated for SPC. A significant increase over the initial LPC indicates the presence of thermoduric psychrotrophs.

**Spore-forming Thermoduric Psychrotrophs:** Some of the most common thermoduric psychrotrophs are spore-formers (e.g. *Bacillus spp.*), which are more heat resistant and may require higher heat to cause spore germination. For their estimation milk is heated at 80°C for 10 minutes followed by rapid cooling. The milk is then plated for psychrotrophs or the milk itself can be stored under refrigeration for 10-17 days or more and then plated for estimating bacterial counts. Any significant growth would indicate potential spoilage by spore-forming psychrotrophs. Heating larger sample volumes (i.e. 200 ml.) for LPC or psychrotrophic spore counts and holding under refrigeration will help detect low-level contamination.

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**Please send your letters to:**

Dr. N.N. Varshney
National Dairy Development Board
PB No.40
Anand 388001
Gujarat

Fax No. (02692) 260157
Email : nnv@nddb.coop