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Quality of manufacturing-grade bulk-tank milk

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QUALITY OF MANUFACTURING-GRADE BULK-TANK MILK

by

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INTRODUCTION

The bulk collection of milk from farms is increasing in importance in Iowa. A limited number of grade A dairies has changed completely to handling milk on the farms in bulk tanks instead of cans. Other plants have shifted in part to bulk handling. This is also true of plants handling milk for manufacturing purposes. Many dairy farmers have converted from handling farm-separated cream to producing manufacturing-grade, bulk-tank milk. This has been due primarily to the possibility of reducing the labor input, coupled with the incentive for increased financial returns.

Because most of the studies have emphasized the economic aspects of grade A bulk milk handling, it was felt that a detailed investigation should be made of the bacteriological quality and technological problems associated with the conversion to handling manufacturing-grade bulk-tank milk.

Producers from four Iowa dairy plants were selected for study. These plants were chosen because they had begun conversion during the time this study was undertaken. The sanitary conditions for production and handling of bulk milk, availability of milk handling equipment and the bacteriological quality of the milk were investigated.

The relationships between a number of quality tests

currently employed for estimation of the number of bacteria in milk were established during the time when proper cooling of milk was a primary problem. Under the present system of bulk-tank milk handling, cooling ordinarily is no longer a problem. Due to the difference in milk cooling conditions between the past and the present, it was decided to compare these quality tests and, if possible, provide the dairy processer with some indication of which bacteriological quality test he should use in order to obtain the most accurate estimation of the quality of milk furnished by his producers milk.

REVIEW OF LITERATURE

Bacteriology of Farm Bulk-Tank Milk

Bulk-tank handling of milk on farms has been thought to be the solution for production of high quality milk. During 1949 a study was made of the logarithmic average of market milk in California by the Milk Inspection Service (61) showing that 501 can samples had an average standard plate count of 9,500 per ml. and 465 bulk-tank samples had an average of 15,300 per ml. Rinses and swabs from cleaned farm equipment showed gross variation in standard plate count and these counts were largely excessive.

Fifty-one comparisons were run by Johnson et al. (44) on raw milk during a 15 month period in which the milk was handled with the bulk system and with the can system on alternate days. Raw milk handled in bulk had an average bacterial count of 17,400 as compared with a count of 54,700 for milk handled in cans. Atherton (5) studied milk operations before and after conversion to bulk-tank milk handling during 1952 and 1954. Bacterial counts showed some improvements for the samples as a whole, but differences were seldom large and one third of the farms delivered higher count milk after converting to bulk tanks.

Pearson (71) indicated that when milk of identical

quality was held in cans and bulk tank, the bulk-tank milk maintained a higher quality on storage than can-stored milk. However, milk that had an initially high bacterial count was of inferior quality after removal from the tank, irrespective of the storage period.

Every-other-day collection has accompanied the use of bulk farm tanks. Prouty (74) collected 628 milk samples from three farm tanks after the fourth milking, incubated them for one and two days at 2.8-3.9° C. and determined the "facultative psychrophilic" colony count in five days at 17° C. and standard plate count at 35° C. The psychrophilic counts increased more rapidly than did the standard counts, but were not excessive after 24 hours storage. After 48 hours storage, one fourth of the samples had psychrophilic counts exceeding 100,000 per ml. The temperature Prouty used for plate incubation is considerably in excess of that normally considered proper for psychrophilic colony counts.

The plate counts made by Marth et al. (58) on milk of low initial bacterial content collected at the end of two days storage indicated that every-other-day pickup did not adversely affect the bacteriological quality of the milk stored in the bulk tanks. There was, however, no assurance that good results could always be obtained with milk produced on different farms.

Raw milk samples from eight farms using different methods and conditions of production were studied by Marth and Frazier (55). The milk samples were held at 36, 38 or 45° F. (2.2, 3.3 or 7.2° C.) for four days and tested daily by the standard colony count. Little increase in bacterial numbers took place in three days at 36° F. (2.2° C.). At 38° F. (3.3° C.) plate counts showed, on the average, rapid and continuous increases in numbers of bacteria. A 45° F. (7.2° C.) storage temperature was too high, since significant increases occurred in some samples within one or two days.

Marth and Frazier (56) also reported that raw milks were more likely to retain high quality in bulk tanks if they had low initial bacterial contents and if the storage temperature did not exceed 38° F. (3.3° C.).

Colony counts at 32° C. were used by Atherton and Bradfield (6) to evaluate the bacteriological quality of milk received at two creameries from farms on every-other-day pickup. Milk produced on farms where recommended equipment cleaning methods were practiced could be collected every-other-day and satisfactory raw quality maintained when held at 3.3° C. All samples, regardless of original bacterial content, gave colony counts over 1,000,000 per ml. after five days storage at 5° C.

Van Demark and March (99) also found that every-other-day pickup resulted in no greater increases in the rate of

bacterial multiplication than did every day pickup. They also found that blend temperatures of 40, 50 and 60° F. (4.4, 10 and 15.5° C.) showed no significant difference in bacterial growth rates.

Psychrophilic Bacteria in Raw Milk

The literature on psychrophilic microorganisms in milk and dairy products was thoroughly reviewed by Thomas (85). Davis (22) considered the general effect of cold on microorganisms in relation to dairying. No attempt will be made in this study to present a complete review of the literature concerning psychrophilic bacteria. Many of these reports cover areas that would contribute little to this study.

Definition of psychrophilic bacteria

Various species of gram-negative bacteria are able to grow at a moderate rate at temperatures employed in refrigerated storage of dairy products. Many of these bacteria grow equally well or even more rapidly at temperatures approaching 30° C. While there is no rigid definition of psychrophilic bacteria, the tenth edition of Standard Methods for the Examination of Dairy Products (3) describes bacteria that may be detected and enumerated by incubating plates at 5° C. for 7 days as psychrophilic. For the purpose of this study this interpretation will be used.

In determining the psychrophilic count of farm bulk-tank milk Atherton and Bradfield (6) also incubated plates at 5° C. for 7 days. However, Prouty (74) determined the "facultative psychrophilic" colony count at 17° C. in 5 days. Marth and Frazier (55) considered bacteria capable of colony formation on plates incubated at 10° C. for 14 days as psychrophilic. However, Boyd et al. (12) pointed out that incubation of agar plates at 10° C. resulted in the inclusion of a group of thermoduric organisms which were not found when plates were incubated at 5° C. These organisms were not considered to be psychrophilic.

Types of bacteria growing in raw milk

The bacteria considered to be psychrophilic because they can reproduce in milk held just above the freezing point and thus may be responsible for spoilage of refrigerated milk, appear to be largely gram-negative, non-spore-forming rods, largely of the Pseudomonas group, according to Sherman et al. (81).

Several investigators have made extensive studies of psychrophilic types isolated from raw milk. These include Thomas and Chandrasekhar (87), who found isolates to be gram-negative rods, the majority of which appeared to be Achromobacter. Others, they thought, were undoubtedly members of the genera Alcaligenes and Pseudomonas. Later Chandrasekhar (16) isolated cultures which included

Achromobacter, Flavobacterium, Alcaligenes, Pseudomonas and a few Micrococcus species.

The psychrophilic bacteria investigated in raw milk by Thomas et al. (95) included species of Achromobacter, Flavobacterium, Pseudomonas and Alcaligenes. Abd-El-Malek and Gibson (1) found 19 samples of raw milk yielding Alcaligenes viscosus, 6 yielding Alcaligenes tolerans, 6 yielding coli-aerogenes group, 12 yielding fluorescent Pseudomonas and 12 yielding other gram-negative rods.

Rogick and Burgwald (78) inoculated litmus milk with psychrophilic cultures isolated from raw milk. They observed that with growth at 4-7° C., 28.2 percent were acid-forming organisms, 17.4 percent alkali-forming and 54.4 percent were inert.

A series of cultures of psychrophilic bacteria isolated by Thomas and Rowland, as cited by Thomas (85), were all gram-negative rods and were mainly Pseudomonas, Achromobacter and Alcaligenes species. However, two were strains of coli-aerogenes bacteria which produced gas from lactose in 5 days at 30° C. but not in 2 days at 37° C. Thomas and Elson, as cited by Thomas (85), isolated strains of the genus Citrobacter /Escherichia, (13)/ and of the genus Klebsiella from raw milk.

The psychrophilic strains of bacteria isolated by Thomé and Ljunggren (97) belonged to the genera Alcaligenes and Pseudomonas.

Marth and Frazier (57) classified the predominant bacteria they isolated from farm raw bulk-tank milk as members of the Achromobacter, Aerobacter, Alcaligenes, Flavobacterium and Pseudomonas genera.

Sources of psychrophilic bacteria in raw milk

Psychrophilic bacteria are part of the normal flora of raw farm milk. It is believed that milk produced under excellent sanitary conditions contains very few psychrophilic bacteria but milk produced when the sanitary conditions are poor may contain a large number.

Parker et al. (70) indicated that the psychrophilic bacteria found in raw milk and other dairy products are common soil and water types and these are the original sources of contamination.

According to Jezeski and Macy (37), water supplies which otherwise are satisfactory may be a major source of psychrophilic bacteria. Thomas (86) reported that water contains a natural microflora composed of Pseudomonas, Flavobacterium and Micrococcus species. Morris (63) studied the water used to rinse milk cans on return to the farm and found that the contaminated water used introduced psychrophilic bacteria to the equipment in large numbers. Water from shallow wells on the farms that were studied contained Pseudomonas strains that grew actively in pasteurized, aseptically drawn milk held overnight at 4° C.

Even though water supplies may contribute psychrophilic bacteria to milk, Thomas and Rowland, as cited by Thomas (85), and Erdman and Thornton (27) indicated that the main avenue of infection of milk with psychrophilic bacteria is unsterile farm utensils.

Thomas and Rowland, as cited by Thomas (85), used steam sterilization or immersion cleaning in two percent caustic soda for the treatment of milking machine clusters and found that the psychrophilic colony count at 3-5° C. was less than 1,000 per cluster. However, when the clusters were treated in warm detergent solution, colony counts ranged between 750,000 and 280,000,000 per cluster.

The swab contact test of farm bulk tanks made by Marth et al. (58) showed that the flat, open surfaces of the tank assemblies were maintained in a satisfactory condition, but valve surfaces required special care in cleaning and sanitizing. Otherwise they could contribute large numbers of psychrophilic organisms to milk passing through them.

The psychrophilic colony count of the air during milking, made by Thomas et al. (95) in a fairly clean cow house, ranged from 1,000 to 15,000 per square foot of sterile milk surface exposed for 10 minutes. Swabbings of the flanks and udders of cows released from 880,000 to 5,000,000 colonies per square foot.

Growth of psychrophilic bacteria in raw milk

Numbers of psychrophilic bacteria depend on the conditions under which milk is produced and handled, the temperature of holding and the length of time before processing.

Research published by Conn and Esten (18), Pennington (72) and Ayers et al. (9) indicated the presence in milk of bacterial types that were capable of growth at temperatures slightly above 0° C.

More recent work by Babel (10) has shown that high quality raw milk generally shows a significant increase in total bacteria after 2 or 4 days at 4.4° C.

Lawton and Nelson (49) reported that if a milk supply is contaminated with organisms capable of low-temperature growth with as little as 1,000 per ml., they will tend to increase, even at 5° C., to about 10 million after only 3 to 4 days. Olson et al. (69) indicated, however, that with maintenance of temperatures below 5° C., growth will be greatly retarded.

Prouty (74) collected 628 raw milk samples from three farm tanks after the fourth milking, incubated them for 1 and 2 days at 2.8-3.9° C. and determined the "facultative psychrophilic" colony count in 5 days at 17° C., rather than 5° C. for 7 days. These counts were not excessive after 24 hours storage. After 48 hours storage 25 percent

of the samples had psychrophilic counts exceeding 100,000 per ml.

Raw milk samples from eight farms using different methods and conditions of bulk milk production were studied by Marth and Frazier (55). Plates were incubated at 10° C. for 14 days. Milk held at 38° F. (3.3° C.) showed, on the average, rapid and continuous increases in numbers of "psychrophilic" bacteria.

Swedish workers Thomé and Ljunggren (97) determined the number of psychrophilic organisms in mixed raw milk. They found that nearly half of the bacteria present were able to grow at 5-10° C.

Thomas and Rowland, as cited by Thomas (85), examined raw milk produced under varying hygienic conditions and indicated that there was a wide range in the psychrophilic count. Milk produced under good conditions and in which very few psychrophilic bacteria were detected on initial examination may sometimes show the presence of appreciable numbers after holding for a few days at refrigerator temperatures. Milk handled in nonsterile equipment invariably shows a very rapid increase in count.

Growth of psychrophilic bacteria isolated from raw milk

Chandrasekhar and Walker (17) studied the optimum temperature for growth of 69 strains of psychrophilic bacteria isolated at 3-5° C. from milk. The majority

produced maximum turbidity in nutrient broth at 24° C. and 66 grew best on yeastrel milk agar at this temperature. Very few strains showed growth at 37° C., while 58 showed good growth in 6 days at 30° C.

Kennedy and Weiser (47) isolated cultures from plates incubated at 10° C. Seven of the 15 cultures had an optimum temperature closer to 10° C., five near 20° C., and three grew best between 25-27° C.

Canadian workers Erdman and Thornton (28) isolated 722 cultures from milk and cream by picking colonies from plates incubated at 4.5 or 10.5° C. All of these cultures grew at 10.5° C. and 27° C. but none grew at 35.5° C.

In contrast Rogick and Burgwald (78) found that all the cultures isolated from plates incubated at from 4-7° C. for 12 days grew at 35° C.

Lawton and Nelson (49) isolated from milk held at 3° C. a Flavobacterium species which had an optimum temperature for growth around 10° C. but failed to grow at 32° C. The Pseudomonas species they isolated all grew more readily at temperatures of 21-32° C. than at 5-10° C.

Three of four strains of Pseudomonas isolated by Van Der Zant and Moore (100) were able to grow over a wide range of temperatures, with 21° C. and above giving more nearly optimum growth. The fourth culture was Ps. fluorescens. It grew better at 5 and 10° C., and not at all at 32 and 35° C.

Pseudomonas cultures isolated by Marth and Frazier (57) grew rapidly and steadily in raw milk at 3.3° C. for the first 2 days and then at a slower rate for the next 3 days. Achromobacter and Alcaligenes isolates grew rapidly for the first 3 days and more slowly during the fourth. An Aerobacter culture isolated grew during the first day, stopped for 2 days and then resumed growth. The Flavobacterium culture failed to grow appreciably at 3.3° C.

A series of 45 cultures of psychrophilic organisms was isolated by Thomas and Rowland, as cited by Thomas (85), from raw milk held for 3 days at 3-5° C. by picking colonies at random from Yeastrel milk agar plates incubated for 7 days at 3-5° C. They all grew in 3 days at 30° C. but only two grew in 2 days at 37° C.

There is evidence that some members of the coli-aerogenes group can grow at refrigeration temperatures. However, early work by Ayers and Clemmer (8) indicated that organisms of this group do not grow at 45° F. (7.2° C.). When milk was kept at temperatures above 50° F. (10° C.), they found rapid growth of this group of organisms. These results were confirmed by Sherman and Wing (82) and Dahlberg (20,21).

An Aerobacter aerogenes strain isolated by Greene and Jezeski (32) was reported to be psychrophilic.

An increase in count of strains of Escherichia coli

and A. aerogenes was detected by Robinton and Genung (77) after incubation at 8° C. for 3 to 6 hours in milk and cream inoculated with these organisms.

Thermoduric Bacteria in Raw Milk

Bacteria that survive laboratory pasteurization have been thoroughly studied with respect to their source, factors affecting their numbers and their significance. The problem of these bacteria in raw milk has been reviewed by Foster et al. (29), while Hileman (35) considered the types.

Early work by Anderson and Meanwell (4) showed that under ordinary farm conditions and without utensil sterilization, more thermoduric bacteria were present in machine-produced milk than in hand-produced milk. These authors indicated that thermoduric bacteria frequently originated from the surfaces of unsterilized utensils. These findings were later confirmed by Hileman and Leber (36), Thomas and Hobson (91), Murray (66), and Thomas et al. (89).

Thomas et al. (90) found milking machines were the major contributor of thermoduric bacteria when the equipment was poorly cleaned. They also found that milk stone served to harbor bacteria of all types, but more particularly thermoduric types. It provided both moisture and nutrient material for growth and multiplication of the bacteria

between milkings. Later, Thomas et al. (96) reported that the thermoduric flora increased more rapidly and attained greater numbers on rough surfaces of a galvanized pail than on a smooth, seamless surface.

According to Thomas and Roberts (94), surface water used on dairy farms may act as a carrier of thermoduric bacteria derived from soil and sewage and it may be one of the contributory sources of heat-resistant flora of dairy utensils. They determined the thermoduric counts on 161 farm water samples; 21 percent exceed 100 per ml. and none was entirely free of these bacteria.

Thermoduric bacteria in milk do not increase at temperatures near the freezing point, according to Sherman et al. (81). Thomas and Hobson (91) and Murray (66) reported that the method and efficiency of cooling had no significant influence on the thermoduric content of milk.

Hileman and Leber (36) considered that laboratory pasteurization counts of raw milk exceeding 5,000 per ml. may be regarded as unsatisfactory. However, McKenzie et al. (59) thought that counts exceeding 10,000 per ml. provided evidence of unsatisfactory production methods.

Resazurin Dye Reduction Test

When proper cooling of milk was a major problem in

milk production, the resazurin test could pick out low quality samples. The temperature of the milk in many cases exceeded 10° C. and a microflora that was able to reduce resazurin predominated. Jones and Davis (45) indicated that in practically every case the organisms capable of souring milk reduced resazurin rapidly.

Early work by Keenan et al. (46), Frayer (30) and later by Golding and Jorgensen (31) using a one-hour resazurin test and Johns and Howson (42) using a three-hour test indicated that milk may be graded simply and accurately by means of the resazurin test. They found that there was good correlation between the standard plate method and the resazurin test.

However, Barrett et al. (11) reported that the resazurin test cannot be used as a means of obtaining an accurate or semi-accurate bacteria count because resazurin is very sensitive to milk that is abnormal from causes other than bacteria. Leucocytes encountered in colostrum and in milk from mastitis-infected udders causes rapid decolorization. Davis and Jones (23) stated that the body cells are able to reduce blue resazurin to pink resorufin very readily, but do not reduce resorufin to colorless dihydroresorufin unless present in exceptionally large numbers. They indicated that milk which is badly affected by mastitis and has a cell count in the range of 3,000,000

per ml. will give a lower reading than if the cells were present in numbers less than 500,000 per ml. Thomas and Probert (93) concluded that at least 1,000,000 leucocytes per ml. are necessary to produce active reduction of resazurin in bulk herd milk samples examined when 18-28 hours old.

The effect of holding milk at low temperatures and of psychrophilic bacteria on the resazurin test

Davis and Ward (24), Thomas and Phillips (92), Morgan (62), Rowland et al. (79) and Hempler (34) all have shown that the resazurin test and the plate count have less correlation in winter than in summer. The milk was cooled in most cases to the atmospheric shade temperature. In winter this temperature was between 40 and 50° F. (4.4 and 10° C.) according to Thomas and Phillips (92).

Johns (40) reported that there was no statistical difference in correlation between resazurin reduction times and plate counts at 32° C. on raw milk during sampling periods of May and June and March and April. However, during the cooler period of March and April a much higher percentage of samples with plate counts exceeding 200,000 per ml. failed to reduce resazurin in 3 hours, even though the coefficient of correlation was fairly high.

Atherton et al. (7) found in refrigerated pasteurized milk that resazurin reduction was very slow, even when

psychrophilic counts reached levels of 1,000,000 per ml. or greater.

Smillie, as cited by Thomas (85), declared that resazurin was quite unsuitable for the bacteriological control of bulk milk collection in Scotland. Watt (101) indicated that as a basic raw milk test, resazurin is inadequate. He reported further that at best it is a rough screening test which is hardly a good foundation on which to build a sound quality milk program. It bears no relationship to the plate count and allows many unsanitary practices to escape detection. Figures he compiled from two milk sheds during April to December, 1955, and involving 104 producers, support his statements. Of 955 samples tested, 294 (30.8 percent) had standard plate counts of 200,000 or more per ml. Of these 294 samples, 154 (52.3 percent) were reported as resazurin grade 1.

Johns (41) recently asserted that with well cooled milk bacterial growth is inhibited and the bacteria present in the milk are in a dormant state and resazurin reduction may be unduly delayed. He thinks that results of the resazurin test following preliminary incubation at 12.8° C. for 18 hours are a much more reliable indication of bacterial quality than those obtained without preliminary incubation. Milks carelessly produced showed much greater bacterial growth during preliminary incubation than those

more carefully produced.

Johns (39) also has stated that resazurin reduction is slowed down by refrigerated storage of the milk samples over night before testing. Revallier-Warffemius (76) also obtained similar results but felt that this phenomena was due to a loss of reduction power of the leucocytes rather than of bacteria. However, Thomas and Davies (88) found an increase of only 3 to 14 minutes in mean reduction times after samples were held under refrigeration, an increase which they regarded as insignificant.

The effect of thermoduric bacteria on the resazurin test

Johns and Howson (43) made potentiometric and resazurin reduction studies using representative cultures of thermoduric organisms inoculated into milk. Only a gradual decline in Eh was found when the direct microscopic count at the start showed five million individual bacteria per ml. The color changes in the dye-milk mixtures were correspondingly gradual. Chalmers (15) also indicated that thermoduric bacteria cause a very slow decline in the oxidization-reduction potential and are not readily detected by the resazurin reduction test.

Little (54) declared that the resazurin test was ineffective in weeding out milk containing large numbers of thermoduric bacteria. He added that heat-resistant bacteria as a group are very poor reducing bacteria, and

even when present in extremely large numbers have little effect on the resazurin test.

About one quarter of the milk samples tested by Thomas and Hobson (91) had a high incidence of thermoduric bacteria, although the results with resazurin were satisfactory. They felt that this might be due to the relatively inert or slow fermentative activity in raw milk of certain types of thermoduric bacteria. Earlier, Morris (64), Morris and Edwards (65), Procter (73) and McKenzie and Morrison (60) reported that most thermoduric organisms present in raw milk are types which only reduce resazurin very slowly.

Direct Microscopic Count

The standard plate count method was found by Jezeski et al. (38) to be a much more rigorous and discriminating test for the presence of bacteria in raw milk than the direct microscopic method. The overall ratio of samples with counts of 200,000 per ml. and over by standard plate count to samples with similarly high counts by the direct microscopic method was 3.5:1. On the basis of the total number of unsatisfactory samples by either criterion, the standard plate count detected 81 percent, whereas the direct microscopic count detected 22 percent.

The Committee on Applied Laboratory Methods (75) also found the direct microscopic count was not as efficient as the standard plate count in picking out unsatisfactory raw milk samples with counts exceeding 200,000 per ml. Of 8,496 raw samples studied, the standard plate count determined that 1,236 were unsatisfactory, while only 362 samples were unsatisfactory by the direct microscopic count.

Recently, Levowitz (51) asserted that the microscopic method can never provide the order of numerical accuracy normally achieved by the agar plate method.

Little (54) found that the microscopic examination of samples of raw milk failed to detect samples with large numbers of thermoduric bacteria.

Tetrazolium Reduction Test

Laxminarayana and Iya (50) used a 0.1 percent solution of 2,3,5-triphenyltetrazolium bromide as a method of estimating the bacterial content of raw milk. The procedure was similar to the methylene blue and resazurin reduction tests. They indicated that reduction occurred solely by the action of bacteria and that the color change was from colorless to an irreversible color which was not affected by exposure to the atmosphere.

Neal and Calbert (67) employed 2,3,5-triphenyltetrazolium chloride as an indicator for inhibition of a test

organism in the presence of antibiotic substances in milk. The salt was incorporated into culture media, since the compound when reduced by the growing cells imparts color to the developing colonies. Liska and Calbert (53) indicated that triphenyltetrazolium chloride is reduced within the bacterial cell. They think the lactic dehydrogenase enzyme and its coenzyme system are involved in the reduction.

This salt was used by Seeleman and Wegener (80) in a medium they tested for estimation of coliform organisms in milk. This medium also permitted the development of alkali-producing and fluorescent gram-negative rods. They suggested that counts on tetrazolium medium might be more valuable than the standard coliform count as an index of pasteurized milk quality.

Day and Doan (25) used 3,5-diphenyltetrazolium chloride in a reduction test for keeping quality of pasteurized milk. The test gave evidence of bacterial action leading to flavor spoilage, but did not correlate with bacterial populations. This dye was found to be more sensitive than either resazurin or methylene blue.

Csenge and Doan (19) compared 3,5-diphenyltetrazolium chloride with 2,3,5-triphenyltetrazolium chloride. Inasmuch as the results with 2,3,5-triphenyltetrazolium chloride showed no definite advantage and because this dye was found

to be more light sensitive, they concluded that 3,5-diphenyltetrazolium chloride was the better reagent.

Pure cultures of psychrophilic bacteria were grown by Broitman et al. (14) on Tryptone Glucose Extract Agar medium containing 2,3,5-triphenyltetrazolium chloride as a coloring agent. All psychrophilic bacteria reduced the dye without exception. They stated that this dye was much more sensitive to reduction by psychrophilic organisms than was resazurin. They also employed tetrazolium in a solution with Nacconol NRSF[1] as a tube keeping quality test for pasteurized milk. The Nacconol NRSF was used to inhibit gram-positive bacteria. They used an incubation temperature of 20° C. The time of the appearance of a pale pink to rose red color in the milk-dye solution was dependent upon the activity of psychrophilic organisms.

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[1]Nacconol NRSF is a surface active agent which was furnished by courtesy of Allied Chemical and Dye Corporation.

EXPERIMENTAL METHODS

Bacteriological Examination by Standard Methods

Samples of bulk-tank milk of manufacturing-grade were obtained during various seasons of the year from producers at four representative Iowa creameries. The samples were collected and tested in accordance with procedures outlined in the tenth edition of Standard Methods for the Examination of Dairy Products (3). They were stored at 1.7-4.4° C. for not over 24 hours before examination. These samples were examined for total, thermoduric and psychrophilic counts. Raw milk to be plated for thermoduric counts was heated to 61.7° C. for 30 minutes in screw-cap tubes containing approximately 5 ml. of sample and then cooled by immersion in ice water. Plates for the standard plate count and thermoduric count were incubated at 32° C. Plates for the psychrophilic count were incubated at 5° C. for 7 days.

Direct microscopic counts were made on many of these samples. The staining technique developed by Levowitz and Weber (52) was used.

The resazurin reduction test was run on most of the samples. The methylene blue reduction test also was run on a limited number of samples. The standards recommended by the Agricultural Marketing Service of the United States

Department of Agriculture (2) for bacterial estimation, using the resazurin and methylene blue tests, were followed in classifying the milk samples. Table 1 presents the classification of milk using the direct microscopic count, resazurin test and methylene blue test.

The resazurin reduction test was run soon after arrival at the laboratory in the late afternoon and before overnight storage at 3° C. on 17 milk samples with standard plate counts ranging from less than 30,000 to 28,000,000 per ml. The resazurin test was run again on these samples the next morning to determine if overnight storage of the samples at low refrigeration temperature before testing has a retarding effect on the reducing ability of the bacteria present in the milk. All samples were placed in the same resazurin class with both procedures, indicating that overnight refrigeration storage before resazurin testing had no appreciable effect on the classification placed on the samples. Therefore, the overnight storage was adopted as a routine.

Studies on Psychrophilic Organisms

Isolation of psychrophilic cultures

Single colonies were picked from plates incubated for 7 days at 5° C. The colonies were picked onto Plate Count Agar (P.C.A.) slants and grown at 23° C. for 24 hours.

Table 1. Classification of milk with the direct microscopic count, resazurin test and methylene blue test

Bacterial estimation classification	Direct microscopic (clump) count per ml.	Methylene blue test, not decolorized in:	Resazurin test, not decolorized beyond P-7/4 in:
Class 1	200,000	5.5 hours	2.75 hours
Class 2	3,000,000	2.5 hours	1.5 hours
Class 3	10,000,000	1.0 hours	0.75 hours

Cultures were stored at 5° C. and transferred bimonthly. Cultures to be characterized were picked from a 24 hour P.C.A. slant into a carbohydrate-free broth and grown at 23° C. for 20 hours. The broth contained 2.5 g. Bacto Yeast Extract, 5.0 g. Bacto Tryptone and distilled water to make 1,000 ml. of medium (26). A broth culture was used for inoculation purposes throughout this work.

Reducing and staining ability of psychrophilic cultures

Milk of known low bacterial content was obtained by aseptic means from the Iowa State College dairy farm bulk tank and immediately taken to the laboratory in a refrigerated container. Approximately 100 ml. of the milk were transferred to a sterilized 6 ounce prescription bottle. One drop of a 20 hour broth culture of test organism was added to the milk and thoroughly mixed. Initial bacterial counts were made on the inoculated milk and an uninoculated control by plating on P.C.A. and incubating the plates at 23° C. for 72 hours. The inoculated and control bottles were incubated at 5° C. for 72 hours. The bottles were gently mixed each day.

After incubation, plate counts were made using incubation at 23° C. for 72 hours. Resazurin and 2,3,5-triphenyltetrazolium chloride reduction tests were run at 28 and 35.5° C. The tetrazolium test solution proposed by Broitman *et al.* (14) did not contain Nacconol NRSF. Direct

microscopic counts were made using the Levowitz and Weber (52) staining technique.

Methods and media used for culture identification

The methods and media used for identification were suggested mainly by the Manual of Microbiological Methods (84). Identification was made on the basis of morphology and biochemical characteristics. Morphological tests included the gram stain and motility determination by the hanging drop method from a 20 hour broth culture.

Biochemical tests were performed at 23° C., unless otherwise indicated. These tests included observations on litmus milk reactions, ability to liquefy gelatin, reduction of nitrate, production of indole, fermentation of glucose and lactose and lipase production, as suggested by Knaysi (48). Cultures demonstrating ability to ferment glucose and lactose with the production of acid and gas were examined further by the methyl red and Voges-Proskauer tests. The slants for growth temperature range were tempered to 5, 23 and 35° C. before streaking and subsequently incubated at these temperatures. Pigmentation of colony growth on P.C.A. was noted.

Litmus milk reactions were noted in a medium of skim milk plus one percent added non-fat dry milk. Litmus was added to the desired color and the dispensed medium was autoclaved for 15 minutes at 15 pounds steam pressure.

Phenol red broth base (26) was used in the fermentation studies. One percent solutions of glucose and lactose were used as carbohydrate substrates. The medium was dispensed in culture tubes and Durham fermentation tubes added. Sterilization was by autoclaving for 15 minutes at 15 pounds steam pressure.

The Voges-Proskauer test was performed as suggested by Hammer (33) by adding a small amount of creatine and a volume of 40 percent sodium hydroxide equal to the volume of the culture.

Modification of Reduction Tests

Use of 28° C. as well as 35° C. for the resazurin test

Procedures used were those of Standard Methods for the Examination of Dairy Products (3) modified by using an incubation temperature of 28° C. as well as 35.5° C. for performing the resazurin test on a limited number of milk samples.

Use of 2,3,5-triphenyltetrazolium chloride

A dye reduction test employing 2,3,5-triphenyltetrazolium chloride rather than resazurin or methylene blue was tested to determine if the results obtained could be correlated with the bacterial populations obtained by other procedures.

One test solution contained an inhibitor of gram-positive bacteria as proposed by Broitman et al. (14) for detecting keeping quality of pasteurized milk. This solution contained 0.1 g. 2,3,5-triphenyltetrazolium chloride, 1.0 g. Nacconol NRSF (a surface active agent produced by Allied Chemical and Dye Corp.), 5.0 g. K_2HPO_4, 0.1 g. KH_2PO_4, and distilled water to make 100 ml. A solution containing all of the above compounds except Nacconol NRSF also was employed. The solutions were put in amber colored bottles and autoclaved for 15 minutes at 15 pounds steam pressure. They were stored at room temperature in the dark.

One ml. of the tetrazolium solution was added to each of two ml. portions of raw milk in screw-cap test tubes. The tubes were inverted three times and one incubated at 28° C. and the other at 35.5° C. The tubes were inverted hourly and examined for the appearance of a pale pink color. The time required to reach this color was noted. The results obtained were compared with results of other bacteriological tests run on the same milk sample. Data were subjected to regression analyses, according to methods outlined by Snedecor (83).

Organoleptic Examination of Milk Samples

The procedures of Nelson and Trout (68) were used for

placing organoleptic grades on each milk sample. The samples were flavor scored after all bacteriological examinations were completed.

Evaluating Installations of Manufacturing-Grade Bulk-Tank Milk Producers

A rating sheet was made for evaluating the production and the milk handling conditions on farms selling manufacturing-grade bulk-tank milk to the four Iowa creameries cooperating in this study. A rating sheet was completed when a farm was visited.

The rating sheet (Appendix) is composed of 13 groups of separate items normally thought to be important in quality milk production. Each of the 13 factors was numerically evaluated from zero up to three points, depending upon the extent the milk producer fulfilled the factor requirements. There were 39 possible points on the rating sheet. A total score for each producer was determined.

In the statistical treatment of the data, according to methods of Snedecor (83), analysis of variance was used to determine if there was more variation in the producer's milking facilities score between producers of the four creameries than within the rating scores for the producers of any one creamery. A similar analysis was made using

the mean logarithmic standard plate count results of the milk of each producer. A coefficient of correlation was determined between the producer's milking facilities scores and their mean logarithmic standard plate count results where analysis of variance indicated more variation between the producers' mean logarithmic standard plate counts than within individual producer's logarithmic standard plate count results.

RESULTS

Bacteriological Quality of Manufacturing-Grade Bulk-Tank Milk

Standard and psychrophilic plate counts, as well as laboratory-pasteurized counts, were determined on 701 samples of bulk-tank milk. The counts were segregated into the four periods of the year to observe any evidence of seasonal differences. The results also were divided into three bacterial count ranges.

The standard plate count results are presented in Table 2. The samples with counts below 200,000 per ml. and above 1,000,000 per ml. made up 37.2 and 37.7 percent, respectively, of the total.

The March-May period had the highest percentage of samples with counts exceeding 1,000,000 per ml. The December-February period had the lowest percentage of samples in this high bacterial count range and the highest percentage of samples with counts of less than 200,000 per ml. There was very little difference between the June-August and September-November periods in the percentage of samples falling in low, medium, or high count ranges. In these summer and fall periods, slightly more than one third of the samples had counts in excess of 1,000,000 per ml., one third had less than 200,000 per ml. and the remaining

Table 2. Standard plate counts (32° C.) on milk samples

Period	Total samples	Samples with standard plate count of:					
		Less than 200,000/ml.		200,000 to 1,000,000/ml.		Greater than 1,000,000/ml.	
		No.	%	No.	%	No.	%
Mar.-May	223	80	35.9	40	17.9	103	46.2
June-Aug.	152	50	32.9	48	31.6	54	35.5
Sept.-Nov.	204	67	32.8	60	29.4	77	37.8
Dec.-Feb.	122	64	52.5	28	22.9	30	24.6
Total	701	261	37.2	176	25.1	264	37.7

had counts in the mid-count range of 200,000 to 1,000,000 per ml.

Table 3 gives the results of the psychrophilic plate counts on the samples of manufacturing-grade bulk-tank milk. Psychrophilic plate counts were in excess of 1,000,000 per ml. in 26.5 percent of the cases. More samples had psychrophilic plate counts in the low bacterial count range than had standard plate counts in this lower range.

The March-May season was the period with the highest percentage of samples with psychrophilic counts that exceeded 1,000,000 per ml. The 39.5 percent of samples in this bacterial count range during the spring period was considerably higher than the next highest period of September-November. The spring season also had the lowest percentage of samples with psychrophilic counts of less than 200,000 per ml. The summer months of June, July and August had the lowest percentage of psychrophilic counts in the high range and the highest percentage in the low count range.

Thermoduric counts on the laboratory-pasteurized samples are summarized in Table 4. Thermoduric counts were in excess of 10,000 per ml. in 43.6 percent of the samples. The months of March, April and May had the lowest percentage of samples with thermoduric counts of over 100,000 per ml.

Table 3. Psychrophilic plate counts (5° C.) on milk samples

Period	Total samples	Samples with psychrophilic plate counts of:					
		Less than 200,000/ml.		200,000 to 1,000,000/ml.		Greater than 1,000,000/ml.	
		No.	%	No.	%	No.	%
Mar.-May	223	99	44.4	36	16.1	88	39.5
June-Aug.	152	118	77.6	12	7.9	22	14.5
Sept.-Nov.	204	121	59.3	32	15.7	51	25.0
Dec.-Feb.	122	79	64.7	18	14.8	25	20.5
Total	701	417	59.5	98	14.0	186	26.5

Table 4. Thermoduric counts on laboratory-pasteurized samples

Period	Total samples	Samples with thermoduric counts of: Less than 10,000/ml. No.	%	10,000 to 100,000/ml. No.	%	Greater than 100,000/ml. No.	%
Mar.-May	223	151	67.7	50	22.4	22	9.9
June-Aug.	152	76	50.0	54	35.5	22	14.5
Sept.-Nov.	204	83	40.7	77	37.7	44	21.6
Dec.-Feb.	122	85	69.7	23	18.8	14	11.5
Total	701	395	56.4	204	29.1	102	14.5

The fall period had the highest percentage of samples with thermoduric counts greater than 10,000 per ml.

Standard plate count results on samples from the four creameries are shown in Table 5. Each creamery varied, in general, as to the amount and quality of field work and as to requirements made of their producers at the time of initial bulk tank installation. This should reflect on the general quality of the milk produced. Creamery C had the highest percentage of samples with standard plate counts of less than 200,000 per ml. and only 6.8 percent with counts in excess of 1,000,000 per ml. The remaining three creameries had at least 40.0 percent of their samples with counts exceeding 1,000,000 per ml. The policy of creameries A, B, and D was, in general, to allow a producer to install a bulk tank under conditions, in many cases, which were not favorable for reasonable quality milk production or to allow tank installation with the promise that the milkhouse, milking area and truck driveway area improvements would be made at a later date.

Composite Bacteriological Results of Several Producers

Standard plate counts determined on the milk of nine producers of a bulk milk route are summarized in Table 6. The order of pickup is as shown in the table. The pounds of milk delivered, as well as the percentage of the total

Table 5. Standard plate counts (32° C.) on samples from four different creameries

Creamery	Total samples	Plate count at 32° C. for 48 hours: Less than 200,000/ml. No.	%	200,000 to 1,000,000/ml. No.	%	Greater than 1,000,000/ml. No.	%
A	345	139	40.3	68	19.7	138	40.0
B	217	59	27.2	63	29.0	95	43.8
C	74	45	60.8	24	32.4	5	6.8
D	65	17	26.2	22	33.8	26	40.0

pounds delivered for each producer, also are included in the table.

Milk of six of nine producers had standard plate counts within the accepted bacteriological quality range of grade A raw milk. These six producers contributed 77.7 percent of the total pounds of milk collected on this route. Producers number 6 and 7 had milk with standard plate counts which were slightly in excess of twice that of the highest limit for grade A raw milk. The milk of producer number 3 had a count of 12,000,000 per ml. This producer delivered slightly less than 9 percent of the total pounds of milk picked up by the bulk-milk tank truck. A sample of the composite milk, obtained just prior to unloading at the creamery, had a standard plate count of 1,100,000 per ml. This count on the composite milk is considerably higher than the results on the milks of all but one of the nine producers.

As is noted in Table 6, the found and calculated plate counts on the composite milk are identical. Similar results were obtained on other routes when the truck composite milks were tested. In a few instances the found plate count was slightly higher than the calculated. This may be due to the truck pump breaking up bacterial clumps as the milk is pumped from the farm bulk tank into the tank truck.

Table 6. Composite bacteriological quality of manufacturing-grade bulk-tank milk from several producers

Producer number	Standard plate count/ml.	Pounds of milk	% of total milk delivered
1	39,000	357	6.9
2	29,000	787	15.1
3	12,000,000	452	8.7
4	190,000	336	6.5
5	26,000	1,104	21.3
6	410,000	271	5.2
7	520,000	438	8.4
8	39,000	704	13.5
9	18,000	748	14.4
Truck comp.	1,100,000	5,197	100.0
Calc. comp.	1,100,000		

Comparisons of Certain Bacteriological Quality Tests

Specific examples of the bacteriological results obtained on the manufacturing-grade bulk-tank milk are illustrated in Table 7. Sample 1 represents reasonably good quality milk that bacteriologically would meet the grade A requirements for raw milk. Sample 2 illustrates the situation in which the resazurin test placed the milk in the highest quality class, but the standard plate count revealed a lower quality with a count of 7,300,000 per ml. The direct microscopic count of only 350,000 per ml. would have placed the milk in resazurin class 2. The psychrophilic count approximated the standard plate count, while the thermoduric count was not extreme.

Sample 3, according to the resazurin test, should be placed in class 1. However, the standard plate count of 1,300,000 per ml. indicated a class 2 quality. The psychrophilic count was low in relation to the standard plate count, but the thermoduric count was 480,000 per ml., which is considerably in excess of the 30,000 per ml. count used in this study to explain poor agreement between results of the several tests for "total" population.

The fourth sample also was placed in class 1 by the resazurin test. However, the standard plate count of 2,300,000 per ml. revealed a lower quality milk. The

Table 7. Comparisons of certain bacteriological quality tests on representative samples of manufacturing-grade bulk-tank milk

Sample	Resazurin class	Counts per ml. determined by: SPC[a]	PPC[b]	TPC[c]	DMC[d]
1	1	90 T[e]	50	3 T	100 T
2	1	7.3 M[f]	6.3 M	<300 T	350 T
3	1	1.3 M	47 T	480 T	500 T
4	1	2.3 M	<30 T	200 T	2 M
5	2	12 M	13 M	100 T	1.9 M
6	3	7 M	<30 T	8 T	6.5 M

[a]SPC = Standard plate count.

[b]PPC = Psychrophilic plate count.

[c]TPC = Thermoduric plate count.

[d]DMC = Direct microscopic count.

[e]T = Thousand.

[f]M = Million.

psychrophilic count was less than 30,000 per ml., but the thermoduric count of 200,000 per ml. agreed closely with the results of the standard plate count. The direct microscopic clump count agreed well with the standard plate count.

Sample 5 was placed in class 2 by the resazurin test, but the standard plate count of 12,000,000 per ml. would have placed the sample, by this criterion, in resazurin class 4. The psychrophilic count of 13,000,000 per ml. was slightly higher than the standard plate count, and the thermoduric count was high. The direct microscopic count was what would have been expected from the results of the resazurin test.

Sample 6 is another example of poor quality bulk-tank milk. The resazurin test placed this sample in class 3 which agreed in quality classification with the standard plate count results of 7,000,000 per ml. The psychrophilic count of less than 30,000 per ml. and the thermoduric count of 8,000 per ml. were both low in relation to the standard plate count. The direct microscopic count of 6,500,000 per ml. agreed well with the standard plate count and resazurin test results.

Comparison of the Resazurin Test and Standard Plate Count

Both the resazurin reduction test and the standard plate count were performed on 670 samples of the bulk-tank

milk. The standards recommended by the Agricultural Marketing Service of the United States Department of Agriculture (2) for bacterial estimation using the resazurin reduction test were employed.

Table 8 presents a comparison of the results of the resazurin reduction test and the standard plate count on the milk samples. Of 670 samples, 454 or 67.8 percent of the total were placed in class 1 by the resazurin reduction test. These samples failed to reduce the resazurin dye beyond Munsell color standard P 7/4 in 2.75 hours. The Agricultural Marketing Service's standards for bacterial estimation indicated that these samples had bacterial counts of less than 200,000 per ml. However, 49.3 percent of all samples placed in resazurin class 1 actually had standard plate counts exceeding 220,000 per ml. and 38.6 percent of the samples placed in this class had standard plate counts greater than 400,000 per ml. A limit of 220,000 per ml. was used to allow a 10 percent margin for borderline situations. The figure of 400,000 permitted comparison with a value double the limit indicated for the class.

During June-August, 77.6 percent of the samples were placed in resazurin class 1, the highest percentage of any of the four seasons. However, a greater percentage of the samples placed in resazurin class 1 had standard plate

Table 8. Ability of the resazurin reduction test to detect bulk-tank samples with standard plate counts in excess of 220,000 per ml.

Period	Total samples	Samples placed in resazurin class 1 (failed to reduce dye beyond P 7/4 in 2.75 hours)		Resazurin class 1 samples with SPC:			
				>220,000		>400,000	
		No.	%	No.	%	No.	%
Mar.-May	192	113	58.9	52	46.0	47	41.6
June-Aug.	152	118	77.6	69	58.5	54	45.8
Sept.-Nov.	204	141	69.1	72	51.0	52	36.9
Dec.-Feb.	122	90	73.8	31	34.4	22	24.4
Total	670	454	67.8	224	49.3	175	38.6

counts exceeding either 220,000 or 400,000 per ml. than in any other season.

During the spring season 58.9 percent of the samples were in class 1. This was the season with the lowest percentage of samples in resazurin class 1. The percentage of samples in resazurin class 1 that had plate counts exceeding 400,000 per ml. was the second highest of any of the seasonal groups.

The December-February period had a relatively high percentage of the samples in resazurin class 1; the percentage of samples placed in this resazurin class and still having high plate counts was the lowest for the four seasons.

Table 9 demonstrates a similar comparison between the results of the resazurin reduction test and the standard plate count in classes in which bacteria were indicated to be more numerous. The comparison concerns samples placed in resazurin class 2 and the corresponding standard plate count grouping. Class 2 samples failed to reduce the resazurin dye beyond Munsell color P 7/4 in 1.5 hours. Resazurin class 2 would indicate, according to the Agricultural Marketing Service's standards, that the estimated bacterial count would range from greater than 200,000 to 3,000,000 per ml. A 10 percent margin of safety was added to the 3,000,000 per ml. limit of resazurin class 2 to

Table 9. Ability of the resazurin reduction test to detect bulk milk samples with standard plate counts in excess of 3,300,000 per ml.

Period	Total samples	Samples placed in resazurin class 2 (failed to reduce dye beyond P 7/4 in 1.5 hours)		Resazurin class 2 samples with SPC:			
				>3,300,000		>6,000,000	
		No.	%	No.	%	No.	%
Mar.-May	192	36	18.7	10	27.8	8	22.2
June-Aug.	152	23	15.1	11	47.8	7	30.4
Sept.-Nov.	204	32	15.7	17	53.1	10	31.3
Dec.-Feb.	122	19	15.6	8	42.1	4	21.0
Total	670	109	16.3	46	42.2	29	26.6

allow for borderline cases.

Sixteen and three-tenths percent of all samples were placed in resazurin class 2. All seasonal periods of the year had a similar percentage of each season's samples placed in resazurin class 2. However, 42.2 percent of all samples placed in resazurin class 2 had standard plate counts exceeding 3,300,000 per ml. and 26.6 percent of all samples in resazurin class 2 had counts exceeding 6,000,000 per ml.

Table 10 shows a further comparison between the results of the resazurin reduction test and the standard plate count with data presented on other tests to help explain discrepancies encountered. Of 670 samples, 221 (33.0 percent of the total) had standard plate counts which were at least twice as high as what would have been expected on the basis of the results of the resazurin tests if Agricultural Marketing Service standards were employed. Of the 221 samples in which there was lack of agreement, 99 had psychrophilic counts that were 75 percent or more of the standard plate counts. Sixty-four of the 221 samples in this group had thermoduric counts exceeding 30,000 per ml. Thirty-one of the 221 samples had both a high psychrophilic and a high thermoduric count. Twenty-seven samples had neither a high psychrophilic nor a high thermoduric count, giving no obvious explanation for the discrepancies in classification. Of the samples which were

Table 10. Comparisons of resazurin tests with standard plate counts on bulk-tank milk

Period	Total samples	Resazurin and SPC not in agreement[a]		Milk samples not agreeing when:							
				PPC high[b]		TPC high[c]		PPC and TPC high		Neither PPC nor TPC high	
		No.	%[d]	No.	%[e]	No.	%[e]	No.	%[e]	No.	%[e]
Mar.-May	192	62	32.3	42	67.8	8	12.9	9	14.5	3	4.8
June-Aug	152	65	42.8	19	29.2	26	40.0	7	10.8	13	20.0
Sept.-Nov.	204	67	32.8	20	29.9	24	35.8	13	19.4	10	14.9
Dec.-Feb.	122	27	22.1	18	66.7	6	22.2	2	7.4	1	3.7
Total	670	221	33.0	99	44.8	64	29.0	31	14.0	27	12.2

[a]SPC = Standard plate count at least twice as high as resazurin class indicates.

[b]PPC = Psychrophilic plate count 75 percent or more than SPC.

[c]TPC = Thermoduric plate count in excess of 30,000 per milliliter.

[d]Percent of total samples in period not agreeing.

[e]Percent of samples not agreeing which fell in this group.

not placed in the same class by the resazurin and standard plate count results, 58.8 percent had high psychrophilic counts and 43.0 percent had high thermoduric counts, some samples falling in both groups.

The greatest lack of agreement between the results of the resazurin test and those of the standard plate count was found during the summer period. The spring and fall seasons had much the same percentage of their samples failing to show agreement. The samples tested in the cool months of December, January and February had best agreement between these two tests.

In both the winter and spring periods, two thirds of the samples which showed lack of agreement had high psychrophilic counts. However, less than one third of the summer and fall samples on which agreement was poor had high counts of psychrophilic bacteria. More discrepancies between the two tests for these latter seasons occurred when the thermoduric count was excessively high. The spring period had a particularly low percentage of samples with thermoduric counts exceeding 30,000 per ml. among the samples for which there was poor agreement between results of the two tests.

Several of the samples which showed lack of agreement in the spring, summer and fall periods had both high psychrophilic and high thermoduric counts. During the

summer one fifth of the samples upon which results did not agree had neither a high thermoduric nor a high psychrophilic count. Only a small percentage of samples in this category was encountered in the winter and spring seasons.

The methylene blue test results were compared with the results of the resazurin test on 77 milk samples. The classification system proposed by the Agricultural Marketing Service of the United States Department of Agriculture (2) was followed for placing the samples in a particular class estimating a bacterial count range. Forty-seven of the 77 samples were placed in class 1 by both tests. However, of 17 samples placed in class 2 by the resazurin test, five were put in the higher quality class 1 by the methylene blue test. Eight samples were put in resazurin class 3 but four of these were placed in class 2 by the methylene blue test.

Comparison of the Direct Microscopic Count and Standard Plate Count

The standard plate count and the direct microscopic count were compared on 586 milk samples. Table 11 summarizes the results of this comparison. A lack of agreement between the two methods was considered to exist when the standard plate count was at least twice as high as the

Table 11. Comparisons of direct microscopic counts with standard plate counts on bulk-tank milk

Period	Total samples	DMC and SPC not in agreement[a]		Milk samples not agreeing when:						Neither PPC nor TPC high	
				PPC high[b]		TPC high[c]		PPC and TPC high			
		No.	%[d]	No.	%[e]	No.	%[e]	No.	%[e]	No.	%
Mar.-May	113	51	45.1	30	58.9	7	13.7	9	17.6	5	9.8
June-Aug.	147	78	53.0	22	28.2	30	38.5	5	6.4	21	26.9
Sept.-Nov.	204	83	40.7	32	38.5	26	31.4	15	18.1	10	12.0
Dec.-Feb.	122	33	27.0	19	57.6	6	18.2	4	12.1	4	12.1
Total	586	245	41.8	103	42.1	69	28.1	33	13.5	40	16.3

[a]SPC at least twice as high as DMC.

[b]PPC 75 percent of or higher than the SPC.

[c]TPC in excess of 30,000 per milliliter.

[d]Percent of total samples in period not agreeing.

[e]Percent of samples not agreeing which fell in this group.

direct microscopic count. Of the 245 samples (41.8 percent of the total) showing lack of agreement, 103 or 42.1 percent had psychrophilic counts that were 75 percent or more of the standard plate count. Sixty-nine samples or 28.1 percent had thermoduric counts that were in excess of 30,000 per ml., 33 or 13.5 percent had both high thermoduric and psychrophilic counts and 40 or 16.3 percent had neither a high thermoduric nor a high psychrophilic count.

The three summer months had the highest percentage of samples that showed lack of agreement between the standard plate and direct microscopic count results, while the winter months of December, January, and February had the lowest percentage of samples in this situation. The psychrophilic counts were high during the summer months on the lowest percentage of the samples on which plate counts and direct microscopic counts did not agree. However, this was the season with the highest percentage of samples with high thermoduric counts.

The spring period had the highest percentage of samples of any period with high psychrophilic counts and had the lowest percentage of samples of any period with high thermoduric counts when the results of both the direct microscopic count and standard plate count did not agree.

Over one fourth of the summer samples on which the direct microscopic count was lower than anticipated from

plate count results had neither a high psychrophilic nor a high thermoduric count.

Most of the samples on which standard plate count and direct microscopic count results failed to agree also had resazurin test results that did not agree too closely with standard plate count results.

Use of 28° C. as well as 35° C. for the Resazurin Test

A bath temperature of 28° C. rather than 35.5° C. was investigated for performing the resazurin test. Many milk samples which were well cooled in farm bulk tanks appear to have a predominately psychrophilic type of microflora. A portion of these bacteria are unable to grow at 35.5° C. and most of them have an optimum growth temperature near 28° C. The results of the resazurin test made on 17 samples indicated that 28° C. incubation temperature retarded the reduction of the dye by the bacteria present in the milk. As a result, the three samples placed in resazurin class 2 with 35.5° C. incubation temperature were put in class 1 when incubated at 28° C. One sample was placed in class 3 with 35.5° C. incubation but was put in class 2 with 28° C. incubation. Another was a class 4 sample at 35.5° C. incubation but was placed in class 3 when incubated at 28° C.

2,3,5-Triphenyltetrazolium Chloride Reduction Test

2,3,5-Triphenyltetrazolium chloride was employed in a reduction test to determine if the standard plate count of milk could be estimated from the time necessary to reduce this chemical from a colorless state to a pale pink color. Temperatures of 35.5 and 28° C. were used for incubating the 10 ml. of milk plus 1 ml. of test solution. One solution contained the surface active agent Nacconol NRSF, while the other solution did not.

The time necessary to reduce 2,3,5-triphenyltetrazolium chloride to a pale pink color was noted. These results were compared with the results of the standard plate counts determined on the same milks. Statistical analyses of correlation were made involving the standard plate count results expressed in logarithms and the reduction time in hours. Results on milk samples that required longer than 8 hours at 35.5° C. incubation to reduce the tetrazolium solution without Nacconol NRSF were not used in the statistical analyses. When the tetrazolium solution did contain Nacconol NRSF and incubation was at 35.5° C. and also when the tests were performed at 28° C. for these same samples, a few which had reduced in 8 hours in the first test required longer than 8 hours to reduce the compound. These few samples were also included in the respective analysis. The value of a test for milks requiring longer than 8 hours

for reduction is limited for the creamery operator because the test would not be completed within a normal work day. Milk samples with bacterial counts high enough to be placed in an undergrade category can be detected within 8 hours.

There is a positive relationship between the standard plate count results and the reduction time of 2,3,5-triphenyltetrazolium chloride, as indicated by the coefficients of correlation. When 55 milk samples were employed using an incubation temperature of 35.5° C. and the test solution containing Nacconol NRSF, the coefficient of correlation was -0.855, with significance at the one percent level. When the 55 milk samples were used employing an incubation temperature of 35.5° C. and the test solution did not contain Nacconol NRSF, the coefficient of correlation was -0.784, with significance at the one percent level. Forty-three milk samples were employed using an incubation temperature of 28° C. and the test solution contained Nacconol NRSF. The coefficient of correlation was -0.839, with significance at the one percent level. The same 43 milk samples discussed above were used when the incubation temperature was set at 28° C. and the test solution did not contain Nacconol NRSF. The coefficient of correlation was -0.845, with significance at the one percent level.

These results indicate a positive relationship between

the standard plate count results and the number of hours necessary for the milk to reduce the tetrazolium solution from a colorless state to a pale pink color within an 8 hour limit. The coefficients of correlation between this test and the standard plate count, with and without Nacconol NRSF, and with incubation at 28° C. were very similar. When comparing the incubation temperature of 35.5° C., the tetrazolium test solution containing Nacconol NRSF had a slightly higher coefficient than when Nacconol NRSF was not present. However, this difference is not great enough to suggest a strong preference for the test solution containing Nacconol NRSF.

Figures 1, 2, 3, and 4 illustrate the relationship found between the results of the standard plate count per ml. in logarithms and the time required to reduce the 2,3,5-triphenyltetrazolium chloride, with and without Nacconol NRSF, and with incubation temperatures of 35.5 and 28° C. The encircled dots represent results that were not included in the statistical analyses because of the time limitation imposed.

The 2,3,5-triphenyltetrazolium chloride solution that contained Nacconol NRSF and employed an incubation temperature of 35.5° C. in performing the test had the highest calculated coefficient of correlation. To determine if the standard plate count per ml. on raw milk can be pre-

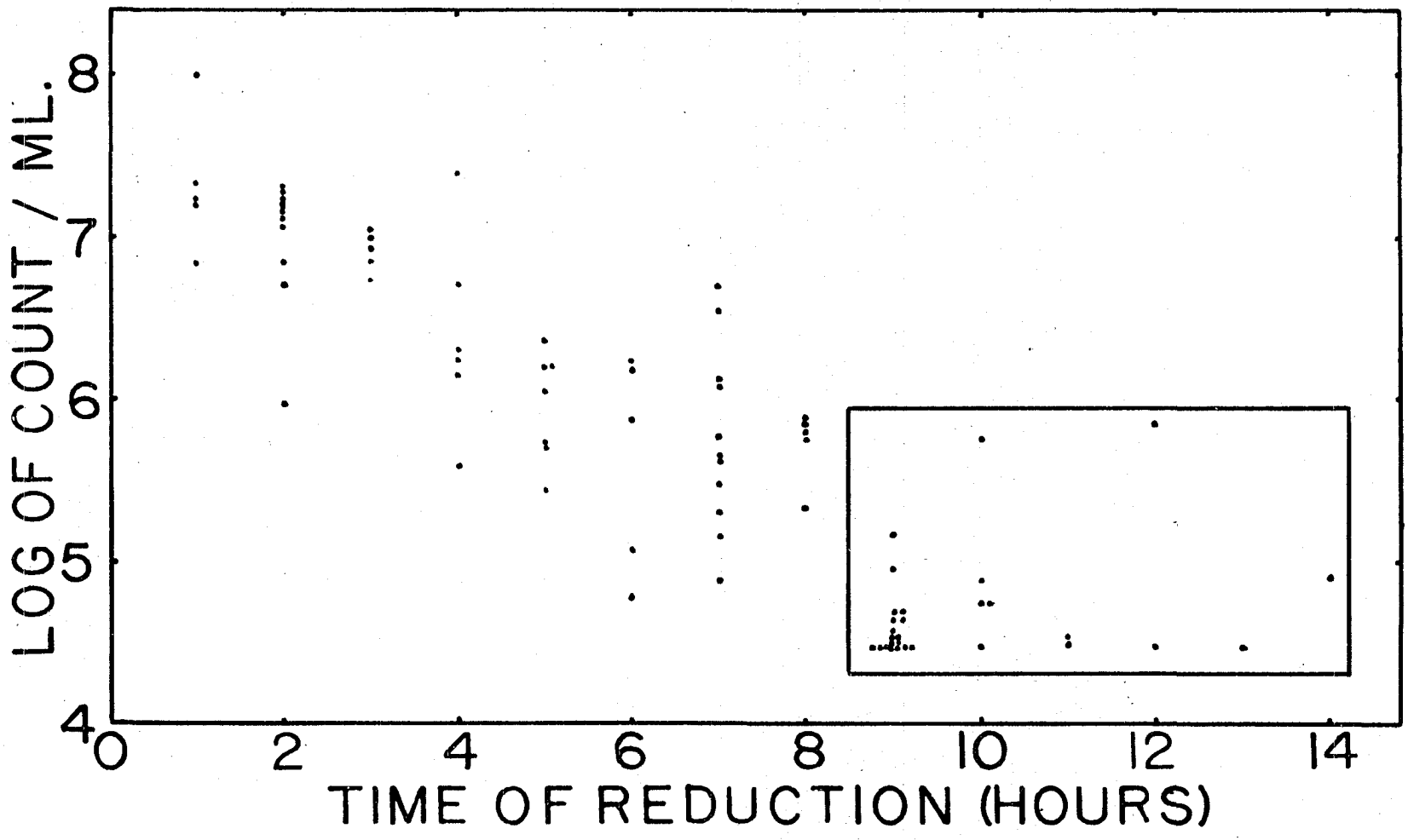

Figure 1. Relationship between the logarithm of the standard plate counts of 84 raw milk samples and the reduction time using 2,3,5-triphenyltetrazolium chloride without Nacconol NRSF with incubation at 35.5° C.

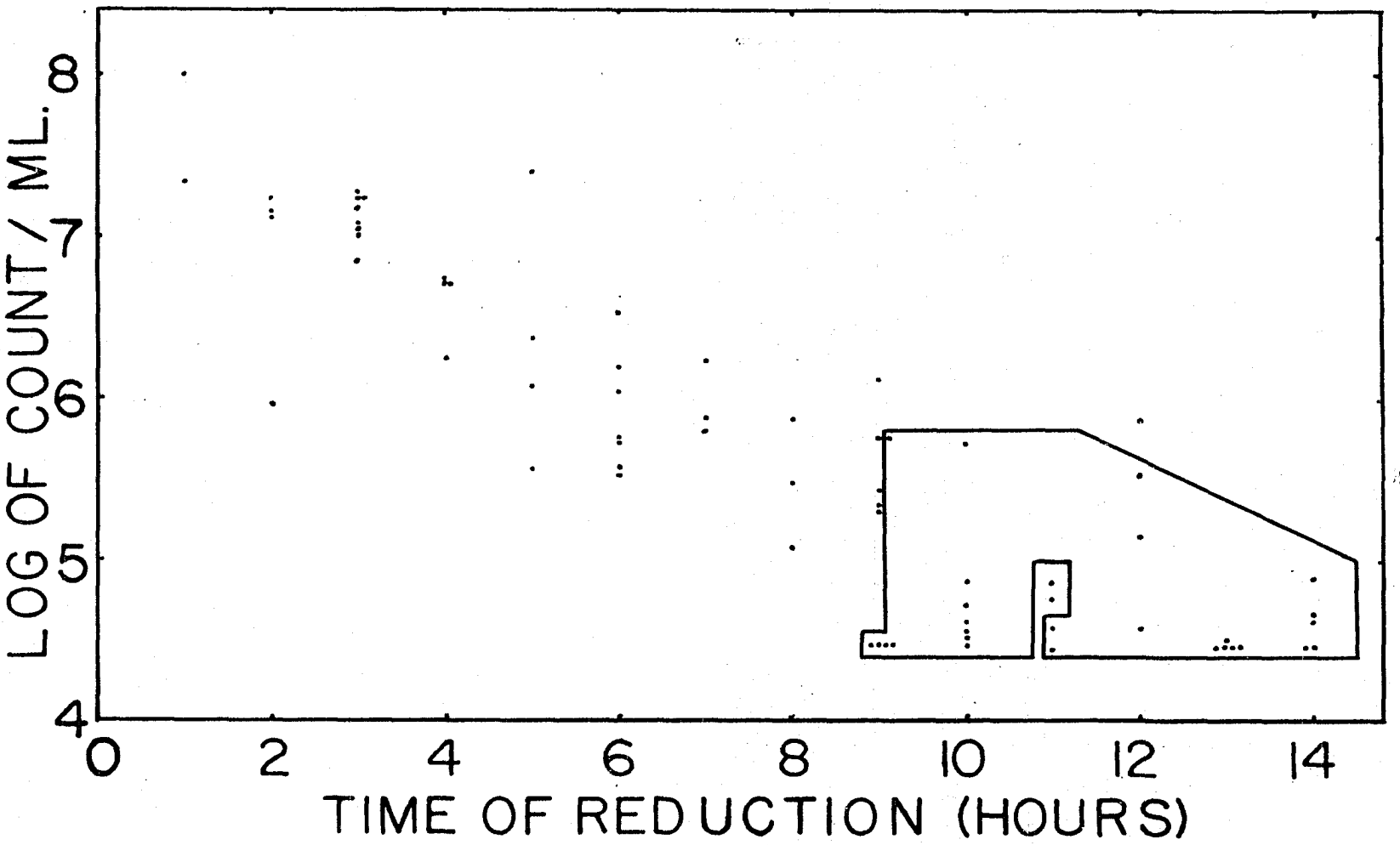

Figure 2. Relationship between the logarithm of the standard plate counts of 70 raw milk samples and the reduction time using 2,3,5-triphenyltetrazolium chloride without Nacconol NRSF with incubation at 28° C.

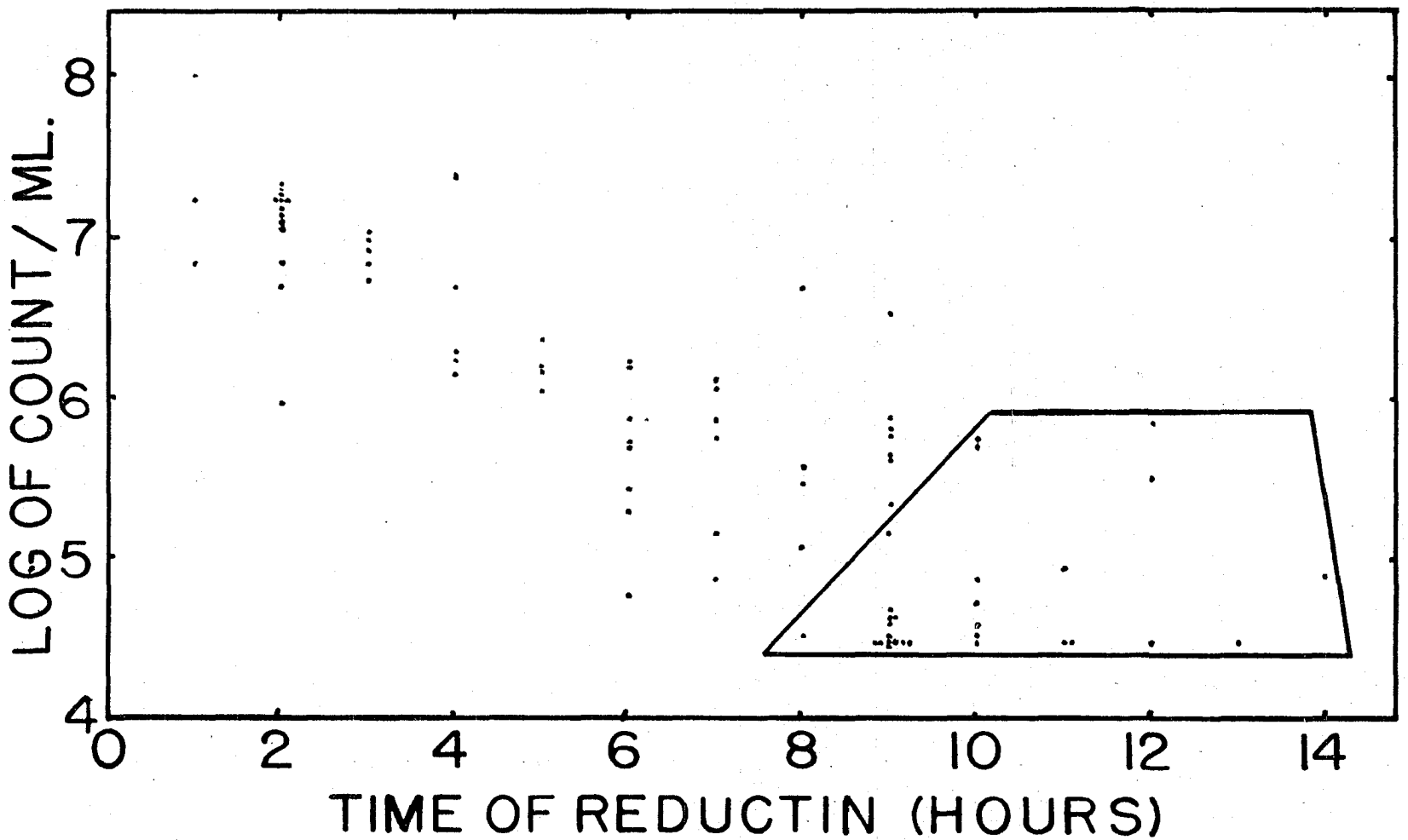

Figure 3. Relationship between the logarithm of the standard plate counts of 84 raw milk samples and the reduction time using 2,3,5-triphenyltetrazolium chloride plus Nacconol NRSF with incubation at 35.5° C.

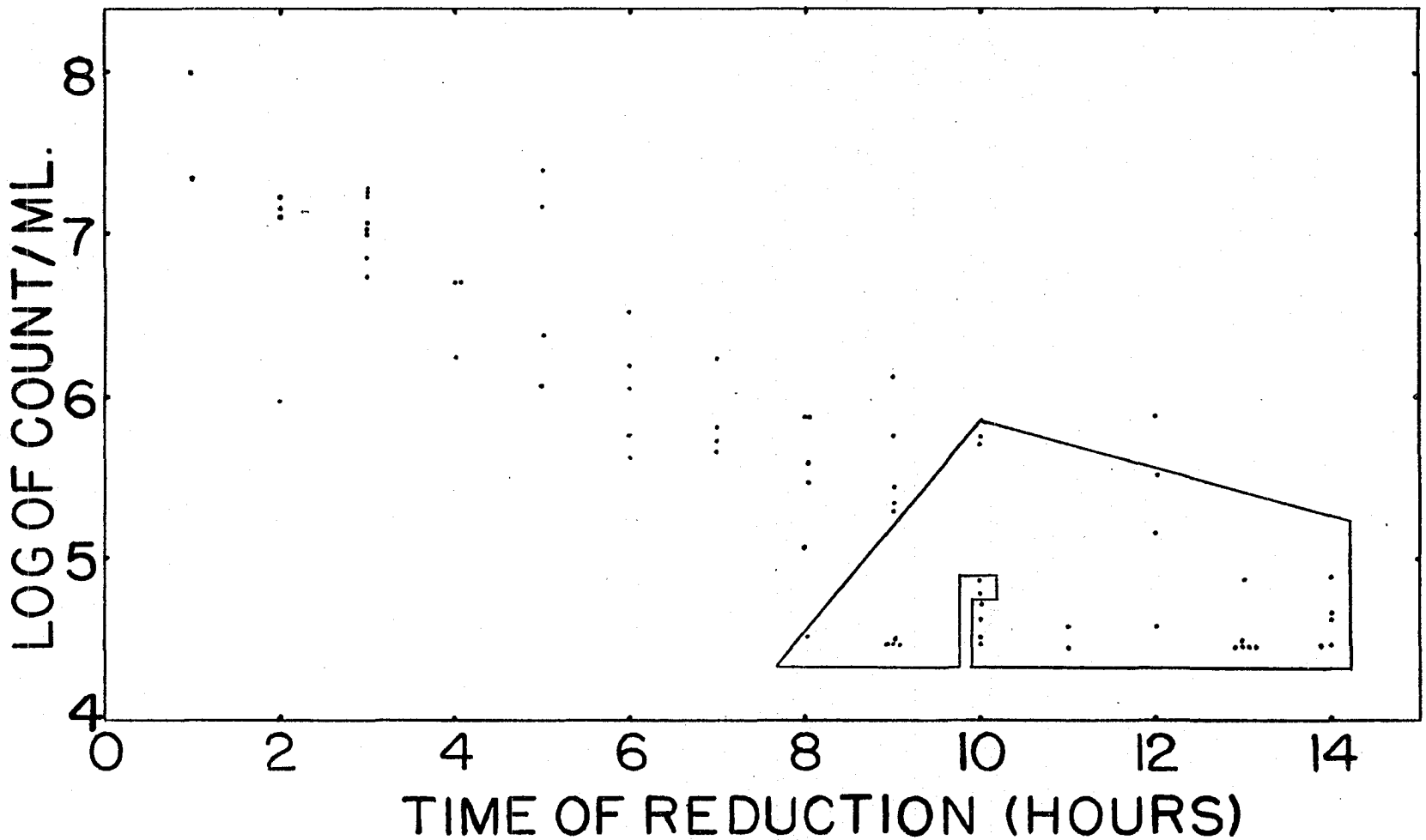

Figure 4. Relationship between the logarithm of the standard plate counts of 70 raw milk samples and the reduction time using 2,3,5-triphenyltetrazolium chloride plus Nacconol NRSF with incubation at 28° C.

dicted from the results of the reduction test using the above incubation temperature and test solution, a sample regression equation was calculated. The sample regression line in Figure 5 is represented by the equation $Y = 7.508 - 0.243 X$. The 95 percent confidence interval estimates also are shown. The sample regression coefficient is -0.243 units of logarithmic plate count per ml. per one hour of reduction time with significance at the one percent level. The 95 percent confidence interval estimate for the sample regression coefficient was calculated to be -0.203 to -0.283.

Flavor Defects in Milk Attributed to High Bacterial Numbers

Organoleptic scores were placed on 701 milk samples to determine the general flavor characteristics of manufacturing-grade bulk-tank milk. The majority of the samples had some degree of feed flavor. Fifty-five samples had flavor defects which would appear to be the result of excessive numbers of microorganisms in the milk. Milk samples with apparent bacterially caused defects included 25 with a slight to distinct high acid flavor, 14 with a malty flavor, 7 with an unclean flavor, 8 with a combination of unclean and high acid flavor and 1 with a distinct putrid flavor. The standard plate counts determined on these milk samples ranged from 930,000 to 210,000,000 per ml.

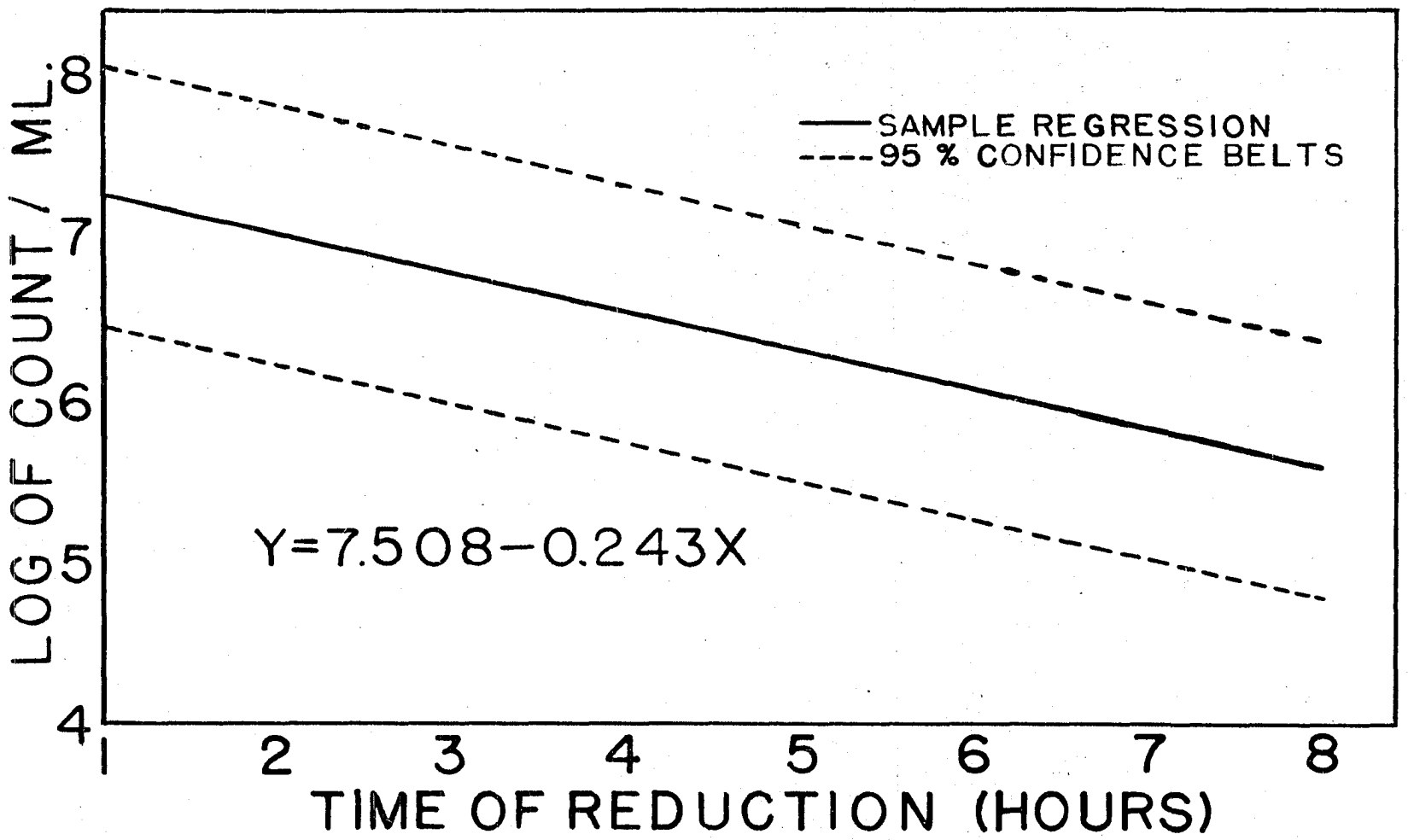

Figure 5. Sample regression and 95 percent confidence belts of the logarithm of the standard plate count of raw milk on the time to reduce 2,3,5-triphenyltetrazolium chloride solution containing Nacconol NRSF at 35.5° C.

The psychrophilic plate counts were within the same numerical range as the standard plate counts.

Table 2 reveals that 264 of the 701 milk samples had standard plate counts in excess of 1,000,000 per ml. Thus 209 samples with plate counts exceeding 1,000,000 per ml. had no apparent flavor defect that could be directly attributed to bacteria. One milk sample with standard and psychrophilic plate counts of 74,000,000 and 73,000,000 per ml., respectively, had only a slight feed flavor defect. There were many other samples with plate counts of several million that had no flavor defect attributable to bacterial growth.

Statistical Analysis of Milking Facilities Scores

A rating score of milk production facilities was made on each of 172 producers of four creameries. The scores ranged from 6 to 37 points, out of a possible 39. There was similar distribution of scores for producers of the individual creameries. The average score for all producers was 23.4. The producers of creameries A, B, C and D had average scores of 24.9, 20.6, 24.0 and 23.5, respectively.

On 68 of the 172 farms there was no water under pressure in the milkhouse. Eighty-six farms had no utensil wash vat and 96 farms had no water heater in the milkhouse. On 30 farms there was no wire screening or other separation of

the bulk tank from the milking area. These 30 farms also had no water heater, wash vat or water under pressure in the area.

An investigation was conducted to determine if the score could be used to estimate the average standard plate count result for an individual producer's milk. Standard plate counts were converted for purposes of analyses to logarithms. An analysis of variance was carried out according to methods outlined by Snedecor (83) on 539 standard plate count results from the milk produced by 172 producers.

The results of the analysis indicated, however, that there was significantly more variation within each individual producer's several plate count results than when each producer's average plate count result was analysed for variation between the 172 producers. This result precluded an analysis of correlation between the average plate count results of the 172 individual producers and the score placed on each producer's milking facility.

An analysis of variance was made of scores placed on the facilities of 172 producers. The producers of the four creameries were grouped according to the creamery to which they sold milk. The results revealed more variation in facility scores between the four producer groups than within the scores of the several producers associated with any one

creamery ($0.10 < P < 0.05$).

A similar analysis of variance was performed using the plate count results of the milk producers when the producers were grouped by creamery. The results indicated that there was significantly more variation in the average plate count results between the four producer groups than within the several plate count results of individual producers associated with any one creamery ($P > 0.005$).

An analysis of variance was made on the plate count results of the milk producers of each creamery. When producer plate count results of creamery D were grouped, the analysis result indicated that there was more variation in the average plate count results between producers of this creamery than within the several plate count results of the creamery's individual producers ($P > 0.005$).

However, when producer results of creameries A, B and C were grouped, the analyses results revealed that there was more variation within the several plate count results of the individual producer than between the average plate count results of the producers grouped according to creamery. These latter results prevented an analysis of correlation for the producers of creamery A, B and C between the individual producer's average plate count results and his milking facility score.

A statistical correlation was made on the scores placed

on the facilities of producers of creamery D and the individual producers' average plate count results. The calculated sample coefficient of correlation was -0.54. This accounts for 0.2916 of the variance, a value significant at the five percent level.

Taxonomy of the Psychrophilic Bacteria Isolated

Colonies were picked from plates incubated at 5° C. for 7 days. The plates were chosen to furnish isolates from milk samples with high, medium and low psychrophilic counts. Samples for which the resazurin test and the standard plate count results did not agree on bacteriological quality classification also were selected as sources for psychrophilic isolates. Normally the colony type that appeared dominant on the plate was picked. Also colonies with unusual colony size, shape, chromogenesis, or other differentiable characteristics were picked, even though the particular colony type was not dominant.

Table 12 summarizes the results of the taxonomic studies of 79 psychrophilic cultures. Sixty-nine cultures were gram-negative rods and 10 were gram-negative cocci or coccobacilli as determined by smears from the growth on Plate Count Agar slants grown at 23° C. Cultures number 21, 45 and 61 were methyl red negative and Voges-Proskauer positive. Culture 44 was methyl red positive and Voges-

Table 12. Characteristics of isolates from psychrophilic plates of manufacturing-grade bul

Number	Cell description	Chromogenesis on P.C.A.	Gelatin liquefaction (days)	Motility	Litmus milk				Gl
					Reaction	Coag.	Proteolysis (days)	Redn.	
1	G-rod	Gray	Complete 4	+	Alk.[a]	-	Complete 3	-	
2	G-rod	Gray	Complete 3	+	Alk.	-	Complete 4	-	
3	G-rod	Gray	Complete 4	+	Alk.	-	Complete 4	-	
4	G-rod	Gray	Complete 4	+	Alk.	-	Complete 4	-	
5	G-rod	Gray	Complete 2	+	Alk.	-	Complete 4	-	
6	G-rod	Gray-white	1/3 in 14	+	Acid	-	-	+	
7	G-rod	Gray-white	1/3 in 14	+	Alk.	-	-	-	
8	G-rod	Gray-white	1/3 in 14	+	Acid	+	-	+	
9	G-rod	Gray	Complete 2	+	Alk.	-	Complete 4	-	
10	G-rod	Yellow	Complete 5	-	Alk.	-	Complete 10	-	
11	G-rod	Gray-white	Complete 2	+	Alk.	-	Complete 4	-	
12	G-rod	White	-	-	Acid	-	-	+	
13	G-rod	Gray	Complete 4	+	Alk.	-	Complete 3	-	
14	G-rod	Gray	Complete 21	+	Alk.	-	Complete 3	-	
15	G-rod	Gray	Complete 8	+	Alk.	-	Complete 3	-	
16	G-rod	Yellow	Complete 4	-	Acid	-	Complete 8	-	
17	G-coccus	Cream-gray	-	-	Alk.	-	-	-	
18	G-coccus	Gray	-	-	Alk.	-	-	-	
19	G-coccus	Gray	-	-	Alk.	-	-	-	
20	G-rod	Cream-gray	Complete 4	+	Alk.	-	Complete 4	-	
21	G-rod	Gray	Complete 12	+	Acid	+	-	+	
22	G-rod	Gray-white	1/4 in 12	+	Neut.	-	-	-	
23	G-rod	Gray	-	+	Alk.	-	-	-	
24	G-rod	Gray	-	+	Alk.	-	-	-	
25	G-rod	Gray	1/2 in 12	+	Alk.	-	Complete 9	-	

[a]Alk. = Alkaline.

[b]A = Acid.

[c]AG = Acid and gas.

(Continued)

bulk-tank milk

Ferm. of Glucose	Ferm. of Lactose	Growth on P.C.A. at 5°C.	Growth on P.C.A. at 23°C.	Growth on P.C.A. at 35°C.	Nitrate redn.	Indole prod.	Lipase prod. 122 hr.	Genus
A[b]	-	+	+	+	-	-	+	Pseudomonas
A	-	+	+	+	-	-	+	Pseudomonas
A	-	+	+	+	-	-	+	Pseudomonas
A	-	+	+	+	-	-	++	Pseudomonas
A	-	+	+	+	-	-	++	Pseudomonas
A	-	+	+	-	-	-	-	Achromobacter
A	-	+	+	-	-	-	+	Pseudomonas
A	-	+	+	-	-	-	-	Achromobacter
A	-	+	+	+	-	-	++	Pseudomonas
-	-	+	+	-	+	+	-	Flavobacterium
A	-	+	+	+	-	-	++	Pseudomonas
A	A	+	+	+	-	-	-	Achromobacter
A	-	+	+	+	-	-	+++	Pseudomonas
A	-	+	+	+	+	-	++	Pseudomonas
A	-	+	+	+	+	-	++	Pseudomonas
-	-	+	+	-	-	+	-	Flavobacterium
-	-	+	+	+	-	-	+	Alcaligenes
-	-	+	+	+	-	-	+	Alcaligenes
-	-	+	+	+	-	-	+	Alcaligenes
A	-	+	+	+	-	-	+++	Pseudomonas
AG[c]	AG	+	+	+	+	-	-	Aerobacter
A	-	+	+	+	-	-	+	Pseudomonas
A	-	+	+	-	+	-	-	Pseudomonas
A	-	+	+	-	+	-	-	Pseudomonas
A	-	+	+	-	+	-	-	Pseudomonas

Table 12. (Continued)

Number	Cell description	Chromogenesis on P.C.A.	Gelatin liquefaction (days)	Motility	Litmus milk			
					Reaction	Coag.	Proteolysis (days)	Redn.
26	G-rod	Gray	Complete 2	+	Alk.	-	Complete 4	-
27	G-rod	Gray	Complete 4	+	Alk.	-	Complete 4	-
28	G-rod	Gray	4/5 in 21	+	Alk.	-	Complete 4	-
29	G-rod	Gray	4/5 in 21	+	Alk.	-	Complete 4	-
30	G-rod	Gray	-	-	Neut.	-	-	-
31	G-rod	Gray	-	-	Neut.	-	-	-
32	G-rod	Gray	1/3 in 21	+	Alk.	-	Complete 6	-
33	G-rod	Gray	-	-	Neut.	-	-	-
34	G-rod	Gray	Complete 3	+	Alk.	-	Complete 3	-
35	G-rod	Gray	Complete 3	+	Alk.	-	Complete 3	-
36	G-rod	Gray	Complete 3	+	Alk.	-	Complete 6	-
37	G-rod	Gray	Complete 3	+	Alk.	-	Complete 6	-
38	G-rod	Yellow	-	+	Neut.	-	-	-
39	G-rod	Gray	Complete 3	+	Alk.	-	Complete 6	-
40	G-coccus	Gray-white	-	-	Neut.	-	-	-
41	G-rod	Gray	-	-	Acid	+	-	+
42	G-rod	Gray	-	-	Acid	-	-	+
43	G-rod	Gray-white	Complete 2	+	Alk.	-	Complete 4	-
44	G-rod	Gray-white	-	+	Acid	-	-	+
45	G-rod	Gray-white	-	+	Acid	-	-	+
46	G-rod	Gray	Complete 4	+	Alk.	-	Complete 4	-
47	G-rod	Gray	Complete 3	+	Alk.	-	Complete 4	-
48	G-rod	Yellow	Complete 21	+	Neut.	-	-	-
49	G-rod	Gray	Complete 5	+	Alk.	-	Complete 4	-
50	G-rod	Gray	Complete 21	+	Alk.	-	Complete 3	-
51	G-coccus	Gray	-	-	Alk.	-	Complete 10	-
52	G-coccus	Gray	-	-	Alk.	-	Complete 10	-
53	G-rod	Gray	Complete 2	+	Alk.	-	Complete 4	-
54	G-rod	Gray	Complete 4	-	Alk.	-	Complete 4	-
55	G-rod	Gray-white	Complete 2	+	Alk.	-	Complete 4	-

(Continued)

dn.	Ferm. of Glucose	Ferm. of Lactose	Growth on P.C.A. at 5°C.	Growth on P.C.A. at 23°C.	Growth on P.C.A. at 35°C.	Nitrate redn.	Indole prod.	Lipase prod. 122 hr.	Genus
-	A	-	+	+	+	-	-	++	Pseudomonas
-	A	-	+	+	+	-	-	++	Pseudomonas
-	A	-	+	+	+	+	-	++	Pseudomonas
-	A	-	+	+	+	+	-	++	Pseudomonas
-	-	-	+	+	-	-	-	+	Achromobacter
-	-	-	+	+	-	-	-	+	Achromobacter
-	A	-	+	+	+	-	-	-	Pseudomonas
-	-	-	+	+	-	-	-	++	Achromobacter
-	A	-	+	+	+	+	-	+++	Pseudomonas
-	A	-	+	+	+	+	-	+++	Pseudomonas
-	A	-	+	+	+	-	-	++	Pseudomonas
-	A	-	+	+	+	-	-	++	Pseudomonas
-	A	-	+	+	-	-	-	+	Flavobacterium
-	A	-	+	+	+	-	-	+	Pseudomonas
-	A	-	+	+	-	-	-	++	Pseudomonas
+	A	A	+	+	+	-	-	-	Achromobacter
+	A	A	+	+	+	-	-	-	Achromobacter
-	A	-	+	+	+	-	-	-	Pseudomonas
+	AG	AG	+	+	+	+	-	-	Escherichia
+	AG	AG	+	+	+	+	-	-	Aerobacter
-	A	-	+	+	+	-	-	+++	Pseudomonas
-	A	-	+	+	+	-	-	+++	Pseudomonas
-	-	-	+	+	-	-	-	-	Flavobacterium
-	A	-	+	+	-	+	-	++	Pseudomonas
-	A	-	+	+	-	+	-	++	Pseudomonas
-	-	-	+	+	-	-	-	++	Alcaligenes
-	-	-	+	+	-	-	-	++	Alcaligenes
-	A	-	+	+	+	-	-	+++	Pseudomonas
-	A	-	+	+	+	-	-	++	Pseudomonas
-	A	-	+	+	+	-	-	+++	Pseudomonas

Table 12. (Continued)

Number	Cell description	Chromogenesis on P.C.A.	Gelatin liquefaction (days)	Motility	Litmus milk				F
					Reaction	Coag.	Proteolysis (days)	Redn.	Glu
56	G-rod	Cream gray	Complete 2	+	Alk.	-	Complete 4	-	A
57	G-rod	Yellow	Complete 21	+	Acid	+	-	+	AG
58	G-rod	Gray	-	+	Alk.	-	Complete 4	-	A
59	G-rod	Gray	Complete 2	+	Alk.	-	Complete 4	-	A
60	G-rod	Cream gray	Complete 3	+	Alk.	-	Complete 3	-	A
61	G-rod	Cream gray	Complete 3	+	Acid	+	-	+	AG
62	G-rod	Gray	-	-	Neut.	-	-	-	-
63	G-rod	Gray-white	1/2 in 9	+	Alk.	-	-	-	A
64	G-rod	Gray	Complete 4	+	Alk.	-	Complete 4	-	A
65	G-rod	Cream gray	Complete 4	+	Alk.	-	Complete 4	-	A
66	G-rod	Gray cream	Complete 3	+	Alk.	-	Complete 4	-	A
67	G-rod	Gray cream	Complete 3	+	Alk.	-	Complete 3	-	A
68	G-rod	Gray cream	-	+	Alk.	-	-	-	A
69	G-rod	Gray	Complete 3	+	Alk.	-	Complete 7	-	A
70	G-rod	Gray	Complete 8	+	Neut.	-	-	+	A
71	G-coccus	Gray	-	-	Alk.	-	-	-	-
72	G-coccus	Yellow	1/2 in 10	+	Acid	-	-	+	A
73	G-coccus	Gray-white	-	-	Alk.	-	-	-	-
74	G-rod	Gray	Complete 5	+	Neut.	-	-	-	A
75	G-rod	Gray-white	-	+	Neut.	-	-	-	A
76	G-rod	Yellow	-	-	Neut.	-	-	-	A
77	G-rod	Gray-white	1/2 in 10	+	Alk.	+	-	-	A
78	G-rod	Gray	-	-	Alk.	-	-	-	-
79	G-rod	Gray	Complete 6	+	Acid	+	-	+	A

	Ferm. of		Growth on P.C.A. at			Nitrate	Indole	Lipase	Genus
1.	Glucose	Lactose	5°C.	23°C.	35°C.	redn.	prod.	prod. 122 hr.	
	A	-	+	+	+	+	-	+++	Pseudomonas
	AG	A	+	+	+	+	-	-	Flavobacterium
	A	-	+	+	-	+	-	+	Pseudomonas
	A	-	+	+	+	-	-	+	Pseudomonas
	A	-	+	+	+	-	-	++	Pseudomonas
	AG	AG	+	+	+	+	-	++	Aerobacter
	-	-	+	+	-	-	-	++	Achromobacter
	A	-	+	+	-	-	-	+	Pseudomonas
	A	-	+	+	+	-	-	+++	Pseudomonas
	A	-	+	+	+	-	-	++	Pseudomonas
	A	-	+	+	+	-	-	+	Pseudomonas
	A	-	+	+	+	-	-	++	Pseudomonas
	A	-	+	+	-	+	-	-	Pseudomonas
	A	-	+	+	+	-	-	++	Pseudomonas
	A	-	+	+	+	-	-	-	Pseudomonas
	-	-	+	+	+	-	-	++	Alcaligenes
	A	A	+	+	+	+	-	-	Flavobacterium
	-	-	+	+	-	+	-	+	Alcaligenes
	A	-	+	+	-	+	-	++	Pseudomonas
	A	-	+	+	-	+	-	-	Pseudomonas
	A	-	+	+	-	-	-	-	Flavobacterium
	A	-	+	+	-	-	-	-	Pseudomonas
	-	-	+	+	+	-	-	++	Alcaligenes
	A	-	+	+	-	+	-	++	Pseudomonas

Proskauer negative.

The isolates included 51 Pseudomonas, 9 Achromobacter, 8 Alcaligenes, 7 Flavobacterium, 3 Aerobacter and 1 Escherichia species.

Twenty-eight of the 79 isolates, or 35.5 percent of the total, failed to grow at 35° C. These included 6 of 9 Achromobacter, 14 of 51 Pseudomonas, 4 of 7 Flavobacterium and 3 of 8 Alcaligenes species. All others were able to show visible growth on Plate Count Agar slants within 48 hours.

Fifty-six of the 79 isolates, or 70.9 percent of the total, showed evidence of lipase production. These included 42 Pseudomonas, 8 Alcaligenes, 4 Achromobacter, 1 Flavobacterium and 1 Aerobacter.

Reduction of Resazurin and 2,3,5-Triphenyltetrazolium Chloride by Psychrophilic Cultures

Colonies picked from plates incubated for 7 days at 5° C. were used to study the reducing ability of pure cultures of psychrophilic organisms. One drop of a 20 hour broth culture incubated at 23° C. was added to 4 ounces of raw milk of known low bacterial content. The inoculated bottles were incubated in 5° C. for 72 hours. After incubation, direct microscopic counts and resazurin and 2,3,5-triphenyltetrazolium chloride reduction tests were

made on the milk.

Table 13 summarizes the results of the reduction of the two dyes by 31 psychrophilic cultures. The cultures differed in growth rate in milk at 5° C. However, all but five cultures had plate counts in excess of 1,000,000 per ml. at the time the reduction tests were performed. Culture number 44, one of five cultures with a plate count of less than 1,000,000 per ml., was able to reduce the resazurin to Munsell P 7/4 in 2.75 hours, placing the samples in resazurin class 2. The plate count of 700,000 per ml. was in the bacterial estimation range of resazurin class 2. The other four cultures giving limited growth caused no reduction.

The 26 cultures with plate counts exceeding 1,000,000 per ml. varied in their ability to reduce resazurin. Eleven of these cultures were placed in resazurin class 1 and 15 in class 2. All of these cultures placed in class 1 had counts ranging from 1,500,000 to 42,000,000 per ml. These counts were considerably in excess of the expected count estimation, based on the results of the resazurin test. The cultures placed in class 2 had counts ranging from 6,300,000 to 100,000,000 per ml. These counts also are in excess of what would be expected from the results of the resazurin test.

Some of these psychrophilic cultures apparently are

Table 13. Reduction of resazurin and 2,3,5-triphenyltetrazolium chloride by several psychrophilic cultures grown in raw milk

Culture number and genus	Plate count after 3 days at 5°C.[a]	Resazurin class		Hours to reduce TTC[b]	
		35.5°C.	28°C.	35.5°C.	28°C.
Control	4 T[c]	1	1	>12	>12
2 *Pseudomonas*	40 M[d]	2	1		2.5
5 *Pseudomonas*	21 M	2	1	2.0	3.0
8 *Achromobacter*	67 M	2	1		2.5
10 *Flavobacterium*	1.5 M	1	1		6.0
12 *Achromobacter*	320 T	1	1	>10	>10
15 *Pseudomonas*	34 M	2	1	1.0	3.0
17 *Alcaligenes*	250 T	1	1	4.0	6.0
19 *Alcaligenes*	300 T	1	1		6.0
21 *Aerobacter*	27 M	2	1		3.5
23 *Pseudomonas*	25 M	1	1	2.0	4.0
29 *Pseudomonas*	60 M	2	1	1.0	3.0

[a]One drop of a 20 hour broth culture in 4 ounces of raw milk before incubation. Plates held at 23° C. for 3 days.

[b]Hours to reduce 2,3,5-triphenyltetrazolium chloride (TTC) to slight pink.

[c]T = Thousand.

[d]M = Million.

(continued)

Table 13. (Continued)

Culture number and genus	Plate count after 3 days at 5°C.		Resazurin class		Hours to reduce TTC	
			35.5°C.	28°C.	35.5°C.	28°C.
31 Achromobacter	6.8	M	1	1	8.0	7.0
33 Achromobacter	12	M	1	1	4.0	4.0
36 Pseudomonas	16	M	2	1		2.5
38 Flavobacterium	7.8	M	1	1	2.0	4.0
40 Pseudomonas	8.3	M	1	1	7.0	5.5
42 Achromobacter	560	T	1	1	7.0	>8
44 Escherichia	700	T	2	1		6.0
46 Pseudomonas	16	M	1	1	2.0	4.0
48 Flavobacterium	24	M	1	1		13.5
49 Pseudomonas	65	M	2	1	1.0	3.0
52 Alcaligenes	14	M	2	1	2.0	4.0
54 Pseudomonas	26	M	2	1		2.5
56 Pseudomonas	100	M	2	1	2.0	2.0
58 Pseudomonas	19	M	2	1	3.5	5.5
61 Aerobacter	6.3	M	2	1	2.0	4.0
62 Achromobacter	8	M	1	1	3.5	4.0
63 Pseudomonas	42	M	1	1	2.0	2.5
67 Pseudomonas	39	M	1	1	2.0	2.5
69 Pseudomonas	60	M	2	1		2.5
75 Pseudomonas	75	M	2	1	2.0	2.0

able to reduce resazurin but are limited as to the rapidity of their action. Only the Escherichia culture was able to reduce the resazurin fast enough to place it in the resazurin class with the presently accepted corresponding bacterial count. No obvious pattern as to the ability of organisms of any particular genus to have more or less resazurin reducing ability than another is apparent. Cultures 8, 10, 23, 31, 33, 38, 40, 48, 49, 52, 58, 62, 63, and 75 did not show visible growth on Plate Count Agar slants at 35° C. However, cultures 8, 49, 52, 58, and 75 showed some evidence of reducing ability even at a temperature at which they apparently were unable to grow. The remaining cultures that did not grow at 35° C. gave no evidence of resazurin reducing ability, even though the plate counts were all in excess of 1,500,000 per ml. at the time the reduction tests were performed.

Methylene blue test results were compared with the resazurin test results on 11 of the 31 psychrophilic cultures. The 11 cultures tested by both methods were placed in the same bacterial estimation class. Five were placed in class 1 and six in class 2.

The time of reduction of 2,3,5-triphenyltetrazolium chloride by these psychrophilic cultures also is noted in Table 13. The 35.5° C. incubation temperature appears to give more rapid reduction by most psychrophilic cultures

than does incubation at 28° C. Those that were tested of the 14 cultures unable to form visible colonies when streaked on Plate Count Agar slants incubated at the higher temperature were able to reduce 2,3,5-triphenyltetrazolium chloride at 35.5° C.

Direct Microscopic Counts on Psychrophilic Cultures Grown in Raw Milk

The microflora of bulk-tank milk may, in many instances, be made up to a large extent of psychrophilic bacteria. Since the direct microscopic count is used extensively to assess the bacteriological quality of milk, the staining ability and cell distribution of various psychrophilic organisms were studied. Table 14 presents the results of the direct microscopic count on 31 psychrophilic cultures grown in low count raw milk at 5° C. for 72 hours.

All psychrophilic cultures grown in raw milk had higher plate count results than direct microscopic counts. Cultures with a wide difference between the results of the two tests tended to grow in clumps, except culture number 48. This culture did not stain as readily as the other cultures and it was difficult to see in the stained preparations. All other cultures appeared to stain well enough for relatively easy observation of the cells.

Number 46 is an example of a culture in which there

Table 14. Microscopic counts on several psychrophilic cultures grown in raw milk

Culture number and genus	Plate count after 3 days at 5° C.[a]		Direct microscopic clump count[b]		Remarks[c]
Control	4	T[d]	40	T	
2 Pseudomonas	40	M[e]	16.0	M	Clumps
5 Pseudomonas	21	M	5.0	M	Clumps
8 Achromobacter	67	M	54	M	Pairs
10 Flavobacterium	1.5	M	440	T	Small clumps
12 Achromobacter	320	T	60	T	
15 Pseudomonas	34	M	10	M	Clumps
17 Alcaligenes	250	T	200	T	

[a]One drop of a 20 hour broth culture in 4 ounces of raw milk before incubation. Plates held at 23° C. for 3 days.

[b]Levowitz-Weber staining technique, clumps counted.

[c]Predominate cell distribution.

[d]T = Thousand.

[e]M = Million.

(Continued)

Table 14. (Continued)

Culture number and genus	Plate count after 3 days at 5°C.	Direct microscopic clump count	Remarks
19 Alcaligenes	300 T	220 M	Pairs
21 Aerobacter	27 M	16.0 M	Pairs and singles
23 Pseudomonas	25 M	24.0 M	Pairs and singles
29 Pseudomonas	60 M	12.0 M	Pairs and clumps
31 Achromobacter	6.8 M	1.6 M	Clumps and pairs
33 Achromobacter	12 M	8.9 M	Pairs and singles
36 Pseudomonas	16 M	2.6 M	Clumps
38 Flavobacterium	7.8 M	4.4 M	Small clumps
40 Pseudomonas	8.3 M	780 T	Clumps
42 Achromobacter	560 T	260 T	Small clumps
44 Escherichia	700 T	420 T	Small clumps
46 Pseudomonas	16 M	3.0 M	Clumps
48 Flavobacterium	24 M	11.0 M	Stained lightly
49 Pseudomonas	65 M	16.0 M	Clumps
52 Alcaligenes	14 M	240 T	Clumps
54 Pseudomonas	26 M	4.4 M	Clumps

(Continued)

Table 14. (Continued)

Culture number and genus	Plate count after 3 days at 5°C.	Direct microscopic clump count	Remarks
56 Pseudomonas	100 M	23.0 M	Pairs, individuals, clumps
58 Pseudomonas	19 M	4.0 M	Clumps
61 Aerobacter	6.3 M	1.6 M	Clumps
62 Achromobacter	8 M	1.1 M	Clumps
63 Pseudomonas	42 M	25.0 M	Pairs and individuals
67 Pseudomonas	39 M	6.7 M	Small clumps
69 Pseudomonas	60 M	13.0 M	Clumps
75 Pseudomonas	75 M	72.0 M	Pairs and individuals

was a wide difference between the plate count and the direct microscopic count. When the milk film was stained and observed, the bacteria appeared to grow predominately in clumps. Since the direct microscopic count is actually a clump count, each clump is counted as one. When the milk sample is plated out, the necessary dilutions of a sample and the subsequent shaking of these dilutions prior to further plating would tend to break up a bacterial clump into more than one clump or source. As a result, the organisms in a clump that would be counted as one with the direct microscopic count, produce more than one countable colony. The plate count would then be greater than the direct microscopic count.

Cultures that did not grow in clumps but rather were observed in pairs or as single cells had direct microscopic counts that were within the approximate range of the plate counts. Culture number 23 is an example of this situation. The plate count was 25,000,000 per ml. and the direct microscopic count was 24,000,000 per ml. The bacterial cells were observed to be either in pairs or single cells in the stained smear.

DISCUSSION

Comparison between various methods for determining "total" bacterial populations are hazardous because the different methods estimate populations by using different criteria of microbial activity. The plate count enumerates those individual cells or aggregates of cells which are able to produce a countable colony under the conditions used for making that particular count. Medium composition and temperature of incubation are two of many variables which may influence the results. The direct microscopic clump count is an enumeration of those single cells or aggregates of cells which take up enough stain to be differentiable from the background material. The type of stain used, the ability of the cell to take up the specific stain, and the extent to which the cells grow in aggregates and the aggregates can be broken up during preparation of the smear are among the factors influencing the direct microscopic count. The dye reduction tests measure the ability of a population to change a dye from the oxidized form to the reduced form with a resultant change in color which may be detected by the person making the test. Dyes vary in the oxidation-reduction potential at which color change occurs and this characteristic determines the extent to which the system must be reduced before the dye changes color. Dif-

ferent bacteria have various abilities to bring about reduction, so the same level of reduction may be the result of widely different populations. Also, the metabolic activity of an organism, as influenced by factors such as temperature and growth phase, has a considerable effect upon reducing ability. Additional factors such as light, amount of air incorporated in the medium and extent to which organisms are concentrated in the cream layer have considerable effect on rate of dye reduction.

Since each procedure for population estimation may be influenced considerably by so many different factors, interpretation of one test in terms of other tests is subject to many hazards. Because one must have a point of departure, the standards for manufacturing-grade milk as employed by the Agricultural Marketing Service of the United States Department of Agriculture (2) have been employed in this study. This choice was based upon relatively widespread usage, rather than upon any information that these relationships were more accurate or useful than others might be. Obviously relationships which were established between tests when milk was handled in cans and frequently not well cooled would not necessarily apply when milk was handled in bulk tanks and cooled to temperatures 10 to 20° F. below those used for milk in cans. To mention only one factor, the types of organisms developing might be distinctly different.

The standard plate count with incubation at 32° C. for 48 hours was chosen as the basis of comparison for the other procedures for determining "total" population in the samples of bulk-tank milk, as well as cultures of psychrophilic bacteria. In no instance did the direct microscopic count or any of the reduction tests run at 35.5° C. indicate a lower quality of the milk than was shown by the plate count. Had an incubation temperature of 35° C. been used for the plate count, the comparative results might have been somewhat different; however, specific data on the relative value of the two incubation temperatures were not obtained. The relatively high proportion of bacteria which gave countable colonies after 7 days at 5° C. found in these samples and the inability of many of the isolates from such plates to grow at 35° C. when inoculated onto agar slants would indicate many of them would not have grown had plates for the standard plate count been incubated at 35° C. Whether any other modifications of the plate count procedure would have given higher counts can not be answered from the results of these studies, because of variables of this type were not examined.

In the present studies, the standard plate count with incubation at 32° C. was shown to classify more samples in a poorer group bacteriologically than did either the resazurin reduction test (Table 10) or the direct microscopic count (Table 11). In many instances where agreement

in results was not obtained, psychrophilic bacteria made up a large proportion of the total population. In other instances the thermoduric population was much higher than was desirable. Both psychrophilic and thermoduric organisms are generally poor reducers of either resazurin or methylene blue.

The ability of psychrophilic cultures to reduce the resazurin and methylene blue dyes was studied (Table 13). All but one culture reduced the resazurin dye at a slower rate than would be expected from the results of the standard plate count. Morris (64), Morris and Edwards (65), Procter (73), and McKenzie and Morrison (60) reported that most thermoduric organisms present in raw milk are types which reduce resazurin very slowly. Reducing ability of this type of organism was not investigated in the present study.

Many dairy plant managers in the United States, including Iowa, rely upon the resazurin or methylene blue dye reduction tests to give them an indication of the quality of the milk bought from their producers. However, the results indicate that these tests are unreliable in detecting the bacterial content of the milk when judged against the results of the standard plate count as applied to manufacturing-grade milk handled in bulk tanks on the farm.

The methylene blue and resazurin tests were compared on a number of bulk milk samples. In many of the higher

count milk samples the resazurin test placed the samples in a lower bacteriological classification than did the methylene blue test. On the higher quality samples both tests were similar in rating the samples. The resazurin test appears to be, to some extent, more sensitive to higher bacterial population of the type encountered in this study in milk than is the methylene blue test.

When milk is not properly cooled the reduction test easily picks out low quality samples because of the type of bacteria that predominate under these conditions. Jones and Davis (45) indicated that bacteria capable of souring milk will reduce resazurin rapidly. When proper cooling of milk was a problem in milk production, Johns and Howson (43), Golding and Jorgensen (31), Keenan et al. (46) and Frayer (30) found good agreement between the standard plate method and the resazurin test. When proper cooling of milk was assured by mechanical means, workers such as Watt (101) and Smillie as cited by Thomas (85) declared that the resazurin test was inadequate as a basic raw milk test.

Manufacturing-grade bulk-tank milk that has been properly cooled but produced and handled in unclean and poorly sanitized equipment may have a high bacterial count, predominantly of psychrophilic types. Because the bulk tank is kept at refrigeration temperature most of the time, the bacteria in that environment would be expected to be

predominantly psychrophilic. The time between emptying and refilling would not be a long enough interval at a higher temperature that one would expect the character of the microflora to change to any great extent, even when washing had been delayed or had been inadequate. With the factors of time and warmer atmospheric temperatures the psychrophilic organisms might increase in numbers very rapidly in the unwashed or poorly washed tank. In spite of the low holding temperatures of the milk subsequently placed in the bulk tank, these residual organisms would slowly increase in numbers and make up a large share of the microflora of the milk.

When the producer fails to clean and sanitize his milking equipment which is held at milkhouse temperatures between uses, there is an opportunity for gram-positive thermoduric organisms to grow on the equipment, especially during the warmer months. This equipment would become a prime source for contaminating the milk. If there was the combination of poor care for the bulk tank and of the milking and milk handling equipment, the microflora of the milk might include large numbers of both psychrophilic and thermoduric bacteria.

The low level of reducing ability of both thermoduric and psychrophilic bacteria probably explains much of the lack of agreement between the results of the resazurin test

and the standard plate count. This can lead to erroneous conclusions concerning the bacteriological quality of milk samples being tested. When the results of the resazurin test and standard plate count do not agree on raw milk, psychrophilic, thermoduric or a combination of both types of bacteria probably constitute a high proportion of the microflora of the milk.

Johns (39) has indicated that resazurin reduction is slowed down by refrigerated storage of the milk samples overnight before testing. However, resazurin tests were run on 17 milk samples both before and after overnight refrigerated storage with no difference in ratings of the milk samples.

The variable results noted by Johns (39) about overnight refrigerated storage of milk undoubtedly reflect differences in the previous histories of the milk. Milk which has been cooled and held at temperatures below 5° C. for 48 to 72 hours in bulk tanks and maintained at this temperature after sampling for a period not to exceed 24 hours should not show an improvement in resazurin classification. However, when milk has not been well cooled and microflora of the milk is predominantly bacteria which reduce resazurin rapidly, then overnight storage before resazurin testing could improve the resazurin rating by decreasing the metabolic activity of the non-psychrophilic types present

in the milk. Revallier-Warffemius (76) also noted a slowing down of resazurin reduction in milk refrigerated overnight, but he attributed this loss of reducing power to the leucocytes in storage over 28 hours, rather than to bacteria. However, with the advent of handling milk in bulk tanks, the age of much of the milk before bacterial examination would be considerably in excess of 28 hours. The leucocytes might thus be too old to actively reduce resazurin.

The direct microscopic count is widely used in evaluating the bacteriological quality of raw milk, as well as pasteurized and non-fat dry milk. Over 42 percent of the samples tested had standard plate counts that were at least twice as high as the microscopic count (Table 11). The majority of the samples lacking agreement between the two tests had a high psychrophilic plate count or a high thermoduric plate count, or both counts high.

Since over 55 percent of the samples in which the results of the microscopic and standard plate count failed to agree had high psychrophilic counts, the staining ability of psychrophilic isolates was studied (Table 14). Only one of 31 isolates grown in raw milk for three days at 5° C. did not stain readily. All other cultures appeared to take up the Levowitz-Weber stain so that the bacterial cells were easily seen. The primary factor responsible for the

wide discrepancies between the two tests appears to be the tendency for many of the psychrophilic organisms to grow in clumps. Since the microscopic count is actually a clump count, each clump is counted as one. When dilutions are made for the standard plate count, many of these bacterial clumps may be broken up and result in more than one countable colony. The plate count result would be greater than the result of the microscopic count. A few psychrophilic cultures did not grow in clumps but appeared under the microscope as individuals and pairs. This type of culture had microscopic counts that were comparable to the standard plate count. The comparability of these two tests when the milk contained a large proportion of psychrophilic bacteria would depend on whether the majority of the psychrophilic bacteria grew in clumps or existed more as individual cells and possibly pairs.

Over 41 percent of the samples that were not properly classified by the direct microscopic count as judged by the standard plate count, had thermoduric plate counts in excess of 30,000 per ml. (Table 11). Although the staining ability of thermoduric isolates was not studied, it would appear that many thermoduric organisms do not readily stain and are thus poorly defined for clear viewing under the microscope. Little (54) found that the microscopic count was not accurate in determining the bacteriological quality of

raw milk when the samples contained large numbers of thermoduric bacteria.

It would appear that the value of the direct microscopic count is very limited for estimating the bacterial population of raw milk, particularly manufacturing-grade bulk-tank milk where the predominate microflora could well be made up of large numbers of psychrophilic and/or thermoduric organisms.

The 2,3,5-triphenyltetrazolium chloride reduction test was made on a limited number of samples. The results, as shown in Figures 1, 2, 3, and 4, indicated that some correlation existed between this test and the standard plate count. However, the 95 percent confidence interval had a range of approximately 1.6 logarithmic units. This is an excessively wide bacterial range estimate that would not be narrow enough to be of practical value in accurately estimating the bacterial population of raw milk.

This test might be of value as a screening test for manufacturing-grade bulk-tank milk in which there are, in many cases, a large proportion of high count samples that may not be detected by the resazurin or methylene blue reduction tests. The tetrazolium test can be run with the same equipment, except for the test solution, presently used by the creameries for the resazurin and methylene blue tests. Incubating in a light-proof container could be carried out in a warm room, since there was a high

correlation even at 28° C. between standard plate count and reduction time.

Psychrophilic isolates were tested to determine their ability to reduce the tetrazolium compound (Table 13). All were able to reduce the tetrazolium chloride, but there was some variation in rate of reduction.

There appears to be no obvious difference between the genera isolated in their ability to reduce tetrazolium chloride. Time of reduction was shortened by use of the 35.5° C. incubation temperature rather than 28° C. Liska and Calbert (53) feel that the lactic dehydrogenase enzyme and its coenzyme system are involved in tetrazolium reduction. Neal and Calbert (67) have indicated that the compound is reduced by the growing cells. However, even at temperatures that prohibit or limit the growth of many psychrophilic cultures the reduction of tetrazolium chloride took place. The 35.5° C. temperature may have accelerated enzyme activity to a greater extent than incubation at 28° C.

Modification of the resazurin test was attempted using an incubation temperature of 28° C. This did not accelerate the dye reduction and in several cases slowed the rate.

A numerical score was placed on several milk producers' production and handling facilities. The producer results of one creamery were such that a statistical correlation would be made between the individual producer's average standard plate count and his facility score. It would

appear that such a score sheet (Appendix) actually is of little value in predicting the quality of a producers bulk-tank milk. Possibly another type of score sheet, which might include other items or a different emphasis on the various items, may be of more value in estimating the milk quality.

A few of the samples which had standard plate counts in the range for grade A milk were produced under conditions approximating those of a grade A installation, but most were produced where conditions did not fulfill these standards. However, several instances of high count milk were detected where milk production and handling conditions would approximate the grade A requirements. A number of producers carried warm water to their milkhouses to wash the bulk tank. They washed and stored the other milking equipment in the house. Several of these producers marketed higher quality milk than farmers with facilities approximating those of grade A requirements. Apparently the bacteria content of the milk can not be estimated by the quality of the producer's milk production and handling conditions.

Statistical analysis of variance of individual producers facility scores and standard plate count results indicated that when producers were grouped according to creamery to which they sold milk there was considerable variation between the creameries. This indicated an actual

difference between producers' facilities and milk quality of the four creameries. Several factors may account for the difference.

One factor is the selection of producers and the pre-installation requirements for these producers in milking area and milkhouse facilities. It was observed that creameries A, B and D were not as discriminating as was creamery C in selection of producers for conversion to a bulk-tank operation. Creamery C normally required that improvements in the milking area, milkhouse and driveway be made before the bulk tank was installed. Twenty-one of the 46 producers of creamery C were able to convert to grade A milk production when a market was available for this milk. Only a small percentage of producers of the other three creameries were in a position to convert to grade A production when there was an opportunity. Most manufacturing-grade installations were such that the capital necessary for conversion to grade A was too great for the producer to assume willingly.

The number of producer contacts as well as the nature of these contacts by the creamery field men also could be a factor in quality milk production and influence the apparent difference between producers of the creameries. In some cases the field men were involved primarily with contacting prospective bulk milk producers, rather than visiting

and advising their present producers. Lack of knowledge concerning factors which influence quality of bulk-tank milk also was evident with some field men. They were willing, but in some cases they did not have the knowledge to render the necessary advice and help to the producer.

Another factor that could affect the quality of manufacturing-grade milk and influence to some extent the difference in general milk quality between creameries was the interest of the bulk-tank truck driver in the quality of the milk produced by his patrons. Initial training of the tank-truck driver played a part in this interest and knowledge. In most instances the truck drivers received very little instruction concerning the rejection of off-flavored milk, collecting and storing milk samples, and cleaning and sanitizing milk handling equipment.

When a creamery allows producers of poor quality milk to continue to sell to the creamery and does not require an improvement in the quality, the efforts of other producers to maintain a quality milk production program on their farms are nullified. One producer, even though contributing a small percentage of the total pounds of milk collected on an individual bulk-tank route, may adversely affect the bacteriological quality of the entire tank truck of milk.

The individual producer's desire, interest and know-

ledge are big factors in quality milk production. These cannot be measured by a milk facilities rating sheet. Even though a producer has all the facilities available that are conducive to quality production, it appears to be no indication that the milk will be of high bacteriological quality. However, producers with limited facilities but with desire to do a good job are able, in many cases, to produce lower count milk than producers with all the equipment normally thought necessary for production of high quality milk but who have little interest and/or knowledge of milk production.

Undoubtedly the season of the year has some effect on the quality of the milk produced. The actual amount of this effect also cannot be specifically measured, but observations in general can be made. During the summer months, atmospheric temperatures are within a range which favors growth of gram-positive bacteria that are responsible for souring uncooled milk. The equipment used in production and handling of milk on the farm may not be properly cleaned and sanitized, as of course could be the case in any period of the year. The milk and soil remaining on this equipment would allow, along with warm summer temperatures, rapid growth of the mesophilic gram-positive bacteria. The summer temperatures are in the growth range of psychrophilic bacteria, but the gram-positive bacteria undoubtedly grow faster at these temperatures. Thus the

relatively smaller number of milk samples with high psychrophilic plate counts in summer could be due to the competitive disadvantage of these organisms under summer contitions (Table 3).

The fall period had the highest percentage of samples with high thermoduric counts (Table 4). No obvious explanation can be offered, unless the fall harvest work would cut into the time normally spent on milking utensil sanitation or the arrival of cooler weather led to some relaxing of sanitation efforts.

Winter months samples were the highest quality from the standpoint of the standard and thermoduric plate counts. A large portion of the psychrophilic plate counts were also low. The cold weather undoubtedly played a part in minimizing bacterial growth on poorly cleaned and sanitized milk producing and handling equipment.

The spring period had the highest percentage of samples of any period with standard and psychrophilic plate counts exceeding 1,000,000 per ml. This situation may be the result of the decreased amount of time spent by the producer in properly cleaning and sanitizing the milk handling equipment because of spring field work and to the general failure to adapt from winter sanitation standards conditioned by low temperatures to the summer sanitation standards which are necessary to minimize growth of contaminants. The

thermoduric plate counts were fairly low in spite of this situation.

A few excessively high plate count results in all seasons of the year can be attributed to failure, advertent or inadvertent, on the part of the producer, to turn on the bulk-tank compressor, resulting in the milk not being properly cooled. In a few cases the blend temperature of the milk in the bulk tank was near 10° C. but the compressor switch had been shut off. The producer had initially cooled the milk to 10° C. and felt a savings could be made by switching off the compressor. A peculiar off-flavor, resembling slight rancidity, was noted in several of these instances. Also a few cases were noted when the thermometer indicated a temperature below 4.5° C. in the tank, but when the agitator was turned on the blend temperature rose to 6-8° C. Before agitation the temperature of the top layer of milk registered approximately 10° C. A layering effect of the milk prevented automatic agitation because the activating switch for the agitator was located near the same area as the thermometer which actually indicated a cool temperature.

The relatively low quality of a considerable portion of the milk samples examined indicates that a bulk tank is far from being the complete answer to milk quality. Even when the tank is operated in such a manner as to cool the

milk very adequately, high counts are encountered too commonly. Proper sanitation must be employed, along with proper cooling, if milk of good bacteriological quality is to be marketed. In addition, management to minimize non-bacterial flavor defects, to keep out extraneous matter and to handle other facets of quality milk production is essential.

SUMMARY AND CONCLUSIONS

Seven hundred and one bulk-tank milk samples of manufacturing-grade were obtained from 267 producers of four different creameries. Standard plate counts (incubation at 32° C.), plate counts of thermoduric organisms, psychrophilic plate counts (incubation at 5° C. for 7 days) and flavor scores were determined on each sample. Direct microscopic clump counts and resazurin reduction times (to the P 7/4 endpoint) were determined on many of the samples and methylene blue reduction tests on a number of the samples. Several possible modifications of the dye reduction tests were evaluated. A numerical score was placed on the production and handling facilities of 172 producers. Seventy-nine isolations were made of typical organisms appearing on the psychrophilic plates. These organisms were identified and typical isolates were studied for their reactions in reduction tests and staining procedures.

The standard plate count was chosen as the basis for comparison for all the other procedures employed. This test frequently detected samples of lower quality that were not detected by other tests and in no case failed to pick out samples which had been of low quality on the basis of other tests. Although no detailed comparisons were made, other

information, such as the inability of many of the isolates from psychrophilic plates to grow at 35.5° C., indicated that plate counts with incubation at 35° C. would have compared less favorably.

The standard plate counts of 37.7 percent of the samples of manufacturing-grade bulk-tank milk exceeded 1,000,000 per ml., as compared to 26.5 percent at this level for the psychrophilic plate count results. Over 43 percent of the samples had thermoduric plate counts that exceeded 10,000 per ml. Fifty-five samples had detectable off-flavors that could be directly attributed to high bacterial counts.

Evaluation of the samples by the direct microscopic count failed to detect nearly 42 percent of the samples with high plate counts, probably because of the presence in the high count milk of many psychrophilic and/or thermoduric organisms. Most psychrophilic isolates stained readily but many appeared to grow in clumps. The clumps probably broke up to some extent in the standard plate count procedure, resulting in considerably higher counts by this latter test. Thermoduric organisms apparently did not readily take up the stain, although isolates were not specifically tested.

The standards for manufacturing-grade milk employed by the Agricultural Marketing Service of the United States

Department of Agriculture (2) were employed in this study to compare the results of the reduction tests with the results of the standard plate count. If the results of the standard plate count were at least twice as high as the results indicated by the resazurin test, the two tests were considered to disagree. The resazurin reduction test failed to properly evaluate 33 percent of 670 samples tested. Forty-three percent of the samples in which the results of the two tests did not agree had thermoduric counts exceeding 30,000 per ml. Over 58 percent of the samples, where the standard plate count and the resazurin reduction test did not agree, had psychrophilic plate counts that were 75 percent of or higher than the standard plate count. Fourteen percent of the samples in which the results of the two tests did not agree had both a high psychrophilic and a high thermoduric count. The poor reducing ability of both types of organisms probably accounts for the discrepancies between the resazurin reduction results and those of the standard plate count.

The methylene blue reduction test and the resazurin reduction test were compared on 77 samples. The resazurin test apparently, to some extent, was the more sensitive to higher bacterial populations in milk of the type examined in this study.

Incubating the reduction tests at 28° C. rather than

35.5° C. failed to improve their relationship with the standard plate count.

A high correlation was found between the results of the standard plate count on a limited number of samples and the times required to reduce 2,3,5-triphenyltetrazolium chloride. A regression analysis revealed, however, that the 95 percent confidence interval was too wide to be used for accurately evaluating the bacterial content of the samples. This test probably could be used more successfully than the other reduction tests to screen undergrade milk.

Taxonomic studies of 79 psychrophilic isolates classified them as 51 Pseudomonas, 9 Achromobacter, 8 Alcaligenes, 7 Flavobacterium, 3 Aerobacter and 1 Escherichia species. Nearly three fourths of the cultures produced lipase and over one third failed to grow at 35° C.

A statistical analysis of variance using 172 individual producer's standard plate count results indicated there was significantly more variation within each individual producer's several plate counts than between their average plate count results of the group of producers. However, when producers were grouped by creamery there was significantly more variation in the average plate count results between the four producer groups than within the several plate count results of individual producers associated

with any one creamery. Difference in selection, pre-installation instructions and requirements made of producers and aid from the creamery field men may account for the differences between creameries.

A numerical evaluation of milking facilities was made on 172 farms. The scores could not be correlated with producer's standard plate count results because of the wide variation within plate count results of individual producers. Many producers did not have equipment and facilities of the types usually considered desirable for producing high quality milk. A few producers were able to consistently produce low count milk, although their facilities for production were poor.

Several producers had facilities approximating those of grade A requirements. A few of these producers marketed milk of low bacteriological quality.

The bulk tank is not the answer to all problems with manufacturing-grade milk. Sanitation and carefulness by the producer, in addition to adequate cooling, are necessary to produce milk of high bacteriological quality.

BIBLIOGRAPHY

1. Abd-El-Malek, Y. and Gibson, T. Studies in the bacteriology of milk. IV. The gram-negative rods in milk. J. Dairy Res. 19: 294-301. 1952.

2. Agricultural Marketing Service, Dairy Division. (U.S.) Minimum specifications for approved plants manufacturing, processing and packaging dairy products. Washington, D. C. U. S. Dept. Agr. 1955.

3. American Public Health Association. Standard methods for the examination of dairy products. 10th ed. New York, N. Y. Amer. Public Health Assn. 1953.

4. Anderson, E. B. and Meanwell, L. J. Studies in the bacteriology of low-temperature pasteurization. Part I. The significance of heat-resisting organisms in raw milk supplies. J. Dairy Res. 4: 213-225. 1933.

5. Atherton, H. V. Observations on raw milk quality before and after conversion to bulk tank pick-up at the farm. J. Dairy Sci. 38: 591. 1955.

6. Atherton, H. V. and Bradfield, A. An evaluation of milk quality as influenced by daily vs. every-other-day pickup of milk cooled in farm bulk tanks. J. Milk and Food Technol. 20: 223-229. 1957.

7. Atherton, H. V., Doan, F. J., and Watrous, G. H. Changes in bacterial populations and characteristics of bottled market milk during refrigerated holding. Penn. Agr. Exp. Sta. Bul. 575. 1954.

8. Ayers, S. H. and Clemmer, P. W. The significance of the colon count in raw milk. U. S. Dept. Agr. Bul. 739. 1918.

9. Ayers, S. H., Cook, L. B., and Clemmer, P. W. The four essential factors in the production of milk of low bacterial content. U. S. Dept. Agr. Agric. Bul. 642. 1918.

10. Babel, F. J. Activity of bacteria and enzymes in raw milk held at 4.4° C. J. Dairy Sci. 36: 562. 1953.

11. Barrett, W. D., Rutan, H., and Keenan, J. A. The resazurin test - its use and practicability as applied to the quality control of raw milk. J. Dairy Sci. 20: 705-709. 1937.

12. Boyd, J. C., Smith, C. K., and Trout, G. M. The effect of the incubation time and temperature on the determination of psychrophilic bacteria in milk by the agar plate method. J. Milk and Food Technol. 17: 365-368. 1954.

13. Breed, R. S., Murray, E. G. D., and Smith, N. R. Bergey's manual of determinative bacteriology. 7th ed. Baltimore, Md., The Williams and Wilkins Co. 1957.

14. Broitman, S., Mallmann, W. L., and Trout, G. M. A simple test for detecting keeping quality for milk. J. Milk and Food Technol. 21: 280-284. 1958.

15. Chalmers, C. H. Bacteria in relation to the milk supply. 4th ed. London, England, Edward Arnold, Ltd. 1955.

16. Chandrasekhar, C. V. Unpublished Ph.D. Thesis. U. of Wales. 1947. (Original not available for examination; cited in Thomas, S. B. Psychrophilic micro-organisms in milk and dairy products. Dairy Sci. Abst. 20: 355-370, 447-468. 1958.)

17. Chandrasekhar, C. V. and Walker, N. Preliminary observations on various temperature characteristics of some facultative psychrophilic bacteria. Proc. Soc. App. Bact. 1: 24-27. 1947.

18. Conn, H. W. and Esten, W. M. The effect of different temperatures in determining the species of bacteria which grow in milk. Conn. (Storrs) Agr. Exp. Sta. Ann. Report. 16: 27-88. 1904.

19. Csenge, J. L. and Doan, F. J. The neotetrazolium test for deterioration of stored cream, concentrated milk, and cottage cheese. J. Milk and Food Technol. 21: 223-225. 1958.

20. Dahlberg, A. C. The keeping quality of pasteurized milk in the New York metropolitan area during cool weather as determined by bacterial counts, presence of coliform bacteria, and flavor scores. J. Dairy Sci. 28: 779-792. 1945.

21. Dahlberg, A. C. The relationship of the growth of all bacteria and coliform bacteria in pasteurized milk held at refrigeration temperatures. J. Dairy Sci. 29: 651-655. 1946.

22. Davis, J. G. The effect of cold on micro-organisms in relation to dairying. Proc. Soc. App. Bact. 2: 216-242. 1951.

23. Davis, J. G. and Jones, V. E. The differentiation of cells from bacteria in the resazurin test. Proc. Soc. Agr. Bact. (Abstracts) 1944: 40-43. 1944.

24. Davis, J. G. and Ward, S. J. A rapid electrical method for estimating the keeping quality of milk. Proc. Soc. Agr. Bact. (Abstracts) 1944: 32-37. 1944.

25. Day, E. A. and Doan, F. J. A test for the keeping quality of pasteurized milk. J. Milk and Food Technol. 19: 63-66. 1956.

26. Difco Laboratories, Inc. Difco manual of dehydrated culture media and reagents. 9th ed. Detroit, Mich., Difco Laboratories, Inc. 1953.

27. Erdman, I. E. and Thornton, H. R. Psychrophilic bacteria in Edmonton milk and cream. I. Numbers. Can. J. Technol. 29: 232-237. 1951.

28. Erdman, I. E. and Thornton, H. R. Psychrophilic bacteria in Edmonton milk and cream. II. Kinds. Can. J. Technol. 29: 238-242. 1951.

29. Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N., and Olson, J. C. Dairy microbiology. Englewood Cliffs, New Jersey, Prentice-Hall, Inc. 1957.

30. Frayer, J. M. The resazurin test - a preliminary study. Vt. Agr. Exp. Sta. Bul. 435. 1938.

31. Golding, N. S. and Jorgensen, S. I. A correlation of the resazurin grade with the standard plate count of raw milk. J. Milk Technol. 8: 189-195. 1945.

32. Greene, V. W. and Jezeski, J. J. Influence of temperature on the development of several psychrophilic bacteria of dairy origin. App. Microbiol. 2: 110-117. 1954.

33. Hammer, B. W. The creatine test for acetylmethylcarbinol plus diacetyl in butter cultures. J. Dairy Sci. 28: 579-581. 1935.

34. Hempler, P. Grading of milk supplies by means of resazurin tests. 13th Inter. Dairy Congress. 2: 255-263. 1953.

35. Hileman, J. L. Thermoduric bacteria in pasteurized milk. A review of literature. J. Dairy Sci. 23: 1143-1160. 1940.

36. Hileman, J. L. and Leber, H. High-temperature, short-time pasteurization and its practical application to the dairy industry. J. Milk Technol. 4: 128-137. 1941.

37. Jezeski, J. J. and Macy, H. Cryophilic organisms in water and butter. J. Dairy Sci. 29: 439-452. 1956.

38. Jezeski, J. J., Olson, J. C., and Lawton, W. C. Minneapolis, Minn. Comparative studies of bacteriological methods for the grading of raw milk. Paper presented at Ann. meeting Amer. Pub. Health Assn., Kansas City, Mo. Nov. 1955.

39. Johns, C. K. Influence of refrigerated storage on dye reduction time of milks. J. Milk and Food Technol. 15: 8-12. 1952.

40. Johns, C. K. Relation between reduction times and plate counts of milk before and after pasteurization. J. Milk and Food Technol. 17: 369-371. 1954.

41. Johns, C. K. Value of preliminary incubation in bacteriological milk tests. Can. J. Pub. Health. 49: 339-347. 1958.

42. Johns, C. K. and Howson, R. K. A modified resazurin test for the more accurate estimation of milk quality. J. Milk Technol. 3: 320-325. 1940.

43. Johns, C.K. and Howson, R. K. Potentiometric studies with resazurin and methylene blue in milk. J. Dairy Sci. 23: 295-302. 1940.

44. Johnson, P. E., Olson, H. C., and Von Gunten, R. L. A comparison of the bulk and can systems for handling milk on farms. Okla. Agr. Exp. Sta. Bull. B-436. 1954.

45. Jones, V. E. and Davis, J. G. The effect of pure cultures on the reduction of resazurin in milk. Proc. Soc. Agr. Bact. (Abstracts) 1944: 38-39. 1944.

46. Keenan, J. A., Barrett, W. D., and Rutan, H. The resazurin test - preliminary studies on its practicalities and possibilities. J. Milk Technol. 1: 22-25. 1937.

47. Kennedy, L. and Weiser, H. Some observation on bacteria isolated from milk that grow within a psychrophilic temperature range. J. Milk and Food Technol. 13: 353-357. 1950.

48. Knaysi, G. On the use of basic dyes for demonstration of the hydrolysis of fat. J. Bact. 42: 587-589. 1941.

49. Lawton, W. C. and Nelson, F. E. The effect of storage temperature on the growth of psychrophilic organisms in sterile and laboratory pasteurized skimmilk. J. Dairy Sci. 37: 1164-1172. 1954.

50. Laxminarayana, H. and Iya, K. K. Tetrazolium reduction test for quality control of milk. Indian J. Dairy Sci. 7: 83-92. 1954.

51. Levowitz, D. An inquiry into the usefulness of the standard method direct microscopic count. J. Milk and Food Technol. 20: 288-293. 1957.

52. Levowitz, D. and Weber, M. An effective "single solution" stain. J. Milk and Food Technol. 19: 121. 1956.

53. Liska, B. J. and Calbert, H. E. Observations on the method of reduction of 2, 3, 5-triphenyl tetrazolium chloride by lactic acid bacteria. J. Dairy Sci. 40: 602-603. 1957.

54. Little, L. Bacteriological control of milk quality. J. Milk Technol. 5: 221-226. 1942.

55. Marth, E. H. and Frazier, W. C. Bacteriology of milk held at farm bulk cooling tank temperatures. I. Effect of time and temperature during storage, farm source of milk and season of the year. J. Milk and Food Technol. 20: 72-78. 1957.

56. Marth, E. H. and Frazier, W. C. Bacteriology of milk held at farm bulk cooling tank temperatures. II. Effect of numbers of bacteria in the original milk. J. Milk and Food Technol. 20: 79-83. 1957.

57. Marth, E. H. and Frazier, W. C. Bacteriology of milk held at farm bulk cooling tank temperatures. III. Psychrophiles found and their growth. J. Milk and Food Technol. 20: 93-99. 1957.

58. Marth, E. H., Hunter, J. E., and Frazier, W. C. Bacteriological studies of a farm bulk milk handling system. J. Milk and Food Technol. 17: 86-90. 1954.

59. McKenzie, D. A., Druce, R. G., and Lambert, J. Routine resazurin test results and thermoduric bacterial content of milk samples as a measure of milk production conditions. Proc. Soc. App. Bact. 1: 19-25. 1950.

60. McKenzie, D. A. and Morrison, M. The occurrence of thermoduric organisms in raw milk. Proc. Soc. App. Bact. (Abstracts) 1945: 26-27. 1945.

61. Milk Inspection Service, Division of Environmental Sanitation, Dept. Public Health, Oakland, Calif. A study of the tanker system of collecting market milk. J. Milk and Food Technol. 13: 366. 1950.

62. Morgan, G. F. V. The resazurin test in factory practice. Dairy Ind. 7: 117-120. 1942.

63. Morris, C. S. Cryophilic bacteria as a cause of milk samples failing in the methylene blue test. Proc. Soc. Agr. Bact. (Abstracts) 1941: 33. 1941.

64. Morris, C. S. The detection and sources of thermoduric organisms in raw milk and their effect on the resazurin test. Proc. Soc. Agr. Bact. (Abstracts) 1943: 28-30. 1943.

65. Morris, C. S. and Edwards, M. Thermoduric organisms in milk. Dairy Ind. 8: 550-554. 1943.

66. Murray, J. G. The speed of "build-up" of thermoduric organisms on farm equipment. Proc. Soc. App. Bact. 2: 20-25. 1949.

67. Neal, C. E. and Calbert, H. E. The use of 2, 3, 5-triphenyltetrazolium chloride as a test for antibiotic substances in milk. J. Dairy Sci. 28: 629-633. 1955.

68. Nelson, J. A. and Trout, G. M. Judging dairy products. 3rd ed. Milwaukee, Wis., The Olsen Publishing Co. 1951.

69. Olson, J. C., Barker, R. B., and Mueller, W. S. The nature, significance, and control of psychrophilic bacteria in dairy products. J. Milk and Food Technol. 18: 200-203. 1955.

70. Parker, R. B., Coldwell, A. L., and Elliker, P. R. Psychrophilic bacteria - a sanitation problem. J. Milk and Food Technol. 16: 136-139. 1953.

71. Pearson, A. M. Bulk handling of milk. Can. Dairy and Ice Cream J. 34(5): 35-40. 1955.

72. Pennington, M. E. Bacterial growth and chemical changes in milk kept at low temperatures. J. Bio. Chem. 4: 353-393. 1908.

73. Procter, F. A study of commercial milk in wartime. Dairy Ind. 8: 211-214. 1943.

74. Prouty, C. C. Observations on the presence of facultative psychrophilic bacteria in milk produced under the farm tank system. J. Milk and Food Technol. 18: 250-253. 1955.

75. Report of the Committee on Applied Laboratory Methods. J. Milk and Food Technol. 19: 7-13, 17. 1956.

76. Revallier-Warffemius, J. G. The bacteriological grading of milk by means of the resazurin test. Netherlands Milk and Dairy J. 1: 152-165. 1947.

77. Robinton, E. D. and Genung, E. F. The effect of temperature on coliform organisms in milk and cream. J. Milk and Food Technol. 8: 97-100. 1945.

78. Rogick, F. A. and Burgwald, L. H. Some factors which contribute to the psychrophilic bacterial count in market milk. J. Milk and Food Technol. 15: 181-185. 1952.

79. Rowlands, A., Barkworth, H., Hosking, Z., and Kempthorne, O. Keeping quality and raw-milk grading. J. Dairy Res. 17: 159-191. 1950.

80. Seeleman, M. and Wegener, K. H. Sum Nachweis von Escherichia - Aerobacter - Keimen in Milchproben. Milchwissenschaft. 11: 51-61. 1956.

81. Sherman, J. M., Cameron, G. M., and White, J. C. The bacteriological spoilage of milk held near the freezing point. J. Dairy Sci. 24: 526-527. 1941.

82. Sherman, J. M. and Wing, H. U. The significance of colon bacteria in milk, with special reference to standards. J. Dairy Sci. 16: 165-173. 1933.

83. Snedecor, G. W. Statistical methods. 5th ed. Ames, Iowa, Iowa State College Press. 1956.

84. Society of American Bacteriologists. Manual of microbiological methods. New York, N. Y., McGraw-Hill Book Co., Inc. 1957.

85. Thomas, S. B. Psychrophilic micro-organisms in milk and dairy products. Dairy Sci. Abst. 20: 355-370, 447-468. 1958.

86. Thomas, S. B. The types of bacteria commonly found in farm and creamery water supplies and their action on milk and milk products. J. Soc. Dairy Technol. 2: 224-232. 1949.

87. Thomas, S. B. and Chandrasekhar, C. V. Psychrophilic bacteria in raw and commercially pasteurized milk. Proc. Soc. App. Bact. 1: 47-50. 1946.

88. Thomas, S. B. and Davies, M. Welch J. Agr. 16: 231-247. 1940. (Original not available for examination; cited by Johns, C. K. Influence of refrigerated storage on dye reduction time of milks. J. Milk and Food Technol. 15: 8-12. 1952.)

89. Thomas, S. B., Egdell, J. W., Clegg, L. F. L., and Cuthbert, W. A. Thermoduric organisms in milk. Part I. A review of the literature. Proc. Soc. App. Bact. 1: 27-64. 1950.

90. Thomas, S. B., Griffiths, D. G., Thomas, B. F., Humphreys, M., and Ellison, D. Heat resistant bacteria in raw milk. Part III. Occurrence of thermoduric bacteria in farm milk supplies. J. Soc. Dairy Technol. 4: 51-60. 1950.

91. Thomas, S. B. and Hobson, P. M. A survey of methods of milk production in relation to the incidence of thermoduric organisms in farm milk supplies. Proc. Soc. App. Bact. 1: 7-12. 1948.

92. Thomas, S. B. and Phillips. G. M. The routine resazurin test as an index of the keeping quality of milk. Dairy Ind. 9: 699-701, 724. 1944.

93. Thomas, S. B. and Probert, Dorothy. The cellular content of milk. Dairy Ind. 6: 295-296. 1941.

94. Thomas, S. B. and Roberts, M. Heat resistant bacteria in farm water supplies. Proc. Soc. App. Bact. 1: 44-46. 1946.

95. Thomas, S. B., Thomas, B. F., and Ellison, D. Milk bacteria which grow at refrigerator temperature. Dairy Ind. 14: 921-925. 1949.

96. Thomas, S. B., Thomas, B. F., and Ellison, D. Heat resistant bacteria in raw milk. Part III. The proliferation of thermoduric organisms on dairy equipment. J. Soc. Dairy Technol. 6: 138-144. 1953.

97. Thomé, K. E. and Ljunggren, B. Psychrophilic bacteria in market milk. Rep. Milk and Dairy Res., Alnarp, Sweden. 52. 1957. (Original not available for examination; abstracted in Dairy Sci. Abstr. 20: 511. 1958.)

98. U. S. Public Health Service. Milk ordinance and code. U. S. Public Health Service Publ. No. 229. 1953.

99. Van Demark, P. J. and March, R. P. The influence of the maximum temperature rise in bulk tanks on milk quality. II. The effect on bacterial counts. J. Milk and Food Technol. 20: 348-351. 1957.

100. Van Der Zant, W. C. and Moore, A. V. The influence of certain factors on the bacterial counts and proteolytic activities of several psychrophilic organisms. J. Dairy Sci. 28: 743-750. 1955.

101. Watt, J. E. The quality of fluid milk. Can. J. Pub. Health. 48: 158-163. 1957.

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APPENDIX

Rating of manufacturing-grade bulk-tank installations:

I. a) Concrete floor and good drainage 3

b) Concrete floor but poor condition and/or slope 2

c) Wood or linoleum floor 1 (Container to catch tank washings - 2)

d) Dirt floor or no attempt to provide proper washing disposal 0

II. a) Milkhouse separate from milking area and wire screened 3

b) Partially partitioned but wire screened from milking area 2

c) Partially partitioned but no wire screening 1

d) Open to milking area 0

III. a) Milkhouse well ventilated from outside 3

b) Minimum ventilation from outside 2

c) Ventilation source from within barn 1

d) No ventilation 0

IV. a) Light directed into tank and throughout milkhouse 3

b) Milkhouse fairly adequately lighted (one bulb and windows) 2

c) Some light (one bulb, no windows, or no bulb, some outside light) 1

d) No fixed light source 0

V. a) 30-50 gal. water heater 3

b) "Replacer" type water heater 2

c) Other facilities for heating water in milkhouse 1

d) No hot water available in milkhouse 0

VI. a) Water under pressure in milkhouse 3

b) Water under pressure in barn accessible to milkhouse 1

c) Water carried from house or well 50 ft. or more away 0

VII. a) Two compartment wash vat 3

b) One compartment wash vat 2

c) Wash basin or other facilities for washing equipment 1

d) No washing facilities for equipment 0

VIII. a) Clean, well lighted and ventilated milking area 3

b) Clean, fair light and limited ventilation 2

c) Semi-clean, poor light and ventilation 1

d) Dirty, dark, dank 0

IX. a) All milk handling equipment clean 3

b) Milk contact surfaces of equipment clean 2

c) Equipment rinsed or poorly cleaned 1

d) No partially adequate attempt to wash equipment 0

X. General appearance of milkhouse and milking area:

a) Excellent 3

b) Good 2

c) Fair 1

d) Poor 0

XI. a) Barn floor concrete, in good repair and clean 3

b) Barn floor concrete not clean or not in good repair 2

c) Barn floor wood 1

d) Barn floor dirt 0

XII. a) Milking utensil storage; metal rack properly above floor 3

b) Milking utensil storage; metal rack but improperly located 2

c) Milking utensil storage on table in milkhouse 1

d) No adequate attempt to allow utensil to drain or stored properly above floor 0

XIII. a) Gravel drive, dry working area, milkhouse easily accessible 3

b) No gravel but well drained drive, dry working area 2

c) Good, dry driveway, but poor working area 1

d) Dirt lane, poorly drained working area 0