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A Comparison of Tests Used in the Selection of Milk

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A COMPARISON OF TESTS USED IN THE SELECTION OF MILK

by

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Roger L. Stephens

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INTRODUCTION

The selection of milk has for many years been based upon the use of bacteriological tests such as the standard plate count, methylene blue, and resazurin tests. Such tests denoted the numbers of bacteria in milk and thus were well accepted as a close correlation of sanitary practices on the farm.

However, improvements in equipment, cleaning procedures, sanitizers and handling methods have greatly improved milk quality over the past decade. The farm bulk tank is a much more efficient system of cooling and storing milk. It has been widely adopted and is in common use in many areas. However, its very efficiency in cooling milk poses many new problems for those engaged in the selection of milk. Since the low storage temperatures associated with bulk tanks practically eliminate growth as a cause of high bacterial count, milk producers may neglect various sanitary precautions and still meet current bacterial-count standards. Thus, since efficient cooling can mask faulty production practices, the results of the standard plate count, methylene blue, and resazurin tests as currently conducted no longer portray the true quality of milk supply because they are a measure of number rather than types of bacteria.

Purpose

It is, therefore, the purpose of this study to determine the effectiveness of the Standard Plate Count, Direct Microscopic Count,

Roundy Mastitis Test, Coliform, Crystal Violet-tetrazolium, and Psychrophile tests in the appraisal of different types of bacteria in selecting milk. Also incorporated into the study will be an examination of the effect of different storage temperatures in relation to the tests and the quality of the milk.

REVIEW OF LITERATURE

The selection of milk embraces a variety of aspects. These include such diverse properties as freedom from dirt, antibiotics, off-flavors, pathogenic organisms, and abnormal numbers of body cells; evidence of cleanliness and care in production and handling as indicated by microbiological analysis; possession of desirable flavor and aroma, and of adequate amounts of those constituents of nutritional importance. Thus the selection of milk depends on the anticipated use to which it will be subjected and may vary from product to product. With milk itself, the bacteriological aspect has received the greatest attention. With butter and cheese, on the other hand, flavor is of far more importance. With ice cream, we are interested in both of these aspects as well as in composition.

In dealing with the subject of bacteriological tests, it is considered preferable to take each test in turn and consider its application, where appropriate, to milk and its various products.

Standard Plate Count

Even though there are available several various methods of assessing the quality of a milk supply such as sediment, flavor, odor, etc., the bacteriological methods predominate as the most acceptable. Of these, the Standard Plate Count is generally conceded to be the most precise method (30). It has been in use longer than other methods of estimating bacterial populations and can be used on all types of dairy

products (2, 43). In 1963, this method was accepted by the state delegates at the National Conference of Interstate Milk Shipments as the one acceptable method for determining the bacteriological standard of milk for interstate shipments, thus eliminating the recognition of the methylene blue, resazurin, and direct microscopic counts insofar as interstate shipments are concerned (34).

However, even though this method has received great prominence in the selection of milk, it is not without disadvantages. Hammer (26) listed objections to this method as (1) pathogenic organisms are not detected, (2) counts are not accurate insofar as representing actual numbers of bacteria present, (3) specific information regarding species is not furnished, and (4) period required before results are available is too long. Many other workers have recognized these disadvantages and have performed exhaustive studies to overcome these limitations. Pederson and Yale (42) reported the results of the studies at the Geneva Agricultural Experiment Station concerning the desirability of an incubation temperature lower than 37° C because the lower temperature would more nearly reflect the actual bacterial density of the product. They reported the percentage of the maximum counts obtained in 48 hours varies considerably at 37° C but is quite constant at 32° C. A lower temperature of 35° C or 32° C has now been accepted by *Standard Methods for the Examination of Dairy Products* (2); however the lower temperature of 32° C is recommended by Nelson and Baker (35) and again by Brazis (11). Other workers (2) investigated different types of incubators and nutrient media which under restricted conditions will most consistently support growth of the maximal number of colonies.

Regardless of the limitations and disadvantages of the Standard Plate Count, it is still the most suitable for low count milk (less than 200,000 per ml) (30).

One of the objections to the Standard Plate Count method as listed by Hammer (26) concerns the limitation of providing specific information regarding different species of bacteria present. This limitation may be due to some bacteria unable to grow and form visible colonies in the alloted time because of injury, character of nutrients, amount of oxygen, unfavorable temperature of incubation, or other factors. Consequently, *Standard Methods for the Examination of Dairy Products* (2) suggests the use of additional plates incubated at other temperatures in order to provide a more complete picture of the bacterial condition of the milks.

The advent of the bulk cooling tank and its high efficiency in cooling and storing milk on the farm has encouraged the supplementary need for variations of the standard plate count to provide a more complete picture.

Johns (31) studied the effects of a preliminary incubation of samples at 55° F (12.8° C) for 18 hours prior to determining the standard plate count procedure as outlined in *Standard Methods for the Examination of Dairy Products* (2). He reported that preliminary incubation encouraged the growth of many contaminating species and readily detected careless production practices on the farm.

Davis and Killmeir (18) recommended a bacterial standard of 200,000 per ml. after preliminary incubation. They stated that a bacteria count of 10,000 per ml or less by accepted bacteriological standards

does not mean good sanitary production methods were used but that almost ideal perfect cooling by a bulk farm tank may have masked very poor sanitation practices. Slatter (47) reported the results of work indicating a Standard Plate Count of 50,000 and a preliminary incubation count of 200,000 would eliminate about the same amount of milk; i.e., 37 and 34 percent respectively.

The detection of thermoduric or thermophilic bacteria by laboratory pasteurization of raw milk samples is another variation of the Standard Plate Count used to provide additional information regarding the bacterial condition of the milk.

Thermoduric bacteria are capable of surviving pasteurization but do not grow until the temperature is again decreased. They consist of a few spore formers but largely of nonspore-forming, heat-resistant organisms and are usually found on surfaces of ineffectively washed or improperly sanitized utensils on farms, in milk cans, on preheating equipment, and on inadequately sanitized pasteurizing equipment (2). *Standard Methods for the Examination of Dairy Products* (2) suggests incubation at 32° C for 48 ± 3 hours be used in determining the presence of thermoduric organisms. An incubation temperature of 37° C is too high for some thermoduric organisms.

Barnum (9) reported the value of the laboratory pasteurization test for thermodurics in detecting improperly cleaned milking machines, particularly inflations, air and milk hoses, and pipe lines.

Hammer (26) concludes the presence of thermoduric organisms in milk are of no significant public health importance since their resistance to heat differentiates them from pathogens. However, Corash (16) points out the difficulty in producing a high quality finished product if a

low quality raw product is used. Barnum (9) suggests excessive thermoduric counts in the raw product would make it difficult to obtain low counts in the pasteurized milk, and thus may be responsible for counts in excess of the legal limit for pasteurized products. Olson (40) suggests the shelf life or keeping quality of properly refrigerated pasteurized milk (below 45° F) is not materially affected by thermoduric organisms unless the temperature was allowed to increase. In this case, the quality of the milk could be altered. Also, another factor in evaluating the importance of thermoduric organisms is that nonfat milk solids containing excessive numbers of sporeformers are discriminated against by manufacturers of baby foods and canned and sterilized products. Slatter (47) noted that some dairy plants are using a thermoduric limit of 3,000 per ml as an indication of satisfactory performance. His work indicated that a limit of 1,000 would be quite lenient, since 78 percent of the milk samples met this standard, and that a standard of 500 might be more effective for use as a quality tool.

Thermophilic bacteria are those that grow at 55° C although many are facultative and can grow at 37° C or lower. They are usually sporeforming bacilli gaining entrance to milk on the producing farm (2).

The presence of thermophilic organisms from a public health standpoint appears unimportant (26). These organisms were a serious problem when vat pasteurization was common, but with the trend toward HTST and UHT pasteurization, the importance has diminished.

Direct Microscopic Count

The Direct Microscopic Count is a method of assessing the bacteriological estimates in milk or cream. The method consists of placing a

measured volume of the milk or cream to be examined on a slide, staining, and examining under a compound microscope.

Even though the Direct Microscope Count is no longer recognized as an official test for interstate milk shipments (34), certain characteristics of the method enhance its continued use by quality control men. Hammer (26) compared the Direct Microscopic Count with the Standard Plate Count and listed its advantages as (1) results are quickly available, (2) less work is required, (3) less apparatus is required, (4) less expensive, (5) microscopic preparations give a permanent record, (6) some idea of species present can be obtained, (7) numbers of body cells present can be determined, and (8) preservations can be used with samples. Some of the more important disadvantages also listed are (1) irregulatities are inherent in the procedure, (2) less generally useful for low-count milk, and (3) less generally useful for pasteurized milk.

Other workers have compared the Direct Microscopic Count with the Standard Plate Count. Johns and Fischer (29) reported a poor agreement between the two methods. Hammer (26) found the most frequent ratio between results of the two methods as 4 to 1. However, wide variations may occur from one lot of milk to another. In general, the difference between the results of the two methods is relatively large with low-count milk and relatively small with high-count milk.

Johns (30) reported a personal communication with Black (U. S. Public Health Service) in 1957 wherein he noted the Standard Plate Count to give higher counts than the Direct Microscopic Counts with counts exceeding 50,000 per ml ; below this level the reverse is true.

Johns (31) also concluded that the Direct Microscopic Count grades milk (at the 200,000 per ml limit) more leniently than the Standard

Plate Count and is doubtless one reason why the decline in popularity.

Reinbold (43) further noted the direct microscopic count was not sufficiently accurate for low-count milk and did not enumerate low-temperature microorganisms very well. He also found the majority of organisms that will be present in bulk-tank milk did not stain readily and may be overlooked.

Levowitz and Weber (33) described a single solution stain that they reported to be superior to the Newman-Lampert stain. They reported the advantages of the stain as (1) stain retained lightly and uniformly by only the milk protein, (2) no mottling or network effect, (3) background tinted to reduce eyestrain, (4) almost completely plasmolyzed microorganisms retain stain sufficient to distinguish readily, (5) the cytoplasmic portions of body cells are distinctly darker than the background with cell outlines well defined. Nucleoplasmic areas are stained deeply but individually differentiated (with no "mossy effect"), so that primary leucocyte classification into myelocytes, young and mature polymorphonuclears, etc., is accomplished directly without employing differential stains on special smears, (6) smears remain fixed even if rinsing is energetic. Fat solvent to remove immersion oil does not remove stain. Crystal of dye particles will not form to obscure proper examination.

The Levowitz-Weber stain has since been well recognized and accepted. *Standard Methods for the Examination of Dairy Products* (2) describes three acceptable staining procedures. These consist of (1) the acid- and water-free stain, (2) Levowitz-Weber modification of Newman-Lampert stains, and (3) North's aniline oil methylene blue stain. Johns (31)

compared the Levowitz-Weber stain with the acid- and water-free stain. The Levowitz-Weber stain gave a greater contrast between cells and background than did the acid- and water-free stain. It also has the advantage of not requiring separate treatments to defat and fix the smear before staining. One disadvantage is the necessary care to keep the solution tightly stoppered to prevent evaporation.

Thompson and Shadwick (49) also compared the Levowitz-Weber stain and the acid- and water-free stain. These studies found the Levowitz-Weber stain to show somewhat higher counts, bacteria stained more intensely, and a better contrast with the background. One slight disadvantage was recorded consisting of more precipitated dye than with the acid- and water-free stain.

Coliform

Coliform is a name applied to the Escherichia-Aerobacter group of organisms and a few lactose-fermenting species of other genera. They include all aerobic and facultative anaerobic, Gram-negative, nonspore-forming bacteria capable of fermenting lactose with the production of gas. The Escherichia organisms come largely from fecal material whereas Aerobacter and intermediate organisms come primarily from non-fecal materials.

Standard Methods for the Examination of Dairy Products (2) states that coliform may enter raw milk or cream under normal conditions of production and handling. However, they are more commonly associated as contaminants from poorly sanitized utensils or moist dirt or manure dropping into the milk. Multiplication of coliform organisms in milk is favored by conditions which favor growth of other bacteria. Because

of the varied sources of coliform in raw milk, and because of their potentially rapid multiplication immediately following introduction, results of coliform tests may not furnish complete indices of original contamination unless the sample is tested within 3 or 4 hours after production or have been produced and stored that Standard Plate Counts are under 10,000 per ml. Reinbold (43) added that because bulk tank milk is 2 to 3 days old when collected, the coliform count is completely unreliable as a sanitary index.

Archambault (4) reported work done by Buchbinder and Alff (13) wherein 468 flasks containing approximately 1 liter each of pasteurized milk, all taken before bottling and usually from a sanitary line just before bottling, were incubated overnight. All the samples, except one, yielded negative results to the coliform test even though the original raw milk used ranged from 1,000 to 56,000,000 coliform per 100 ml. These workers concluded coliform organisms do not survive pasteurization in significant numbers. The presence of coliform in pasteurized milk and cream is almost invariably an indication of post pasteurization recontamination.

Hammer (26) noted the effects of Escherichia-Aerobacter organisms as causing objectionable flavors, production of gas, and ropiness in cream. In 1954 Nelson and Baker (35) concluded the retention of the coliform test as a quick index of contamination seemed justified. However, they cautioned that interpretation of negative results should be conservative because of the considerable possibility the test will not detect some important types of contamination.

Johns (30) summarized the value of the coliform test. He reported the use of this test on raw milk in Canada has been, for many years,

confined primarily to raw milk sold for consumption without pasteurization. It was generally felt that high counts represented growth more than direct contamination. He predicted the use of the test would become much more prevalent with the widespread use of farm bulk tanks.

In pasteurized products, the use of the coliform test to detect contamination has been much more generally accepted. At the present time, the Milk Ordinance and Code (51) requires a regular frequency of examination of pasteurized milk for coliform organisms. The maximum standard for coliform is at 10 per ml. Archambault (4) regards this standard as much too lenient, consequently suggesting a practicable standard of 50 per 100 ml.

Two shortcomings of the test should be noted. These are (1) difficulty in deciding whether small red colonies should be counted, and (2) atypical colonies on overcrowded plates. Barber and Fram (8) also report the results of a preliminary study wherein they found that both the fresh fruit (bananas, strawberries, and peaches) and the plain ice cream mix were coliform negative; but when mixed together, positive coliform reactions were obtained. Further study has shown that it apparently is the sucrose which is responsible for the development of large red colonies on the solid test media and the production of gas in the liquid test media. Apparently these organisms make up a large part of the normal bacterial flora of these fruits. Identification is not complete, but it has been proven conclusively that these organisms are not part of the coliform group. Under these circumstances, results must be confirmed.

Thomas (50) studied the presence of coli-aerogenes bacteria in the bovine udder. From his studies, he concluded these bacteria are not

normal inhabitants of the healthy udder, though occasional contaminants of bulk herd milk by coliforms derived from udder infections may occur. Only a small proportion of the cases of bovine mastitis investigated have been found to be caused by coli-aerogenes bacteria, but a high proportion of cows in a few herds are infected with coliform mastitis. He added that it is possible the presence of toxic strains of this nature (coliform mastitis) in bulk herd milk could also be the cause of intestinal disorders in children.

Psychrophile

Standard Methods for the Examination of Dairy Products (2) defines psychrophilic bacteria as those bacteria which are capable of relatively rapid growth at low temperatures, commonly within the range of 1.7 to 10° C (35 to 50° F). Thus, the low temperatures at which raw and pasteurized milk is stored, and the extended storage of these products encourages the growth of these organisms. If not controlled, they may cause slime formation on cottage cheese, and fruity, putrid, and rancid flavors and odors in other dairy products.

The source of psychrophilic bacteria may originate in water supplies used to rinse sanitized utensils and equipment, residues on improperly sanitized equipment, splashings from floors or equipment, condensate drippings, and similar sources.

Rogick and Burgwald (44) found psychrophilic organisms to multiply slowly up to four days, but to increase more rapidly thereafter. Raw milk samples showed mesophilic counts approximately three times as great as psychrophilic counts. One week after pasteurization, the psychrophilic counts were higher than mesophilic counts.

In their discussion, they reported no psychrophiles were found after pasteurization; but after one week storage, the psychrophiles increased appreciably. This could mean either (1) not all psychrophiles were destroyed but the number surviving is so low as to escape detection in the amount of milk used, or (2) some mesophiles develop psychrophilic tendencies. Also, they repoted that the first milk through the system (from pasteurizer to bottler) always contained psychrophiles, but were not found in the last milk.

Nelson and Baker (35) studied the time and temperature of incubation to detect psychrophilic bacteria. They reported truly psychrophilic bacteria have their optimum growth temperature at 15° C or below, whereas many of the bacteria growing under refrigeration will grow better at temperatures of 21° C or above than at the lower temperatures. They found that plates incubated at 5° C for 7 days did not give quite as high counts as 5° C for 10 days.

The eleventh edition of *Standard Methods for the Examination of Dairy Products* (2) recommends incubation of plates at 5 - 7° C for 7-10 days.

Day and Doan (19) concluded after their work that milk would keep under refrigeration temperatures for long periods of time if it was relatively free of psychrophiles. However, due to the extended incubation periods required for enumeration of psychrophilic bacteria and because of the wide variations in numbers of organisms at the time of flavor spoilage, the use of the psychrophilic plate count was not very successful in predicting the "keepability" of milk. They concluded by emphasizing that the specific types of bacteria present in milk was

more important than total numbers in influencing keeping quality.

Hempler (27) studied facultative psychrophiles which grow not only at 7° C but often at 30° C. He found only a slight increase in the psychrophilic count during two days storage at 7° C but rapid increases after the third day. After storage at 7° C, strains of Achromobacter, Pseudomonas, and Alcaligenes predominated. Small numbers of coli-aerogenes were sometimes found even though the freshly pasteurized samples had given a negative test.

Broitman et al. (12) demonstrated the importance of various types of organisms involved in off-flavor production. Their work shows samples with great variation in initial bacterial counts but still developing off flavors very near each other in days.

They found a relationship between the psychrophilic counts of the milk at the time of spoilage and the presence of off-flavors. Milks with initial psychrophilic counts of less than 10 per ml exhibited a greater refrigerated storage life than milks with counts in the range of 10 to 10,000 per ml. However, they concluded it would be very difficult if not impossible to predict shelf-life by psychrophilic population.

Andrey and Frazier (3) studied the different types of psychrophiles in milk. The genera they found in raw milk showed Arthrobacter was predominant most often making up about 37%, Flavobacterium 29%, Pseudomonas 17% and then in smaller amounts Micrococcus, Aerobacter, and Alcaligenes. Arthrobacterium, Micrococcus, and Flavobacterium did not grow in pasteurized milk after 3 days at 38° F. However, fairly rapid growth was observed in *Alcaligenes marshallii*, *Pseudomonas signis*, *Pseudomonas convexa*, and *Pseudomonas cohaerens*, and almost as good, *Aerobacter aerogenes*.

In barn feeding and holding cows, Arthrobacter was predominant, Pseudomonas second, and Micrococcus third, whereas in pasture feeding Flavobacterium was first, Arthrobacter second, and Alcaligenes third. They also found a seven-fold increase in psychrophiles when cows went on pasture after barn feeding. The authors indicated that green plants are a source of Flavobacterium, but the main source is water and soil.

Schultze and Olson (46) suggested that psychrophilic bacteria may occur more frequently in dairy products than has been recognized. This may be due to a misclassification because of their atypical biochemical activities, or because of their rapid growth at 98.6° F.

Work performed by Schultze and Olson (46) on 586 samples of commercial fluid dairy products and cottage cheese stored at 40° F for one week revealed that Pseudomonas made up 60.6 percent of the cultured bacteria, Alcaligenes 7.9 percent, Achromobacter 9.2 percent, Flavobacterium 0.7 percent, yeasts 0.8 percent, and coliforms 10.8 percent. Of those cultures which produced the typical rapid acid fermentations of lactose, four were classified as *Escherichia intermedia*, five as *Aerobacter cloaeae*, thirteen as *Aerobacter aerogenes*, and three were unidentified.

The preponderance of evidence shows that psychrophilic do not survive pasteurization (44, 53, 41, 10). In fact, many of the prominent psychrophiles have been demonstrated to be heat sensitive; viz., *P. fragi* (14), *P. fluorescens* (14), *P. viscosa* (14), and *P. Putrefaciens* (54). Davis and Babel (17) studied bacterial cultures capable of forming slime on cottage cheese and found the most resistant strain was destroyed by a 2.5 minute exposure to 62.8° C (145° F). Hence, the presence

of psychrophiles in pasteurized milk and dairy products is generally considered to be a result of post-pasteurization contamination.

The above evidence and conclusions, however, do not preclude the existence of thermoduric psychrophiles. Erdman and Thornton (22) found four of 722 isolates to be thermoduric. Kennedy and Weiser (32) found that all but one of fifteen pure cultures survived 145° F for 30 minutes and did so in considerable numbers. Jezeski and Macy (28) found only six of 41 cultures of psychrophilic bacteria isolated from fresh butter survived laboratory pasteurization at 150° F for 30 minutes. Abd-el-malek and Gibson (1) identified *Alcaligenes tolerans* as a psychrophile surviving pasteurization in the laboratory. They also noted rapid growth of this organism in various samples after pasteurization.

Psychrophilic bacteria are not of public health significance insofar as they are known to produce disease. Ohye and Scott (36) observed that *Clostridium botulinum* Type E was capable of germination and growth at low temperatures. Hence, while this organism is an obligate anaerobe and probably grows too slowly to be considered a psychrophile, it remains a very unlikely, but potentially dangerous, contaminant in milk pasteurized at ultra-high heat or in improperly sterilized sterile milk.

The biochemical activity of psychrophilic bacteria is summarized as (a) inactive in reducing dyes (25, 6, 43), (b) cause destruction of diacetyl (20), (c) some are active phosphatase producers but do not increase phosphatase in milk samples (6), (d) some are oxidase producers, but not responsible for oxidized flavors (15), and (e) Schultze (46) reported 90 percent of psychrophiles are lipelytic and 66 percent proteolytic or both.

The number of psychrophiles found to cause any detectable physical or flavor changes has been studied. Olson (40) found changes occurring when the count had increased to approximately 2 to 10 million per milliliter. White (52) noted the count must be over 3 million per milliliter to cause any measurable changes. Such high counts are soon realized (44, 12) when the product contains relatively low initial counts. Elliker (21) presented data from a pasteurization plant (Table 1) depicting relatively low initial counts and the rapid growth during a 5 day storage at 45° F. This respective plant was on a regular sanitation program but did not include psychrophilic control as part of the program.

Table 1. Bacterial counts of products from a pasteurization plant not practicing a psychrophilic control program

Product	Fresh		Stored 5 days at 45° F
	SPC	Coliform	
homo	1800	0	28,100,000
half and half	2500	1	52,200,000
cream	400	0	102,000,000
skim	3200	2	23,800,000

Elliker recommended a method for determining the sanitary condition of equipment in the plant. It consists of subjecting the fresh pasteurized products to a Standard Plate Count at 32° C, then storing the samples at 45° F for 5 days. The Standard Plate Count is rerun on the samples after 5 days. He proposed the increase in counts was due to psychrophilic activity and would thus indicate the source of contamination.

Olsen (37) also proposed the same method as Elliker for a reliable test in determining psychrophilic contamination.

Freeman et al. (24) presented a preliminary report on a method for determining the psychrophilic count in 72 hours. This method is based on the proposal that most psychrophiles of practical importance to the dairy industry are Gram-negative. The method was to add a chemical to the milk samples which would inhibit the Gram-positive organisms. Very encouraging results were obtained, but additional study was needed.

Catalase

Hammer (26) recorded some of the enzymes normally present in milk as protease, lipase, peroxidase, catalase, and phosphatase. Additional enzymes are also present but these have been studied more than the others.

Catalase is an oxidizing enzyme. One of its characteristics is the ability to split hydrogen peroxide into water and molecular oxygen according to the reaction $2\ H_2O \rightarrow 2\ H_2O + O_2$. Its presence in milk can be observed by adding a small amount of hydrogen peroxide to milk and observing the liberation of oxygen bubbles.

By careful measurement of the amount of milk used and the amount of hydrogen proxide added, the amount of liberated oxygen can be collected and measured. Thus, a comparison of catalase activity in a given lot of milk can be learned.

Catalase contents of various lots of milk differ considerably with colostrum and milk from cows advanced in lactation containing especially large amounts. Milk from abnormal udders often shows excessive amounts

of catalase because of the presence of many leucocytes (white blood cells).

The relationship of leucocytes and catalase to abnormal milk suggests the possibility of determining the presence of abnormal milk by measuring the amount of catalase present. The catalase test is based upon this principle.

Roundy (45) describes the Catalase Test based upon a determination of catalase activity in a sample of milk. The Roundy Mastitis Test is conducted by adding a determined amount of Solution A to 10 ml of milk. Solution A was developed by Roundy containing a known concentration of hydrogen peroxide. The sample is then incubated at 95° F for 2 hours after which the amount of peroxide is measured by adding Solution B. Solution B is a color reagent which in the presence of peroxide gives a characteristic blue color.

Roundy (45) lists the advantage of this method as: (a) Practical. It can be used to detect abnormality in bulk milk or in milk from individual cows. It can be applied either at the barn or in the laboratory. (b) Simple to run. No special skill or technical training is required to perform the test. (c) Extremely sensitive. This test can be used to detect peroxide in quantities as low as approximately 10 parts per million parts of milk (0.001 %). Therefore, it can be used to detect early stages of infection or abnormality. (d) Accurate. One can determine the catalase activity of milk and obtain reproducible results with a high degree of accuracy.

Spencer and Simon (48) compared the Catalase, California, and Cell Count tests and found the California test to be superior as a barn

test on quarter samples where immediate results were desired. However, the Catalase Test was found to be superior for quality control tests on mixed herd milk.

Frank and Pounden (23) studied the effect of preservatives on the Catalase, California, and Whiteside Mastitis Tests. They found potassium dichromate and formalin greatly reduced the reactions of these three tests. Consequently, these preservatives should not be used in milk samples in which these tests for mastitis are going to be used. They also found that as the period of refrigeration increased, the production of gas in the catalase test was increased. This increase in gas was caused by enzymes produced by contaminating bacteria. Long refrigeration also was found to affect the accuracy of the Whiteside and California test results. They concluded that for most accurate results fresh milk should be used.

Crystal Violet-tetrazolium (CVT)

Olson (39) describes a new plating technique developed to detect post pasteurization contamination in milk. The technique detects the coliforms as well as many of the rod-shaped Gram-negative organisms which could be spoilage types. The basis for the test is that organisms which survive pasteurization are generally Gram-positive while those usually responsible for spoilage in pasteurized milk are Gram-negative.

The procedure consists of adding 1 ml of 0.1% crystal violet solution per liter to standard plate count agar. The crystal violet solution inhibits the growth of the Gram-positive organisms. After sterilization, 1 ml of 0.5% 2,3,5 triphenyltetrazolium chloride (TTC) is added just prior to pouring plates. The plates are incubated at 32° C for 48 hours

and then counted. The Gram-negative organisms generally grow well and in the presence of TTC develop red colonies, whereas the Gram-positive types are less tolerant to TTC and develop little or no color.

This test detects the coliform as well as other Gram-negative organisms and is, therefore, considered much more sensitive than the Coliform Test. Since psychrophiles are mostly Gram-negative, they will also be counted on the plates. Olson (38) reports a fairly close correlation between the CVT and psychrophilic counts.

Olson (38) reports always finding a few hundred colonies on plates poured with raw milkm but additional investigation is needed in this area. He anticipates further study to reveal a fairly close correlation between the CVT counts and production methods because the organisms developing on the CVT plates are not generally the resistant types and would, therefore, be associated with inadequate cleaning and sanitizing methods.

In a comparison of some 20 tests for predicting shelf life, Olson (37) found that the initial psychrophilic count, initial CVT count, and the CVT count after holding for 24 hours at 55° F showed the closest correlation with shelf life at 45° F.

Temperatures

The rate at which organisms increase in milk is determined primarily by two factors, temperature of holding, and species present (26). At temperatures near freezing (0° C), there is a decrease in the plate count for 1 week or more. Multiplication then begins and continues until enormous numbers are present.

As the temperature of holding increases through the 0° to 37° C range, bacteria multiplication also increases. Pseudomonas and other psychrophilic organisms are active at the lower temperatures. Higher in the range, *S. lactis* largely dominates. At the higher temperatures, lactobacilli may develop extensively.

Ayers et al. (7) studied the effect of time and temperature on the growth of bacteria under various conditions of production (Table 2). Their work stressed the importance of good production technique and low storage temperatures in the control of bacteria in milk.

Table 2. Effect of production conditions and temperature of storage on bacterial growth

Production conditions	Storage temp.	Fresh	SPC 24 hr.	SPC 48 hr.
Good sanitation -- clean cows, utensils, environment	40	4,295	4,138	4,566
	50	4,295	13,961	127,727
	60	4,295	1,587,333	33,011,111
Poor sanitation -- clean cows, dirty utensils, environment	40	39,082	88,028	121,864
	50	39,082	177,437	831,615
	60	39,082	4,461,111	99,120,000
Very poor sanitation -- dirty cows, utensils, environment	40	136,533	281,646	538,775
	50	136,533	1,170,546	13,662,115
	60	136,533	24,673,571	639,884,615

Atherton and Bradfield (5) studied the effect of time and temperature in relation to number of milkings in a tank and types of bacteria present. Some of their work appears in Table 3.

Results indicate that milk can be stored three days at 38° F without excessive increases in counts, except when the original sample

Table 3. Effect of storage time and temperature in relation to types of bacteria present

	Milkings in tank	Storage days and temperature				
		0	3 (38° F)	3(41° F)	5(38° F)	5(41°F)
SPC	2	24T[a]	100T	4.8M[a]	5.1M	90M
	4	51T	140T	5.8M	4.4M	90M
Psy.	2	1700	120T	940T	1.0M	10M
	4	3500	120T	830T	930T	10M

[a]T = thousand; M = million

had a high psychrophile count. However, milk stored at 41° F showed strong increases.

These workers reported only one of the 32 samples organoleptic tested after 5 days storage at 38° F failed to retain its normal flavor. This was one of the samples collected after the second milking. On the other hand, 35 of the individual samples from the 26 pairs tasted after 5 days at 41° F gave pronounced off-flavors. All samples, regardless of original bacteriological quality, had counts in the millions after 5 days storage at 41° F (5° C).

Reinbold (43) recently emphasized the value of the cold efficient bulk milk tank when he noted the ease of producing milk containing under 100,000 bacteria per milliliter using abnormally poor equipment, little sanitation, and a lot of cold. For example, a properly cleaned bulk tank can easily have fewer than 130,000 bacteria on its surface after sanitizing. If only 300 pounds of milk were then placed in this bulk tank, the milk would contain fewer than 1 bacterium per milliliter. To exceed 100,000 bacteria per milliliter, there would have to be well over 13 billion bacteria on the surface of the bulk tank.

PROCEDURE

Selecting and Handling of Milk

The milk used in these studies was obtained from Grade A permitted farms in the Cache Valley area. Care was incorporated into the selection of the farms to include those considered to be very good, average, or poor in sanitary milk handling techniques.

The 31 samples collected were spread over a two month spring to summer period in order to incorporate as much as possible the change in weather temperature, feed and handling of cows. A one-quart sample was collected from the bulk tank after the second milking had been added and cooled. Samples were collected and transported to the laboratory according to the procedures outlined in the *Standard Methods for the Examination of Dairy Products*, 11th edition (2). Before collecting the sample, each tank was agitated for five minutes to insure obtaining a representative sample. Sterile paper sampling tubes and bottles were used and the samples were stored on ice while being transported to the laboratory. Examination of the samples was initiated within two hours after sampling.

Preparation and Treatment of Milk

At the laboratory, each sample was thoroughly mixed and divided into three equal portions. The portions were labeled A, B, and C respectively. Portion A was for immediate examination. Portion B was incubated at 40° F for 72 hours, and Portion C was incubated at 50° F

for 72 hours after which they were examined. Seventy-two (72) hours was selected to closely approximate the age of the first two milkings in a tank by the time it would normally be processed in a pasteurization plant.

At the laboratory, the examination of Portion A was initiated by dividing into two equal parts. One part was laboratory pasteurized before the tests were started; the other part was tested raw. Portions B and C were incubated for 72 hours at the respective temperatures before being divided for pasteurization and tested (Figure 1).

Tests on the Milk

Six tests were conducted on both the raw and pasteurized portion of the samples (Figure 1). These tests were the standard Plate Count, Direct Microscopic Count, Coliform, Psychrophile, Crystal Violet-tetrazolium (CVT), and Roundy Mastitis Test (Catalase).

Plating, incubating, and counting procedures outlined in *Standard Methods for the Examination of Dairy Products* (2) were closely followed for the Standard Plate Count, Direct Microscopic Count, Coliform, and Psychrophile tests. Directions as set forth by Olson (39) for the CVT test and Roundy (45) for the Roundy Mastitis Test are found in Appendix A. It should be noted, however, that even though these directions were followed in this work, improvements and revisions for the CVT and Roundy Mastitis Test have subsequently been recommended by Olson and Roundy.

Standard Plate Count dilutions were 1:100 and 1:1000. Incubation was 32° C for 48 hours.

Coliform dilutions were 1:10 and 1:1. Incubation was at 35° C for 24 hours.

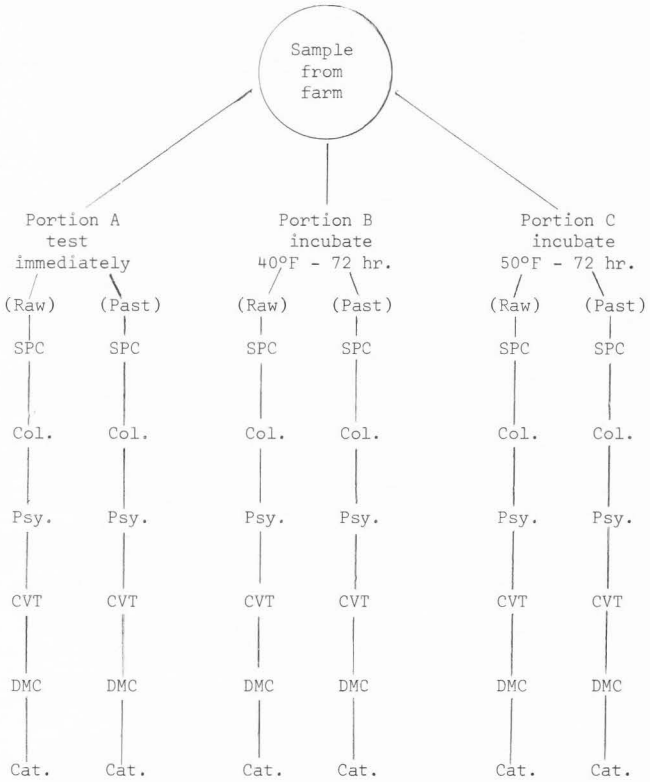

Figure 1. Examination procedure used on each sample received from the farm

The psychrophile dilutions were 1:100 and 1:1000. Incubation was 5° C for 10 days.

Clumps were counted in the Direct Microscopic Counts rather than attempting to count individual bacteria. The Levowitz-Weber modification of the Newman-Lampert stain was used.

RESULTS AND DISCUSSION

Each of 31 samples of milk collected for this study was divided into three equal portions in order to incorporate the effects of storage temperature on the bacterial flora. Portion A was examined within two hours after collection. Portion B was incubated at 40° F for 72 hours and Portion C was incubated at 50° F for 72 hours before examination.

Examination consisted of subjecting each portion to six (6) tests before and after laboratory pasteurization. Five of the tests -- Standard Plate Count, Direct Microscopic Count, Coliform, Psychrophile, and Crystal Violet-tetrazolium -- are designed to measure bacteriological flora in milk and milk products. The sixth test -- Catalase -- measures catalase activity as associated with the presence of leucocytes; however, it may also be slightly affected by catalase producing bacteria.

The Catalase Test was included in the study because of the growing importance and need to eliminate abnormal milk from the milk supply. In addition to the Catalase Test, the National Mastitis Council recognizes the California Mastitis Test, Wisconsin Mastitis Test, the Milk Quality Test, and the Direct Microscopic Count as effective tests in determining the presence of abnormal milk. Some of the reasons for detecting abnormal milk include the loss of productive cows from the producer herd, the decrease in yield and quality of milk and milk products, and the possible seizure of milk products containing staphlococcus organisms associated with abnormal milk by regulatory agencies. However, it should be noted that proper pasteurization will inactivate the staphlococcus organisms.

A total of 155 bacteriological tests on raw milk were performed. Results revealed a bacteriological increase in 74.8 percent of the samples stored at 40° F and 98.0 percent of those stored at 50° F compared to the samples examined immediately after collection (Table 4). The amount of bacteriological increase varied not only between samples, but between test results performed on the same sample which would express the need for more than one test to use in selecting milk. It is very difficult to predict the growth rate of bacteriological organisms in a given sample of milk because of the many different species present and their different abilities to adapt to the environment. Data obtained in this study resulted in an arithmetic average Standard Plate Count increase of 7.1 times the count of milk tested immediately after the second milking as compared to milk stored at 40° F for 72 hours and 39.6 times when compared to milk stored at 50° F for 72 hours. Results of the arithmetic average Direct Microscopic Counts resulted in an increase of 8.7 and 34.8 times the count of milk tested immediately as compared to milk stored at 40° F for 72 hours and 50° F for 72 hours respectively (Tables 4 and 5).

Results in 71 percent of the Standard Plate Counts, 90 percent of the Direct Microscopic Counts, 55 percent of the Coliform counts, 87 percent of the Psychrophile counts, and 71 percent of the CVT counts revealed that storage at 40° F for 72 hours was conducive to bacteriological growth as compared to the immediate use of the milk after the second milking (Tables 4, 5, 6, 7, and 8). Since one of the advantages of a bulk cooling farm tank is every-other-day collection, this study presents the desirability of lower than 40° F storage temperature.

Table 4. Standard Plate Count results and arithmetic average of samples of raw milk divided into three portions for various temperatures of incubation for 72 hours

Sample number	Portion A no incubation	Portion B 40° F incubation	Portion C 50° F incubation
1	6,800	48,000	1.950,000
2	14,000	38,000	1,300,000
3	10,000	9,000	1,300,000
4	4,000	3,400	390,000
5	11,000	3,500	620,000
6	15,000	44,000	1,700,000
7	4,100	5,400	440,000
8	170,000	150,000	12,000,000
9	4,200	5,400	370,000
10	53,000	35,000	3,100,000
11	180,000	150,000	800,000
12	2,900	4,600	460,000
13	110,000	200,000	2,800,000
14	380,000	4,100,000	5,900,000
15	94,000	120,000	2,800,000
16	15,000	470,000	3,700,000
17	4,200	6,800	14,000
18	8,200	910,000	1,200,000
19	9,400	39,000	4,100,000
20	5,600	390,000	3,500,000
21	5,400	7,200	100,000
22	1,800	1,300	15,000
23	1,400	1,800	130,000
24	14,000	60,000	2,100,000
25	9,000	10,500	1,400,000
26	5,400	3,400	5,900,000
27	15,000	13,200	590,000
28	3,000	10,000	520,000
29	4,000	4,600	400,000
30	2,600	4,200	240,000
31	540,000	5,300,000	7,600,000
Arithmetic average	54,935	391,881	2,175,452

Table 5. Direct Microscopic Count results and arithmetic average of samples of raw milk divided into three portions for various temperatures of incubation for 72 hours

Sample number	Portion A no incubation	Portion B 40° F incubation	Portion C 50° F incubation
1	10,000	29,000	4,200,000
2	10,000	110,000	9,500,000
3	<10,000	10,000	6,800,000
4	10,000	21,000	370,000
5	10,000	21,000	440,000
6	10,000	31,000	2,900,000
7	10,000	380,000	400,000
8	42,000	62,000	6,200,000
9	10,000	71,000	380,000
10	42,000	21,000	3,400,000
11	83,000	52,000	2,600,000
12	150,000	380,000	530,000
13	260,000	330,000	3,200,000
14	83,000	1,700,000	4,700,000
15	93,000	130,000	500,000
16	21,000	320,000	420,000
17	52,000	71,000	93,000
18	100,000	490,000	3,600,000
19	21,000	62,000	1,400,000
20	10,000	83,000	1,500,000
21	62,000	42,000	580,000
22	620,000	1,500,000	1,700,000
23	150,000	620,000	260,000
24	42,000	420,000	4,300,000
25	100,000	420,000	580,000
26	110,000	420,000	1,000,000
27	31,000	420,000	2,500,000
28	71,000	100,000	310,000
29	21,000	52,000	150,000
30	62,000	130,000	310,000
31	150,000	13,000,000	21,000,000
Arithmetic average	79,226	693,484	2,758,807

Table 6. Coliform test results of samples of raw milk divided into three portions for various temperatures of incubation for 72 hours

Sample number	Portion A no incubation	Portion B 40° F incubation	Portion C 50° F incubation
1	3	21	845
2	42	5,850	6,500
3	5	780	1,130
4	4	108	1,213
5	11	31	650
6	6	845	455
7	5	5	400
8	1	1	140
9	2	13	8,000
10	12,000	5,000	88,000
11	10	64	14,950
12	44	140	570
13	80	80	1,820
14	488	4,800	24,000
15	7	134	6,400
16	13	6	3,200
17	2	1	7
18	132	3,000	4,000
19	100	210	4,000
20	150	36	5,300
21	9	10	130
22	1	1	150
23	6	1	1
24	180	100	10,400
25	5	30	100
26	6	1	760
27	12	50	340
28	35	20	1,560
29	1	1	1
30	29	20	3,950
31	24,700	13,000	58,500

Table 7. Psychrophile test results of samples of raw milk divided into three portions for various temperatures of incubation for 72 hours

Sample number	Portion A no incubation	Portion B 40° F incubation	Portion C 50° F incubation
1	3,700	45,000	3,000,000
2	4,200	13,000	5,900,000
3	500	7,000	2,700,000
4	200	800	620,000
5	400	600	910,000
6	900	35,000	2,300,000
7	100	100	169,000
8	100	1,900	31,000
9	100	100	140,000
10	200	500	27,500
11	2,500	4,500	1,000,000
12	100	400	715,000
13	100	90,000	1,105,000
14	17,000	1,600,000	7,800,000
15	100	82,000	1,690,000
16	100	585,000	2,990,000
17	100	100	9,000
18	200	1,300,000	5,685,000
19	100	43,000	1,900,000
20	800	195,000	400,000
21	100	200	104,000
22	100	100	7,200
23	100	300	335,000
24	1,000	15,000	1,430,000
25	100	1,000	93,000
26	100	600	14,600
27	100	1,200	585,000
28	100	12,600	1,000,000
29	100	200	200,000
30	100	600	310,000
31	565,000	10,530,000	17,550,000

Table 8. CVT test results of samples of raw milk divided into three portions for various temperatures of incubation for 72 hours

Sample number	Portion A no incubation	Portion B 40° F incubation	Portion C 50° F incubation
1	910	4,238	3,890
2	405	1,872	2,925
3	236	224	9,360
4	215	186	174
5	137	108	22,695
6	198	4,500	14,455
7	364	1,950	TNTC
8	5,460	6,370	TNTC
9	104	585	TNTC
10	6,825	6,760	TNTC
11	2,080	2,535	TNTC
12	152	231	4,540
13	3,510	4,590	6,000
14	2,362	1,982	2,500
15	878	5,850	6,630
16	64	875	1,600
17	780	1,202	2,035
18	262	4,840	6,250
19	255	2,670	4,100
20	942	1,600	5,000
21	142	288	3,100
22	3	29	800
23	46	82	975
24	1,560	1,495	2,014
25	488	715	1,610
26	158	143	1,085
27	383	325	700
28	71	520	1,064
29	82	74	876
30	65	138	2,010
31	4,840	5,000	6,000

A lack of correlation was noted between the results of the Standard Plate Count and the Direct Microscopic Count in 51 percent of the raw and 74 percent of the pasteurized samples (Table 9). Difficulties in comparing results of these tests are enhanced by some organisms capable of growing readily on standard plate count media may not be capable of staining and thus would not be included on a Direct Microscopic Count.

Results of the Coliform Test on the fresh raw samples (Portion A) varied from less than 1 per ml to 24,700 per ml with 77 percent being less than 100 per ml. With only three exceptions, samples Nos. 8, 11, and 15, did a low Coliform count fail to result in a similar low Standard Plate Count. In 58 percent of the samples a low Coliform count (35 per ml or less) was similar to a low Standard Plate Count (30,000 per ml or less), but a high Coliform count (over 35 per ml) was similar to a high Standard Plate Count (over 30,000 per ml) in only 13 percent of the samples. Of the 31 raw samples tested, 35 percent had a Coliform count more than 10 per ml, but the Standard Plate Count was less than 30,000 per ml (Table 10). The inherent nature of the methods examining for different types of bacteria could account for the lack of uniformity, and would again express the need for analyzing the different types of bacteria present to depict the quality of a milk supply.

The growth of psychrophilic bacteria was greatly increased as the storage temperature and time was increased to 40° F and 50° F for 72 hours. Of the 31 raw samples examined, six (6) had a psychrophilic count over 1000 per ml immediately after the second milking. After 72 hours storage at 50° F, four (4) of the six (6) or 66 percent had increased to 3,000,000 per ml or over. Of the remaining 25 samples with initial counts of 1000 per ml or less, only one or 4 percent increased to 3,000,000 per ml or more (Table 7).

Table 9. Comparison results of Standard Plate and Direct Microscopic Counts on raw and pasteurized samples, portion B

Sample number	SPC raw	DMC raw	SPC past.	DMC past.
1	6,800	10,000	400	<10,000
2	14,000	10,000	300	<10,000
3	10,000	<10,000	3,800	<10,000
4	4,000	10,000	400	<10,000
5	11,000	10,000	100	<10,000
6	15,000	10,000	100	<10,000
7	4,100	10,000	300	<10,000
8	170,000	42,000	27,000	10,000
9	4,200	10,000	100	<10,000
10	5,300	42,000	100	<10,000
11	180,000	83,000	700	<10,000
12	2,900	150,000	300	21,000
13	110,000	260,000	5,300	10,000
14	380,000	83,000	3,500	31,000
15	94,000	93,000	100	10,000
16	15,000	21,000	300	10,000
17	4,200	52,000	100	21,000
18	8,200	100,000	100	10,000
19	9,400	21,000	1,300	21,000
20	5,600	10,000	100	31,000
21	5,400	62,000	200	10,000
22	1,800	620,000	<100	42,000
23	1,400	150,000	<100	83,000
24	14,000	42,000	100	71,000
25	9,000	100,000	100	21,000
26	5,400	110,000	400	10,000
27	15,000	31,000	100	62,000
28	3,000	71,000	100	71,000
29	4,000	21,000	<100	31,000
30	2,600	62,000	<100	71,000
31	540,000	150,000	15,000	140,000

Table 10. A comparison of results of the Standard Plate Count, Coliform, Psychrophile, and CVT tests, portion A

Sample number	SPC	DMC	Col.	Psy.	Cat.	CVT
1	6,800	10,000	3	3,700	3	710
2	14,000	10,000	42	4,200	3	405
3	<10,000	10,000	5	500	2	236
4	4,000	10,000	4	200	2	215
5	11,000	10,000	11	400	2	137
6	15,000	10,000	6	900	2	198
7	4,100	10,000	5	100	2	364
8	170,000	42,000	<1	100	2	5,460
9	4,200	10,000	2	100	2	104
10	53,000	42,000	12,000	200	1	6,825
11	180,000	83,000	10	2,500	2	2,080
12	2,900	150,000	44	100	1	152
13	110,000	260,000	80	100	1	3,510
14	380,000	83,000	488	17,000	2	2,632
15	94,000	93,000	7	100	3	878
16	15,000	21,000	13	100	1	64
17	4,200	52,000	2	100	1	780
18	8,200	100,000	132	200	2	262
19	9,400	21,000	100	100	2	255
20	5,600	10,000	150	800	1	942
21	5,400	62,000	9	100	1	142
22	1,800	620,000	<1	100	1	3
23	1,400	150,000	6	100	2	46
24	14,000	42,000	180	1,000	2	1,560
25	8m999	100,000	5	100	1	488
26	5,400	110,000	6	100	1	158
27	15,000	31,000	12	100	1	383
28	3,000	71,000	35	100	1	71
29	4,000	21,000	<1	100	1	82
30	2,600	62,000	29	100	1	65
31	540,000	150,000	24,700	565,000	1	4,840

The CVT test was developed primarily as a test to detect sources of contamination after pasteurization. However, this test was included on the raw samples to determine the possiblity of any correlation between CVT results and results from the other tests. Generally, it was found (Table 10) that a high Psychrophile, Standard Plate Count, or Coliform count would also result in a high (over 500 per ml) CVT count in the raw samples. A close study of the immediate counts (Portion A) revealed a 74 percent correlation between the CVT and Psychrophile counts, an 87 percent correlation between the CVT and Standard Plate Count results, and 71 percent correlation between CVT and Coliform counts. Percentages were established for comparison purposes by assigning arbitrary standards to the tests concerned. Results below these standards were considered low and results above the standards were considered high. The standards chosen were as follows: Standard Plate Count, 30,000 per ml; Coliform, 35 per ml; CVT, 500 per ml; and Psychrophile, 1000 per ml.

Of the 31 samples examined, only 5 (samples 1, 2, 3, 4, and 11) had over 3 mg of hydrogen peroxide decomposed by catalase (Table 11). Furthermore, these results were obtained only after 50° F incubation for 72 hours. This would indicate an increase in catalase due to catalase producing bacteria. Further indication of bacterial activity is presented by studying the results and noting that only 6 percent of the samples increased in catalase activity at 40° F; but when the temperature was increased to 50° F, a 42 percent increase was noted.

Pasteurization had a decided effect on the tests. After pasteurization, negative results were obtained from the Coliform, Psychrophile, and CVT tests. Results thus indicated either (a) complete destruction

Table 11. Catalase test results[a] of samples of raw milk divided into three portions for various temperatures of incubation for 72 hours

Sample number	Portion A no incubation	Portion B 40° F incubation	Portion C 50° F incubation
1	3	3	6
2	3	2	6
3	2	1	5
4	2	2	4
5	2	1	3
6	2	2	3
7	2	1	2
8	2	2	3
9	2	2	2
10	1	2	2
11	2	2	4
12	1	1	1
13	1	1	2
14	2	1	3
15	3	2	3
16	1	1	1
17	1	1	1
18	2	1	2
19	2	1	1
20	1	1	1
21	1	1	1
22	1	1	1
23	2	2	2
24	2	1	2
25	1	1	1
26	1	1	2
27	1	1	1
28	1	1	1
29	<1	<1	<1
30	1	1	1
31	1	3	2

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

of all coliform, psychrophile, and Gram-negative organisms (CVT), and active catalase, or (b) the amount of sample used in the tests was insufficient to produce any reaction. The effect of pasteurization on the Standard Plate Count and Direct Microscopic Count tests was variable. Of the total of 93 Direct Microscopic Counts performed on raw milk, 10 samples (sample Nos. 12, 13, 17, 19, 20, 21, 24, 27, 29, and 30 in Appendix B) or 32 percent increased in bacterial counts after pasteurization. An increase in bacterial counts may be due to the presence of thermophilic and thermoduric organisms; however, the possibility of error in procedural technique should not be overlooked. None of the Standard Plate Count results indicated an increase after pasteurization.

SUMMARY

The Standard Plate Count, Direct Microscopic Count, Psychrophile Count, Coliform Count, Crystal Violet-tetrazolium Count (CVT), and the Roundy Mastitis Test (catalase) were performed on samples of milk before and after pasteurization. Also incorporated into the study was a study of the effect of storing the milk for 72 hours at 40° F and 50° F.

Results emphasized the importance of determining the types of microorganisms in a milk supply rather than determining only the total number. Of necessity, therefore, careful consideration and interpretation should be given to the use of more than one test in the selection of milk.

Each test was found to possess specific advantages and limitations. The results of the Direct Microscopic Count emphasized the value of the method in obtaining prompt information as well as types of organisms present and source of contamination.

It was found that a Coliform Count paralleled a Standard Plate Count in 71 percent of the samples studied. The remaining samples revealed no correlation but would emphasize the importance of determining the types of bacteria present.

Psychrophilic organisms were observed to multiply very rapidly when stored at 50° F for 72 hours. Fresh samples containing 1000 or more psychrophiles per milliliter would multiply to 3,000,000 per ml or more when stored at 50° F for 72 hours. Other workers have reported physical or flavor changes in milk when the psychrophile count approached

3,000,000 per milliliter. Psychrophile growth in samples stored at 40° F were appreciably less than when stored at the higher temperature.

The Crystal Violet-tetrazolium test was developed to be used in locating the source of post-pasteurization contamination in milk plants. The nature of this test helps to measure organisms associated with inadequate cleaning and sanitizing methods. The test was performed on the raw milk samples and results paralleled the psychrophile counts in 74 percent of the tests.

The Roundy Mastitis Test (catalase) was affected by long storage periods and by an increase in temperature. This was probably due to catalase-producing bacteria. Therefore, the need for fresh samples of milk to produce more accurate results is emphasized. The test required a time in excess of two hours to complete; thus the results were not available as soon as may be desired although the test is relatively simple to conduct.

A storage temperature of 50° F was conducive to rapid bacteriological growth. A lower temperature of 40° F was studied and a considerable decrease in bacteriological activity occurred. Growth was still present at 40° F and even lower temperatures should be considered for storage.

CONCLUSIONS

1. The types of bacteria present in a supply of milk are more important than the total number as depicted by any one method.

2. Careful consideration and interpretation should be given to the use of more than one test to determine the quality of a milk supply.

3. The Standard Plate Count is the most widely used and recognized method for assessing the quality of milk; but due to inherent limitations such as failure of some bacteria to form visible colonies or failure of the analyst to follow directions, it should be supplemented by other tests.

4. The Direct Microscopic Count is very adaptable to producing prompt information with a minimum amount of apparatus required, but its greatest advantage is to emphasize the species present and the source of contamination.

5. Psychrophilic organisms can cause many undesirable effects in milk. Care should be exercised in eliminating the source of these organisms and carefully considering the length and temperature of storage. A temperature of 40° F or less will control growth much more efficiently than 50° F. Extremely rapid growth was observed in samples held at 50° F or above for 72 hours.

6. A combination of 40° F temperature and 72 hour storage permitted bacterial growth in 71 percent of the Standard Plate Count samples, 90 percent of the Direct Microscopic Count samples, 55 percent of the Coliform samples, 87 percent of the Psychrophile samples, and 71 percent of the Crystal Violet-tetrazolium samples.

7. Of the 155 bacteriological tests performed on the raw milk, results revealed a bacteriological increase in 74.8 percent of the samples stored at 40° F and 98 percent of those stored at 50° F compared to the samples examined immediately after collection. The increase varied not only between samples but between test results performed on the same sample which would express the need for more than one test in selecting milk as well as lower than 40° F storage temperature to restrict bacteriological activity.

8. Data obtained in this project resulted in an arithmetic average Standard Plate Count increase of 7.1 times the count of milk tested immediately after the second milking as compared to milk stored at 40° F for 72 hours and 39.6 times when compared to milk stored at 50° F for 72 hours. Results of the arithmetic average Direct Microscopic Counts resulted in an increase of 8.7 and 34.8 times the count of milk tested immediately as compared to milk stored at 40° F for 72 hours and 50° F for 72 hours respectively.

9. Coliform counts varied from less than 1 per ml to 24,700 per ml with 77 percent resulting in less than 100 per ml. Results of the Coliform test were similar to the Standard Plate Count results in a total of 71 percent of the samples. No correlation was found in the remaining 29 percent of the samples which would again emphasize the need for analyzing the different species of bacteria present.

10. In 74 percent of the samples it was noted the results of the CVT test were similar to those of the Psychrophile test. The CVT test needs additional study beyond the scope of this thesis to determine its full value to the dairy industry.

11. Catalase activity increased as storage temperature increased, possibly due to the activity of catalase producing bacteria. None of the samples examined exceeded 3 mg of hydrogen peroxide decomposed or an estimated 500,000 leucocytes per ml. The test is simple, but results are not immediately available. Fresh milk samples should be used in this test to preclude the possibility of catalase producing bacteria affecting the actual results.

12. Phosphatase negative pasteurization resulted in destruction of the catalase enzyme, psychrophiles, and coliform organisms. The Gram-negative organisms, usually responsible for spoilage in pasteurized milk, were also destroyed.

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APPENDIXES

Appendix A

Directions for Tests

Appendix A presents the directions for the Crystal Violet-tetrazolium (CVT) test as outlined by Olson and the directions for the Roundy Mastitis test as outlined by Roundy.

Crystal Violet-tetrazolium (CVT)

Materials needed:

Standard Plate Count agar
0.1% crystal violet solution (1.0 g in 1000 ml distilled water = 1000 ppm)
0.5% alcoholic TTC (0.5 g 2,3,5 triphenyltetrazolium chloride in 100 ml 95% alcohol = 5000 ppm)

Preparation of agar:

Prepare agar as per instructions on container, except that 1 ml of 0.1% crystal violet solution is added per liter (1 ppm). Sterilize as usual. Add 1 ml of 0.5% TTC per 100 ml sterile melted agar just before pouring the plates (50 ppm).

Milk dilutions:

Prepare dilution blanks with distilled water (does not need to be buffered) containing 10 ppm of crystal violet (1 part of 0.1% crystal violet solution per 100 parts of water) and dispense in 9 ml quantities in screw capped test tubes or vials, with capacities of about 25 ml and sterilize. Nine (9) ml of milk mixed with this quantity results in a 1:2 dilution. Place 1 ml on each of two plates.

Incubation:

Incubate plates at 32° C for 48 hours.

Counting:

Record the total of the colonies developing on the two plates as the count per ml.

Roundy Mastitis Test (Catalase)

Materials needed:

Water bath suitable to control at 95° F (± 2).
Test tubes (preferably 3/4 x 5 inches).
Test tube racks.
10 ml dipper or pipette.
Thermometer.
Reagents -- Solution A and Solution B obtained from Z.D. Roundy.

Procedure:

Place 10 ml of milk to be tested in each of 5 test tubes and label.
Add 1 drop of Solution A to tube No. 1, 2 drops to No. 2, 3 drops to No. 3, etc.; mix well.
Hold the test tube in the water bath for two hours.
Remove the test tube from water bath and add 6 drops of Solution B.
After 5 minutes, observe color.

Interpretation:

Each drop of Solution A contains approximately one milligram of hydrogen peroxide. The presence of a blue color in the tube indicates the milk has decomposed the peroxide in the Solution A. Thus, the catalase activity and the number of leucocytes in a given sample can be measured.

Milk that decomposes the peroxide in 3 drops of Solution A indicates the presence of approximately 500,000 or more leucocytes. The decomposition of 4 and 5 drops of Solution A indicates the presence of approximately 700,000 and 1,000,000 leucocytes respectively. Usually, leucocyte count in excess of 500,000 is considered to be abnormal milk.

Appendix B

Sample Test Results

Appendix B contains the tabulated results of the various tests performed on 31 samples. Each table presents the results on one sample. Detailed information concerning the selection, handling, preparation, and test procedure of the milk may be found in the body of the thesis.

Sample No. 1. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	6,800	10,000	3	3,700	3	710
B	48,000	29,000	21	45,000	3	4,238
C	1,950,000	4,200,000	845	3,000,000	6	3,890

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	400	<10,000	<1	0	<1	0
B	400	21,000	<1	0	<1	0
C	600	10,000	<1	0	<1	0

Sample No. 2. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	14,000	10,000	42	4,200	3	405
B	38,000	110,000	5850	13,000	2	1872
C	1,300,000	9,500,000	6500	5,900,000	6	2925

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	300	<10,000	<1	0	<1	0
B	100	21,000	<1	0	<1	0
C	100	310,000	<1	0	<1	0

Sample No. 3. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	10,000	<10,000	5	500	2	236
B	9,000	10,000	780	7,000	1	224
C	1,300,000	6,800,000	1130	2,700,000	5	9,360

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	3,800	<10,000	<1	0	<1	0
B	2,800	<10,000	<1	0	<1	0
C	3,400	560,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 4. Tests and results. Raw and pasteurized.

Raw Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	4,000	10,000	4	200	2	215
B	3,400	21,000	108	800	2	186
C	390,000	370,000	1213	620,000	4	174

Pasteurized Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	400	<10,000	<1	0	<1	0
B	100	<10,000	<1	0	<1	0
C	200	31,000	<1	0	<1	0

Sample No. 5. Tests and results. Raw and pasteurized.

Raw Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	11,000	10,000	11	400	2	137
B	3,500	21,000	31	600	1	108
C	620,000	440,000	650	910,000	3	22,695

Pasteurized Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	<10,000	<1	0	<1	0
B	200	<10,000	<1	0	<1	0
C	100	10,000	<1	0	<1	0

Sample No. 6. Tests and results. Raw and pasteurized.

Raw Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	15,000	10,000	6	900	2	198
B	44,000	31,000	845	35,000	2	4,550
C	1,700,000	2,900,000	455	2,300,000	3	13,455

Pasteurized Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	<10,000	<1	0	<1	0
B	600	10,000	<1	0	<1	0
C	300	240,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 7. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	4,100	10,000	5	100	2	364
B	5,400	380,000	5	100	1	1,950
C	440,000	400,000	400	169,000	2	TNTC

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	300	<10,000	<1	0	<1	0
B	400	<10,000	<1	0	<1	0
C	200	31,000	<1	0	<1	0

Sample No. 8. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	170,000	42,000	<1	100	2	5,460
B	150,000	62,000	<1	1,900	2	6,370
C	12,000,000	6,200,000	140	31,000	3	TNTC

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	27,000	10,000	<1	0	<1	0
B	15,000	10,000	<1	0	<1	0
C	17,000	170,000	<1	0	<1	0

Sample No. 9. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	4,200	10,000	2	100	2	104
B	5,400	71,000	13	100	2	585
C	370,000	380,000	8,000	140,000	2	TNTC

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	<10,000	<1	0	<1	0
B	100	10,000	<1	0	<1	0
C	100	10,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 10. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	53,000	42,000	12,000	200	1	6,825
B	35,000	21,000	5,000	500	2	6,760
C	3,100,000	3,400,000	88,000	27,500	2	TNTC

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	<10,000	<1	0	<1	0
B	100	<10,000	<1	0	<1	0
C	100	10,000	<1	0	<1	0

Sample No. 11. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	180,000	83,000	10	2,500	2	2,080
B	150,000	52,000	64	4,500	2	2,535
C	800,000	2,600,000	14,950	1,000,000	4	TNTC

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	700	<10,000	<1	0	<1	0
B	100	10,000	<1	0	<1	0
C	200	580,000	<1	0	<1	0

Sample No. 12. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	2,900	150,000	44	100	1	152
B	4,600	380,000	140	400	1	231
C	460,000	530,000	570	715,000	1	4,540

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	300	21,000	<1	0	<1	0
B	100	540,000	<1	0	<1	0
C	100	930,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 13. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	110,000	260,000	80	100	1	3,510
B	200,000	330,000	80	90,000	1	4,590
C	2,800,000	3,200,000	1,820	1,105,000	2	6,000

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	5,300	10,000	<1	0	<1	0
B	200	550,000	<1	0	<1	0
C	400	440,000	<1	0	<1	0

Sample No. 14. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	380,000	83,000	488	17,000	2	2,632
B	4,100,000	1,700,000	4,800	1,600,000	1	1,982
C	5,900,000	4,700,000	24,000	7,800,000	3	2,500

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	3,500	31,000	<1	0	<1	0
B	2,300	77,000	<1	0	<1	0
C	6,200	48,000	<1	0	<1	0

Sample No. 15. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	94,000	93,000	7	100	3	878
B	120,000	130,000	134	82,000	3	5,850
C	2,800,000	500,000	6,400	1,690,000	3	6,630

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	10,000	<1	0	<1	0
B	100	92,000	<1	0	<1	0
C	100	230,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 16. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	15,000	21,000	13	100	1	64
B	470,000	320,000	6	585,000	1	875
C	3,700,000	420,000	3200	2,990,000	1	1,600

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	300	10,000	<1	0	<1	0
B	100	21,000	<1	0	<1	0
C	100	280,000	<1	0	<1	0

Sample No. 17. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	4,200	52,000	2	100	1	780
B	6,800	71,000	<1	100	1	1,202
C	14,000	93,000	7	9,000	1	2,035

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	21,000	<1	0	<1	0
B	200	830,000	<1	0	<1	0
C	100	1,200,000	<1	0	<1	0

Sample No. 18. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	8,200	100,000	132	200	2	262
B	910,000	490,000	3000	1,300,000	1	4,840
C	1,200,000	3,600,000	4000	5,685,000	2	6,250

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	10,000	<1	0	<1	0
B	100	420,000	<1	0	<1	0
C	100	2,700,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 19. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	9,400	21,000	100	100	2	255
B	39,000	62,000	210	43,000	1	2,670
C	4,100,000	1,400,000	4,000	1,900,000	1	4,100

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	1,300	21,000	<1	0	<1	0
B	200	130,000	<1	0	<1	0
C	500	93,000	<1	0	<1	0

Sample No. 20. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	5,600	10,000	150	800	1	942
B	390,000	83,000	36	195,000	1	1,600
C	3,500,000	1,500,000	5,300	400,000	1	5,000

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	31,000	<1	0	<1	0
B	100	100,000	<1	0	<1	0
C	100	1,100,000	<1	0	<1	0

Sample No. 21. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	5,400	62,000	9	100	1	142
B	7,200	42,000	10	200	1	288
C	100,000	580,000	130	104,000	1	3,100

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	200	10,000	<1	0	<1	0
B	100	83,000	<1	0	<1	0
C	100	100,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 22. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	1,800	620,000	<1	100	1	3
B	1,300	1,500,000	<1	100	1	29
C	15,000	1,700,000	150	7,200	1	800

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	<100	42,000	<1	0	<1	0
B	100	52,000	<1	0	<1	0
C	<100	52,000	<1	0	<1	0

Sample No. 23. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	1,400	150,000	6	100	2	46
B	1,800	620,000	<1	300	2	82
C	130,000	260,000	<1	335,000	2	975

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	<100	83,000	<1	0	<1	0
B	<100	93,000	<1	0	<1	0
C	100	52,000	<1	0	<1	0

Sample No. 24. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	14,000	42,000	180	1,000	2	1,560
B	60,000	420,000	100	15,000	1	1,495
C	2,100,000	4,300,000	10,400	1,430,000	2	2,014

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	71,000	<1	0	<1	0
B	<100	28,000	<1	0	<1	0
C	<100	330,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 25. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	9,000	100,000	5	100	1	488
B	10,500	420,000	30	1,000	1	715
C	1,400,000	580,000	100	93,000	1	1,610

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	21,000	<1	0	<1	0
B	300	42,000	<1	0	<1	0
C	100	100,000	<1	0	<1	0

Sample No. 26. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	5,400	110,000	6	100	1	158
B	3,400	420,000	<1	600	1	143
C	5,900,000	1,000,000	760	14,600	2	1,085

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	400	10,000	<1	0	<1	0
B	800	10,000	<1	0	<1	0
C	1,000	52,000	<1	0	<1	0

Sample No. 27. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	15,000	31,000	12	100	1	383
B	13,200	420,000	50	1,200	1	325
C	590,000	2,500,000	340	585,000	1	700

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	62,000	<1	0	<1	0
B	<100	110,000	<1	0	<1	0
C	100	220,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 28. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	3,000	71,000	35	100	1	71
B	10,000	100,000	20	12,600	1	520
C	520,000	310,000	1,560	1,000,000	1	1,064

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	100	71,000	<1	0	<1	0
B	100	21,000	<1	0	<1	0
C	<100	83,000	<1	0	<1	0

Sample No. 29. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	4,000	21,000	<1	100	<1	82
B	4,600	52,000	<1	200	<1	74
C	400,000	150,000	<1	200,000	<1	876

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	<100	31,000	<1	0	<1	0
B	<100	140,000	<1	0	<1	0
C	<100	200,000	<1	0	<1	0

Sample No. 30. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	2,600	62,000	29	100	1	65
B	4,200	130,000	20	600	1	138
C	240,000	310,000	3,950	310,000	1	2,010

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	<100	71,000	<1	0	<1	0
B	<100	100,000	<1	0	<1	0
C	200	170,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.

Sample No. 31. Tests and results. Raw and pasteurized.

Raw

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	540,000	150,000	24,700	565,000	1	4,840
B	5,300,000	13,000,000	13,000	10,530,000	3	5,000
C	7,600,000	21,000,000	58,500	17,550,000	2	6,000

Pasteurized

Portion	SPC	DMC	Col.	Psy.	Cat.[a]	CVT
A	15,000	140,000	<1	0	<1	0
B	14,000	1,200,000	<1	0	<1	0
C	19,000	1,400,000	<1	0	<1	0

[a]Expressed as milligrams of hydrogen peroxide decomposed by active catalase.