

Microbiological Characteristics of Total Coliforms in Milk

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ABSTRACT

Milk is a nutrient-rich food that supports the growth of many microorganisms, including bacteria used as indicators of sanitary quality. Among these organisms, total coliforms are especially important because their presence may indicate contamination from water, soil, feed, equipment, hands, animal surfaces, or fecal material. Although total coliforms are not themselves a definitive measure of the presence of pathogens, elevated counts suggest weaknesses in hygienic production, handling, storage, or processing. This paper examines the microbiological characteristics of total coliforms in milk, their major sources, methods of detection, significance for milk quality, and relationship with shelf life. The study is based on a review of microbiological literature and practical serial-dilution observations involving poor-hygiene, good-hygiene, and water-adulterated milk samples. Coliforms are generally Gram-negative, non-spore-forming, facultatively anaerobic rods capable of fermenting lactose with acid and gas production under specified test conditions. The practical dataset showed the highest mean count in poor-hygiene milk, followed by water-adulterated milk, while good-hygiene milk had the lowest mean count. However, the reported calculations contain a dilution-factor inconsistency that must be corrected before the results can be treated as definitive. Current analytical guidance emphasizes validated methods, appropriate dilution procedures, confirmation of presumptive colonies, and careful interpretation of results. Effective control requires hygienic milking, clean water, sanitized equipment, rapid cooling, cold-chain maintenance, pasteurization, and routine microbiological monitoring.

Keywords: Coliforms, Milk Hygiene, Escherichia Coli, Serial Dilution, Microbial Quality, Dairy Microbiology, Shelf Life

Introduction

Milk is one of the most widely consumed animal-derived foods and provides proteins, fats, carbohydrates, vitamins, minerals, and water. These same characteristics make

milk an excellent growth medium for microorganisms when it is exposed to contamination or held under unsuitable conditions. Microbial quality is therefore a central component of milk safety, shelf life, and consumer protection. The microorganisms found in milk may originate from the udder, animal skin, feces, bedding, soil, feed, water, milking equipment, handlers, storage containers, transport systems, or processing facilities (Boor et al., 1998; Coorevits et al., 2008).

Total coliforms are among the most frequently used indicator organisms in food and water microbiology. In laboratory practice, “coliform” is a functional rather than strictly taxonomic term. The group is commonly defined as Gram-negative, facultatively anaerobic, non-spore-forming rods that ferment lactose with acid and gas production under specified conditions. The U.S. Food and Drug Administration’s Bacteriological Analytical Manual describes coliforms as organisms that ferment lactose to produce acid and gas within 48 hours at approximately 35°C (U.S. Food and Drug Administration [FDA], 2020). Commonly associated genera include *Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter*, although the exact composition of the group depends on the analytical method used (Leclerc et al., 2001).

Coliform testing is important because milk contamination is often difficult to assess by directly testing for every possible pathogen. Pathogens may occur intermittently, at low concentrations, or in a nonuniform distribution. Indicator organisms provide indirect information about the sanitary condition of a product and the effectiveness of hygienic controls. A high coliform count may indicate poor cleaning, contaminated water, inadequate personal hygiene, post-pasteurization contamination, or insufficient temperature control. Nevertheless, coliform results must not be interpreted as direct proof that a specific pathogen is present. Conversely, an acceptable coliform result does not guarantee that milk is free from all pathogenic microorganisms.

The microbiological condition of milk is influenced by both initial contamination and subsequent microbial growth. Freshly drawn milk may contain microorganisms introduced during milking, but rapid cooling can restrict their multiplication. If milk remains warm, psychotropic organisms and other contaminants may increase during storage, causing souring, off-flavors, proteolysis, lipolysis, coagulation, and reduced shelf life. Thus, coliform enumeration has value not only for assessing hygiene but also for identifying conditions likely to reduce product stability.

The objectives of this paper are

Describe the microbiological characteristics of total coliforms found in milk.

Identify important sources and routes of coliform contamination.

Explain laboratory methods used to detect and enumerate coliforms.

Analyze practical observations from milk samples with different hygiene histories.

Discuss the relationship between coliform levels, milk quality, and shelf life.

Recommend measures for reducing coliform contamination in milk.

Review of Literature

Definition and characteristics of coliforms

Coliforms are a group of bacteria identified primarily by physiological behavior rather than by a single evolutionary lineage. The conventional definition includes Gram-negative, non-spore-forming, rod-shaped bacteria that can grow under aerobic or

facultatively anaerobic conditions and ferment lactose with production of acid and gas. Because the definition is method-dependent, different media, incubation temperatures, incubation periods, and confirmation procedures may recover somewhat different populations (Leclerc et al., 2001; FDA, 2020).

Most coliforms are classified within the family Enterobacteriaceae. Typical genera associated with milk and dairy environments include *Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. Some members are primarily associated with the intestinal tract, whereas others are widespread in soil, water, plants, dust, and processing environments. Consequently, a total coliform result should generally be interpreted as an indicator of general hygienic quality rather than as a definitive fecal test.

Escherichia coli is especially important because it is more strongly associated with the intestinal tract of humans and other warm-blooded animals than many environmental coliforms. Some *E. coli* strains are harmless intestinal commensals, while others are pathogenic and can cause diarrheal disease, urinary tract infections, or systemic illness. Therefore, detection of total coliforms and detection of pathogenic or diarrheagenic *E. coli* are related but distinct laboratory objectives. Coliform cells are generally relatively small rods and do not form heat-resistant endospores. Their lack of spore formation makes them susceptible to properly applied heat treatments, including pasteurization. However, their destruction during pasteurization does not prevent recontamination afterward. A product may therefore satisfy the heat-treatment requirement but still show coliform contamination because of dirty filling equipment, contaminated packaging materials, poor employee hygiene, or leaks in the processing system.

Coliforms in raw milk

Raw milk is rarely sterile. Microorganisms may enter milk from the teat canal, udder surface, animal hide, bedding, feces, soil, feed, water, air, and milking equipment. The microbial profile of raw milk depends on animal health, housing, milking procedures, equipment sanitation, season, water quality, feed, and storage time (Coorevits et al., 2008; Torkar & Teger, 2008).

A study of bulk-tank milk in the United States found that a large majority of samples were positive for coliforms, demonstrating that their presence in raw milk is common and reflects the complex microbial environment of dairy production (Van Kessel et al., 2004). The presence of coliforms in raw milk does not necessarily mean that the milk came into direct contact with feces. Environmental contamination can occur through soil, plant material, water, bedding, or inadequately cleaned equipment.

Milk supports microbial growth because it contains water, lactose, proteins, minerals, and other nutrients. Its high-water activity allows microorganisms to multiply when temperature and competing microbial conditions are favorable. The natural antimicrobial systems of fresh milk can temporarily inhibit some organisms, but these systems do not ensure long-term safety. Cooling is therefore essential immediately after milking.

Poor udder health can also influence bacterial levels. Mastitis, an inflammatory condition of the mammary gland, may increase the microbial load and alter the chemical composition of milk. Although not every coliform-positive milk sample

results from mastitis, environmental coliforms can be associated with udder contamination and intramammary infections. Control of mastitis, teat preparation, fore-stripping, and hygienic milking are therefore important components of coliform reduction.

Sources of contamination

Coliforms can enter milk through several routes. The most important sources include: Animal feces and manure. Fecal material may contaminate teats, udders, bedding, hands, clothing, and milking equipment.

Water. Unsafe water used to wash udders, equipment, containers, or hands may introduce coliforms.

Soil and plant material. Dirt, dust, grass, straw, and feed can carry environmental coliforms.

Milking equipment. Residual milk films and biofilms in pipelines, buckets, teat cups, gaskets, and storage vessels may support bacterial persistence.

Handlers. Poor handwashing, dirty clothing, coughing, sneezing, and improper handling may contaminate milk. Storage and transport. Unclean containers, warm temperatures, prolonged holding, and contaminated transport tanks can increase microbial numbers.

Post-pasteurization environments. Coliforms detected in pasteurized milk may indicate contamination after heat treatment or inadequate cleaning and sanitation.

Farm-level management practices strongly influence coliform levels. Failures in milking-machine washing, poor cluster cleaning, and accidental detachment of milking units may increase contamination (Pantoja et al., 2011). Equipment must be cleaned promptly because milk residues provide nutrients for microbial attachment and biofilm formation. Biofilms can protect microorganisms from routine cleaning and allow repeated contamination of successive batches.

Indicator organisms and public-health interpretation

Indicator organisms are used because direct testing for every pathogen is impractical. A detector organism reflects the microbiological status of a food or environment, whereas an index organism suggests the possible presence of ecologically related pathogens (Chapin et al., 2014). Total coliforms mainly function as indicators of general sanitary conditions. Fecal coliforms and *E. coli* provide more specific evidence of fecal influence, although neither is a perfect substitute for direct pathogen testing.

The interpretation of coliform results depends on the product and stage of production. A raw-milk sample is expected to contain more environmental microorganisms than properly pasteurized milk. In pasteurized milk, coliforms should be low because pasteurization should destroy vegetative bacterial cells. A high count in a pasteurized product may indicate inadequate pasteurization, contamination after pasteurization, or failure of cleaning and sanitation.

The Grade “A” Pasteurized Milk Ordinance has historically used a limit of no more than 10 coliform organisms per milliliter for pasteurized milk and milk products under specified conditions (FDA, 2017). Regulatory requirements vary among jurisdictions and products, so a laboratory result must always be compared with the standard

applicable to the specific country, product category, and sampling plan. The FDA's current analytical guidance also emphasizes that coliform enumeration is method-specific and requires appropriate confirmation of presumptive colonies (FDA, 2020).

Relationship between coliforms and shelf life

Coliforms can contribute to milk spoilage, although they are not the only organisms responsible. Depending on species and storage conditions, coliforms may ferment lactose and produce acids, gases, odors, and other metabolic products. Their activity may cause souring, swelling of containers, curdling, off-flavors, and changes in texture.

Shelf life is also strongly affected by psychotropic bacteria, especially organisms capable of growing at refrigeration temperatures. Some psychographs produce heat-stable proteases and lipases that remain active after pasteurization.

These enzymes may cause bitterness, rancidity, gelation, and other defects during storage. Consequently, coliform counts are useful hygiene indicators but do not fully predict the shelf life of milk.

A high initial coliform load increases the likelihood that milk will deteriorate rapidly if temperature control is poor. However, the relationship is not perfectly linear. The actual shelf life depends on the species present, initial cell numbers, storage temperature, competing microorganisms, oxygen availability, packaging, heat treatment, and time. Milk with a low coliform count can still spoil because of non-coliform psychrotrophs, while milk with a higher count may not show immediate sensory deterioration if it is rapidly cooled and processed.

Materials and Methods

Study design

This paper uses a combined literature-review and practical-laboratory approach. The literature component examines the definition, ecology, significance, and detection of coliforms in milk. The practical component is based on observations supplied in the laboratory record for milk samples categorized as poor-hygiene milk, good-hygiene milk, and water-adulterated milk.

The practical observations should be considered preliminary because the supplied record does not provide complete information about sample collection, replication, medium composition, quality-control strains, plate acceptance criteria, or confirmation of presumptive colonies. The results are therefore interpreted as an educational demonstration rather than as a regulatory compliance study.

Gram staining

A loopful of bacterial culture was transferred to a clean microscope slide and spread into a thin smear. The smear was air-dried and heat-fixed. Crystal violet was applied as the primary stain, followed by Gram's iodine, alcohol decolorization, and safranin counterstaining. The prepared slide was examined under a compound microscope.

Gram staining differentiates bacteria according to the structure of their cell envelopes. Gram-positive organisms retain the crystal violet-iodine complex and appear purple, whereas Gram-negative organisms are decolorized and take up the safranin counterstain, appearing pink to red. Coliforms are expected to appear as Gram-

negative rods, although morphology and Gram reaction alone cannot establish that an isolate is a coliform.

Serial dilution and plating

For each milk sample, a decimal dilution series was prepared by transferring 1 mL of milk into 9 mL of sterile diluent. Additional tenfold dilutions were prepared in the same manner. An aliquot of diluted sample was transferred to an agar plate, followed by addition of molten agar or spreading on a solid medium, depending on the selected plating method. Plates were incubated at approximately 37°C for approximately 24 hours, after which visible colonies were counted. The FDA's standard approach to coliform analysis uses validated media and confirmation procedures. In the Most Probable Number method, serial dilutions are inoculated into broth, gas-positive tubes are examined, and presumptive results are confirmed in selective broth such as brilliant green lactose bile broth (FDA, 2020). For a plate-count approach, the medium and incubation conditions must be appropriate for coliform recovery, and representative colonies should be confirmed rather than assumed to be coliforms solely because they have a particular appearance.

Calculation of colony-forming units

The general formula for a plate count is:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated in mL}}$$

For example, if 210 colonies are counted from a 10^{-6} dilution and 0.1 mL is plated, then:

$$\text{CFU/mL} = \frac{210 \times 10^6}{0.1} = 2.1 \times 10^9 \text{ CFU/mL}$$

This calculation differs from the value recorded in the supplied practical document, which reported 2.1×10^8 CFU/mL. The discrepancy suggests that either the actual dilution was 10^{-5} , the plated volume was 1.0 mL, or the original calculation omitted a factor of ten. Similar inconsistencies occur for the good-hygiene sample. Dilution records and plated volumes should therefore be verified before final statistical interpretation.

Data analysis

The practical record supplied three observations for each milk category. Mean counts were compared descriptively. Because the dataset is small and does not include standard deviations, confidence intervals, statistical tests, or independent sampling details, no inferential conclusion is made. The analysis focuses on the relative pattern among sample categories and on the methodological limitations that affect interpretation.

Laboratory safety and quality control

Milk samples and bacterial cultures should be handled using appropriate microbiological safety procedures. Laboratory personnel should wear protective clothing, disinfect work surfaces, use sterile equipment, minimize aerosol generation, and dispose of cultures according to institutional biosafety requirements. Positive and negative controls should be included when possible. Media should be checked for sterility and performance, incubators should be monitored, and colony counts should be recorded from plates within an acceptable counting range.

Results

4.1 Observed coliform counts

The supplied practical dataset reported the following mean values:

Milk category	Reported mean coliform count
Poor-hygiene milk	1.90×10^8 CFU/mL
Good-hygiene milk	0.93×10^8 CFU/mL
Water-adulterated milk	1.73×10^8 CFU/mL

The poor-hygiene milk had the highest reported mean count. Water-adulterated milk had the second-highest mean, while good-hygiene milk had the lowest. The relative order was therefore:

$$\text{Poor-hygiene milk} > \text{Water-adulterated milk} > \text{Good-hygiene milk}$$

The supplied individual observations were:

Sample	Poor-hygiene milk	Good-hygiene milk	Water-adulterated milk
1	2.1×10^8	1.1×10^8	1.6×10^8
2	1.7×10^8	0.9×10^8	1.7×10^8
3	2.0×10^8	0.8×10^8	1.9×10^8
Mean	1.9×10^8	0.93×10^8	1.73×10^8

Gram-staining observation

The expected Gram-staining result for a coliform isolate is a pink or red rod-shaped cell because coliforms are Gram-negative rods. The practical record identifies *E. coli* and other coliform cultures as organisms examined by Gram staining. However, no photomicrograph, replicate observation, or biochemical confirmation was supplied.

Accordingly, the Gram-staining result supports compatibility with coliform morphology but does not establish species identity.

Calculation issue

The practical document included a count of 210 colonies, a stated dilution factor of 10^6 , and a plated volume of 0.1 mL. Using the standard equation, the result is 2.1×10^9 CFU/mL, not 2.1×10^8 CFU/mL. Similarly, 110 colonies under the same conditions would equal 1.1×10^9 CFU/mL. This tenfold discrepancy is important because it affects comparisons with microbiological standards and conclusions about contamination severity.

Discussion

Interpretation of the observed pattern

The practical results show a plausible relationship between hygiene conditions and coliform counts. Poor-hygiene milk had the highest mean count, which is consistent with contamination from dirty udders, fecal material, contaminated containers, poor hand hygiene, inadequately cleaned equipment, or unsafe water. Good-hygiene milk had the lowest count, suggesting that improved sanitation and handling reduced the transfer of coliform organisms.

The water-adulterated milk showed a high mean count, although it was lower than the poor-hygiene sample. This result may indicate that the added water was microbiologically contaminated. Water used to adulterate or dilute milk can introduce coliforms directly and can also reduce the concentration of natural antimicrobial components or alter the product environment. If the water is unsafe, dilution does not make the milk safer; instead, it introduces additional microorganisms and may increase the risk of disease.

The pattern is consistent with the broader literature, which identifies water, equipment, feed, soil, feces, and environmental surfaces as important sources of milk contamination (Coorevits et al., 2008; Kagkli et al., 2007). Nevertheless, the results cannot prove the exact source of contamination because the experiment did not include microbiological analysis of the water, equipment, udder surfaces, handlers, or environmental samples.

Significance of the high counts

The counts reported in the practical dataset are extremely high when compared with standards commonly applied to pasteurized milk. The Grade “A” Pasteurized Milk Ordinance has historically specified no more than 10 coliform organisms per milliliter for pasteurized milk and milk products under the relevant testing conditions (FDA, 2017). However, the practical samples appear to be raw or intentionally contaminated teaching samples rather than finished pasteurized milk. Direct comparison with a pasteurized-milk standard would therefore be inappropriate unless the product category and regulatory framework are clearly established.

If the reported values accurately represented coliform counts in commercially pasteurized milk, they would indicate a serious quality failure. Possible explanations would include inadequate heat treatment, post-pasteurization contamination, damaged packaging, poor sanitation, or incorrect laboratory technique. If the samples were raw

milk, the high values would still indicate poor hygienic quality and a substantial need for improved controls.

The calculation discrepancy must be resolved before the counts can be used for formal conclusions. A tenfold error may change whether a sample is categorized as moderately or extremely contaminated. Laboratory records should include the dilution of the plate counted, the actual plated volume, the number of replicate plates, the medium used, the incubation conditions, and any confirmation results.

Limitations of total coliform testing

Total coliform testing has several limitations. First, coliforms are not a uniform taxonomic group. The organisms recovered depend on the medium, temperature, incubation time, and definition used. Second, environmental coliforms may be present without fecal contamination. Third, some pathogens may be present when total coliform counts are low or absent. Fourth, injured or stressed cells may fail to grow on selective media. Finally, high counts may result from nonpathogenic organisms and therefore should not be interpreted as proof of a specific disease hazard.

For these reasons, coliform testing should be combined with other analyses when public-health decisions are required. Depending on the product and risk assessment, additional tests may include total aerobic counts, *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, somatic cell count, antibiotic-residue testing, phosphatase testing, pH, and sensory evaluation. The appropriate testing plan depends on whether the sample is raw milk, pasteurized milk, fermented dairy, cheese, powdered milk, or another product.

Coliforms and shelf life

The results support the expectation that milk with a higher initial microbial load may have a shorter useful shelf life, especially if temperature control is inadequate. Coliform metabolism can produce acid and gas, leading to souring and other visible or sensory defects. However, coliforms should not be treated as the sole determinant of shelf life.

Shelf life is often controlled by psychrotrophic organisms that grow during refrigeration. These bacteria can produce extracellular enzymes that degrade milk proteins and fats. Some enzymes may remain active after pasteurization, causing defects later in storage. Therefore, a low coliform count may coexist with poor shelf life if the milk was exposed to psychrotrophic contamination before pasteurization.

Temperature is especially important. Rapid cooling after milking slows the multiplication of many organisms. Repeated warming during transport or retail display can accelerate growth. A properly cooled product with a moderate initial count may remain acceptable longer than a heavily contaminated product that is quickly refrigerated, although neither situation should replace the use of good hygiene.

Importance of method validation

The practical procedure described in the supplied record uses serial dilution, plating, incubation, and colony counting. This is a useful educational method, but a reliable regulatory method requires greater standardization. The selected agar must support

coliform recovery while suppressing unrelated organisms. Incubation temperature and time must match the method. Colonies must be counted on plates within the accepted range. Representative colonies should be confirmed using biochemical or molecular procedures.

The FDA's BAM method describes a multistep process for presumptive and confirmed coliform testing. In the MPN procedure, lactose fermentation and gas production are assessed in broth, positive reactions are confirmed in brilliant green lactose bile broth, and the result is calculated from dilution patterns (FDA, 2020). Plate-based methods may be more convenient for some laboratories, but they require careful selection of medium and confirmation criteria.

A laboratory should also distinguish between a count of presumptive colonies and a confirmed coliform count. Reporting presumptive colonies as confirmed coliforms may overestimate the true value. Conversely, overly selective conditions may underestimate stressed or injured cells. Method performance should be evaluated using reference organisms and quality-control practices.

Public-Health and Quality-Control Implications

Hygienic milking

The first control point is the animal and milking environment. Teats and udders should be visibly clean before milking. Pre-milking preparation should reduce dirt and microbial contamination without irritating the skin. Post-milking teat disinfection can reduce the transfer of organisms and help control mastitis.

Animals with clinical illness or suspected mastitis should be identified and managed according to veterinary and regulatory guidance. Milk from treated animals should not enter the food supply when drug withdrawal requirements have not been met. Clean bedding, proper manure management, and adequate ventilation can reduce environmental contamination.

Water quality

Water used for cleaning, rinsing, udder preparation, equipment sanitation, or milk adulteration must be microbiologically safe. A contaminated water supply can undermine otherwise effective hygiene practices. Wells should be protected from surface runoff, sewage, animal waste, and flooding. Water used in dairy operations should be tested periodically, especially after infrastructure damage or contamination events.

Equipment sanitation

Milking equipment should be cleaned promptly after use. Cleaning generally involves a sequence of rinsing, detergent washing, intermediate rinsing, sanitizing, and appropriate drainage or drying. The exact procedure depends on the equipment and chemical products used. Inadequate temperature, incorrect chemical concentration, insufficient contact time, poor mechanical action, or residual milk deposits can allow coliforms and other bacteria to persist.

Gaskets, joints, rubber components, valves, and dead ends require particular attention because they can harbor biofilms. Equipment should be inspected regularly for cracks, rough surfaces, and worn parts. A clean-looking surface is not necessarily

microbiologically clean, so environmental monitoring and periodic microbiological testing are valuable.

Cooling and storage

Milk should be cooled rapidly after milking and maintained at a consistently low temperature during storage and transportation. Temperature abuse can permit rapid growth of bacteria and reduce shelf life. Storage tanks should be cleaned and sanitized between uses, and milk should not be mixed with older milk unless the process is controlled.

Retail and household handling also matter. Consumers should keep milk refrigerated, avoid leaving it at room temperature, and use clean containers and utensils. Repeated opening and warming can increase contamination and accelerate spoilage.

Pasteurization and post-pasteurization protection

Pasteurization is designed to destroy vegetative pathogens and reduce the microbial load. It is not a substitute for hygienic production because heavy contamination can create quality defects, and post-pasteurization contamination can reintroduce microorganisms. Pasteurized milk should be protected from contaminated raw product, dirty equipment, employees, packaging, and water.

A high coliform count in pasteurized milk should trigger an investigation. The investigation should examine pasteurizer performance, phosphatase results, filling equipment, storage tanks, packaging materials, employee practices, and environmental samples. Repeated positive findings often indicate a persistent sanitation problem or biofilm.

Conclusion

Total coliforms are important microbiological indicators of the sanitary quality of milk. They are generally Gram-negative, non-spore-forming, facultatively anaerobic rods capable of fermenting lactose with acid and gas production under defined test conditions. Commonly associated genera include *Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. Because coliforms may originate from feces, soil, water, feed, animals, equipment, or handlers, their detection must be interpreted in the context of the product and production stage.

The practical observations reviewed in this paper showed that poor-hygiene milk had the highest mean coliform count, water-adulterated milk had an intermediate count, and good-hygiene milk had the lowest count. This pattern supports the importance of hygienic milking, safe water, effective equipment sanitation, and proper handling. However, the results should be regarded as preliminary because the supplied record lacks complete methodological details and contains a tenfold dilution-calculation discrepancy.

Coliform counts are valuable indicators but cannot alone establish the presence or absence of pathogens. Reliable assessment requires validated laboratory methods, confirmation of presumptive colonies, appropriate sampling, quality controls, and comparison with the standard applicable to the specific milk product. The most effective prevention strategy is a comprehensive control system based on clean animals, sanitary water, hygienic milking, properly cleaned equipment, rapid cooling,

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cold-chain control, validated pasteurization, and protection against post-pasteurization contamination.

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