dependent. In addition the actual cost of tuition and fees is paid and some assistance is given toward personal travel costs of the trainee. Traineeship awards generally are for a period of one academic year, although special exceptions may be made.

During the academic year 1961-1962, 33 sanitarians and 12 other workers in the field of sanitation received Public Health Service Traineeships. Table 6 shows this information for other professional categories. All but two of the 33 sanitarians receiving traineeships attended Schools of Public Health. Table 7 shows the distribution of trainees among schools. Sanitarian recipients of the fellowship awards were from 16 states and 2 territories.

### SUMMARY AND CONCLUSION

There are many opportunities for graduate professional education open to sanitarians. During the academic year 1961-1962, 61.8% of the sanitarians applying to Schools of Public Health were accepted. For the same period sanitarians received 6.6% of the Public Health Traineeships awarded. At one School of Public Health, 36% of the students receiving masters degrees with majors in the environmental sciences were sanitarians. Any sanitarian qualified to undertake graduate work in his professional category can find support.

Professional status for the sanitarian, as for any other group, must be built upon a foundation of education. Most professional education today is at the graduate level, and the current trend in that direction is growing stronger all the time. Yesterday, we worked in parts per million, today we cope with micromicrocuries; what will it be tomorrow? Training tomorrow's sanitarians to meet today's problems is not enough. Graduate education prepares the sanitarian to meet tomorrow's problems as well as today's.

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# ENUMERATION OF PSYCHROPHILIC MICROORGANISMS[1]

## A REVIEW

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The importance of psychrophilic microoorganisms in quality control, sanitation and processing of milk products has fostered a growing interest in methods of their enumeration.

The literature on psychrophilic microorganisms was reviewed briefly by Doetsch and Scott *(20)* and comprehensively by Davis *(19)*, Thomas *(43)*, Ingraham and Stokes *(25)*, and Witter *(52)*. The purpose of this paper is to summarize the more pertinent information about the enumeration of psychrophilic microoorganisms.

One of the first problems which arises is that of finding a precise, yet sufficiently comprehensive, definition for these organisms. Many definitions have been proposed, but as yet no single definition is universally accepted. These definitions are discussed by Witter *(52)* and Ingraham and Stokes *(25)*. Methods of enumerating psychrophilic microorganisms are as numerous as their definitions, since definitions are used as a basis for establishing counting procedures. Consequently, the problem of the one "best" method of enumerating psychrophilic microorganisms is still unsolved.

### PLATE COUNT INCUBATION TEMPERATURE AND TIME

Temperature and time of incubation for determination of psychrophiles by the agar plate method seem to be the subjects of greatest debate. The eleventh edition of Standard Methods for the Examination of Dairy Products *(9)* recommends holding plates at 41-44.6 F (5-7 C) for 7-10 days. The

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recommended incubation time and temperature have changed with each edition of this publication, indicating that no single procedure or definition has yet been suggested which has remained acceptable to the majority of workers in this field. The third and fourth editions of Standard Methods of Milk Analysis *(1, 2)* made no provisions for psychrophilic counts. One plate incubation temperature and time combination, 99.5 F (37.5 C) for 48 hours, was recognized as the standard for all counts. The fifth edition *(3)* lowered the temperature to 98.6 F (37 C), but the time of incubation remained the same. As psychrophilic organisms gained more attention, the need for their selective enumeration was seen. The sixth edition *(4)* suggested "tentative" standards of 68 F (20 C) or room temperature incubation for 72 hours. The seventh edition *(5)* known as Standard Methods for the Examination of Dairy Products, recommended holding plates at 77 F (25 C) for 4 days. This temperature was reduced considerably in the eighth edition *(6)*. A temperature of 41-50 F (5-10 C) for time sufficient to permit visible colonies to develop was recommended. The time was usually 10 to 14 days or longer. The time was established at 10 to 14 days in the ninth edition *(7)*, but the temperature remained unchanged. The temperature and time combination suggested in the tenth edition *(8)* in 1953 was 41 F (5 C) for 7 days. As has been mentioned previously, the standards are now 41-44.6 F (5-7 C) for 7-10 days *(9)*.

Many temperature and time combinations for plate incubation other than those noted above can be found in the literature. Thomas *(43)* listed 28 temperature and time combinations as used by various workers; the temperatures range from 32 to 77 F (0 to 25 C), and times range from 3 to 28 days of incubation. Pennington *(37)* in 1908 made a study of milk stored at 29.0 to 31.0 F (-1.67 to -0.55 C). Plates were incubated at 68 F (20 C) for 5 days and at 29.0 to 31.0 F (-1.67 to -0.55 C) for a month to 6 weeks. For milk stored less than 4 weeks, 68 F (20 C) incubation gave the highest counts. If the milk had been stored 4 weeks or longer, the lower temperature of incubation yielded counts almost as high as were obtained at 68 F (20 C). In some cases higher counts were obtained at the lower temperature.

Ingraham and Stokes *(25)* proposed 32 F (0 C) for 2 weeks as a suitable plating temperature and time. Mikolajcik and Burgwald *(30)*, using Tryptone Glucose Extract Milk Agar, found that with plates incubated at 32-35 F (0-1.66 C), 7 days were required for colonies to become visible. At 36-40 F (2.2-4.4 C), 5 days were required. Only 3 days were required to produce visible colonies at 45-50 F (7.2-10 C).

An incubation temperature of 37.4-41 F (3-5 C) has been used frequently for psychrophilic plate counts. Thomas *et al. (46)* used Yeastrel Milk Agar plates incubated for 7 days at 37.4-41 F (3-5 C). Druce and Thomas *(21)* stated that the psychrophilic colony count obtained at 37.4-41 F (3-5 C) for 7 days provides information on the total number of Gram-negative spoilage bacteria. They believed that, since lactic streptococci will not grow at 37.4-41 F (3-5 C), a colony count at this temperature is more useful than one at 86 F (30 C). The possibility of combining psychrophilic and lipolytic counts by using Tributyrin or Triolein Agar incubated for 14 days at 37.4-41 F (3-5 C) was suggested. Thomas and Chandrasekhar *(44)* compared counts obtained at 37.4-41 F (3-5 C) for 7, 14 and 21 days. Geometric means for the respective times were 8,412, 67,190 and 91,190. The 14 day counts were eight times the 7 day counts, and the 21 day counts showed a 50% increase over the 14 day counts. Thomas *et al. (45)* compared counts obtained from plates incubated at 37.4-41 F (3-5 C) for 10 days with counts obtained from plates incubated at 44.6 $\pm$ 0.9 F (7 $\pm$ 0.5 C) for 10 days. The 44.6 F (7 C) counts usually were higher, with only 12 of 177 samples giving lower counts at 44.6 F (7 C) than at 37.4-41 F (3-5 C). Counts at 44.6 F (7 C) were one to five times higher than the counts at 37.4-41 F (3-5 C) for 98 of the samples, including 50 samples whose counts were less than two times higher at 44.6 F (7 C) than at 37.4-41 F (3-5 C). Counts at 44.6 F (7 C) were 10 or more times higher for 40 samples, including nine samples whose counts at 44.6 F (7 C) were more than 100 times the counts at 37.4-41 F (3-5 C). The greater increases were obtained with samples giving low colony counts at 37.4-41 F (3-5 C).

A temperature of 41 F (5 C) for 7 days or longer has been used quite frequently for psychrophilic plate counts. Andrey and Frazier *(10)* and Overcast and Skean *(35)* incubated plates at 41 F (5 C) for 7 days. Boyd *et al. (14)* determined psychrophilic counts using an incubation temperature and time of 40 F (4.4 C) for 20 days. One difficulty encountered with incubation at 41 F (5 C) for 7 days is that colonies sometimes are so small that counting is made difficult. Johns *(27)* stated, however, that after 10 days the colonies are frequently many times larger. Boyd *et al. (15)* compared counts on commercially pasteurized milk from plates incubated at 41 and 50 F (5 and 10 C) for 7, 10, 15 and 20 days. Incubation at 41 F (5 C) for 7 days was discontinued because the colonies were small and difficult to count. In all other cases, 41 F (5 C) yielded lower counts than did 50 F (10 C). Maximum counts at either temperature were not reached in

less than 20 days of incubation. Glenn *(23)* reported that incubation for 14 days at 41 F (5 C) often resulted in considerably higher psychrophilic counts than did incubation for 7 days at 41 F (5 C). In many cases, after 7 days of incubation, the colonies were too small to be counted. After 14 days, the colonies were larger and easier to count in every instance.

Van der Zant and Moore *(48)*, plating on Tryptone Glucose Extract Agar, incubated plates at 77 F (25 C) for 2 and 3 days, 69.8 F (21 C) for 2, 3 and 4 days, and 50 and 41 F (10 and 5 C) for 5, 7 and 10 days. At all temperatures, they found counts to be higher at the maximum incubation time than at the shorter times. At 41 F (5 C) for 5 days, counts averaged 51% of the 10-day counts; the counts at 7 days averaged 70% of the counts after 10 days incubation. At 50 F (10 C) incubation, the counts after 5 days averaged 82% of the 10-days counts; the 7-day counts averaged 89% of the 10-day counts. These workers agreed with other workers previously cited in that incubation at 41 F (5 C) for 7 days frequently resulted in colonies so small as to make counting difficult. They concluded that incubation for 3 days at 77 F (25 C) was best for enumeration of psychrophiles.

Heather and Van der Zant *(24)* plated pure cultures of heat-treated and nonheated psychrophilic bacteria on Tryptone Glucose Extract Agar. Incubation was at 77 F (25 C) for 2 and 3 days, 59 F (15 C) for 3, 5 and 7 days and 41 F (5 C) for 5, 7 and 10 days. Lower numbers were found at the shorter incubation times, especially with the heated cells. Heated psychrophiles were detected best at 77 F (25 C) for 3 days and at 59 F (15 C) for 7 days.

Nelson and Baker *(31)* incubated plates at 95 F (35 C) for 2 days, 89.6 F (32 C) for 2 days, 77 F (25 C) for 2 and 3 days, 69.8 F (21 C) for 2, 3 and 4 days, 59 F (15 C) for 3, 4 and 7 days, 50 F (10 C) for 4, 5 and 7 days and 41 F (5 C) for 5, 7 and 10 days. Of the samples plated at 41 F (5 C), the average count at 7 days was 81% of the count at 10 days. In all cases incubation at 77 F (25 C) for 3 days detected samples giving high counts on plates incubated at 41 F (5 C) for 10 days. Therefore, it was recommended that plates be incubated at 69.8 F (21 C) for 4 days or 77 F (25 C) for 3 days for the detection of milk with a high bacterial count due to growth during refrigeration.

Witter *(52)* stated that psychrophiles are capable of forming visible colonies on plates incubated for 10 days at 44.6 ± 0.9 F (7 ± 0.5 C). Olson *et al. (33, 34)* and Schultze and Olson *(42)* used 44.6 F (7 C) for 10 days. Comparisons of 44.6 F (7 C) for 10 days with other temperatures and times have been reported above. Kaufmann and Andrews *(28)* plated on Tryptone Glucose Extract Agar containing 0.2 per cent yeast extract and incubated at 47 F (8.3 C) for 7 days. Jezeski and Macy *(26)* compared total, lipolytic and caseolytic counts using 98.6 F (37 C) for 2 days, 68 F (20 C) for 5 days and 46.4 F (8 C) for 7 days. They reported that 68 F (20 C) incubation resulted in the highest counts. Burgwald and Josephson *(17)* determined psychrophilic counts on Tryptone Glucose Extract Milk Agar incubated at 46.4-50 F (8-10 C) for 10 days.

Kennedy and Weiser *(29)* plated raw and pasteurized milk and held the plates at 50 F (10 C) for 24, 48 and 72 hours. Their results showed that, as the time of incubation increased, the number of colonies on plates of both raw and pasteurized milk samples increased. This increase was considered to have possibly resulted from either slow growing psychrophiles or mesophilic bacteria which had become adapted to growth at 50 F (10 C) during the 72 hour incubation period.

Erdman and Thornton *(22)* compared counts from plates incubated at 95.9 F (35.5 C) for 2 days, 50.9 F (10.5 C) for 7 days and 40.1 F (4.5 C) for 7 days. The 40.1 F (4.5 C) counts were lower than the 95.9 F (35.5 C) counts while the 50.9 F (10.5 C) counts varied from 36 to 350 per cent of the 95.9 F (35.5 C) counts. The differences never were considered to be greater than the experimental error of the plate method. Subsequent work indicated that incubation at 95.9 F (35.5 C) did not adequately enumerate psychrophilic organisms, because colonies isolated from the plates incubated at 40.1 (4.5 C) and 50.9 F (10.5 C) were inhibited at 95.9 F (35.5 C). Atherton *et al. (12)* found that organisms from commercially pasteurized milk grew equally well on plates incubated at 68, 78.8, and 89.6 F (20, 26 and 32 C), and that the counts at 50 F (10 C) were only slightly lower.

Many workers believe that incubation of plates at 50 F (10 C) does not give a true representation of the psychrophilic content of milk, since thermoduric organisms may grow on these plates. Olson *et al. (34)* stated that incubation at 50 F (10 C) will result in the growth of thermoduric bacteria not encountered at 45 F (7.2 C). Atherton *et al. (11)* found that organisms surviving laboratory pasteurization showed little growth up to 15 days in milk held at 45 F (7.2 C) but that marked growth occurred at 50 F (10 C). Boyd *et al. (14)* picked colonies which had appeared on plates between 10 and 15 days at 50 F (10 C) and compared their pasteurization resistance with that of colonies which appeared before 10 days. Thirty-two and one-half per cent of the isolates appearing before 10 days withstood pasteurization at 141.8 ± 0.9 F (61 ± 0.5 C) for

30 minutes, and 82.5 per cent were able to grow at 41 F (5 C). On the other hand, 100 per cent of the cultures isolated after 15 days incubation withstood pasteurization and only 17.9 per cent were able to grow at 41 F (5 C). It was concluded that the increase in count from 10 to 15 days incubation was due to thermoduric microorganisms and that, therefore, 50 F (10 C) is too high to detect true psychrophiles which are considered to be killed by proper pasteurization. Thomas *et al. (45)* found that no organism from laboratory pasteurized samples grew at 44.6 F (7 C) and concluded that incubation at 44.6 F (7 C) was adequate for detecting post-pasteurization contamination. Other workers *(18, 21, 29, 34, 36, 41, 43, 44, 47, 49)* also thought that the presence of psychrophilic bacteria was due to post-pasteurization contamination rather than to thermoduric microorganisms.

Some workers favor higher temperatures for estimating numbers of psychrophilic microorganisms. Prouty *(38)* recommended holding plates at 62.6 F (17 C) for 5 days for the enumeration of "facultative psychrophiles." Weber *(50)* believes that incubation at 68 F (20 C) was more adequate than 95 F (35 C) for determining bacterial content of milk held in cold storage. Watrous *et al. (49)* stated that incubation at 77 F (25 C) gives the most useful information on refrigerated dairy products. Other workers *(24, 31, 48)*, in determinations previously described, recommend 77 F (25 C) for 3 days, or some comparable temperature and time combination.

### PRODUCT DETERIORATION AND NUMBERS OF PSYCHROPHILIES

Several workers have attempted to correlate numbers of organisms with flavor changes in milk or by observing some metabolic activity of the pyschrophilic bacteria. Olson *et al. (33)* stated that, at a given temperature, the rate of deterioration in a product depends not only on the initial numbers but also on the types of organisms in the product. For this reason, using counts to predict keeping quality or bacterial activity to estimate numbers has not been completely successful. According to Atherton *et al. (12)*, there is little relationship between the psychrophilic population and off-flavor development, but changes in flavor are due more to bacterial types than numbers. However, Boyd *et al. (15)* found a definite relationship between flavor deterioration and bacterial increase. At 40 F (4.4 C) the psychrophilic count was 26,800,000 per ml when the flavor score went below 37, and, at 33 F (0.6 C), the count was 2,500,000 per ml when the score dropped below 37. Pennington *(37)* held milk at 29.0 to 31.0 F (-1.67 to -0.55 C) for as long as 6 weeks and found no off-odor or taste even when psychrophilic counts were in the hundreds of millions. Punch *et al. (40)*, working with pure cultures, determined counts at the time off-flavors were detected. They reported the following ranges of organisms per ml: *Pseudomonas*, 5.2-200 million; *Alcaligenes*, 2.5-14 million; *Flavobacterium*, 8.3-120 million; coliforms, 2.7-150 million; and yeasts, 2.5-14 million. Weese and Henderson *(51)* studied the relationship between flavor deterioration, acidity development and bacterial numbers in refrigerated pasteurized milk. Samples of commercially pasteurized milk were held in home refrigerators varying in temperature from 37 F (2.8 C) to 52 F (11.1 C). There was little decrease in flavor score or little increase in acidity until after 4 days. The number of cells increased rapidly after storing for 3 to 4 days. Burgwald and Josephson *(17)* found acidity development definitely correlated with psychrophilic growth. Atherton *et al. (11)* found little change in acidity or pH of commercially pasteurized milk after storage at 40 F (4.4 C). Change in flavor was slow, even with rapidly increasing bacterial numbers; dye reduction was slow and no significant increase in phosphatase occurred. The first perceptible indication of deterioration appeared to be a loss of stability of casein. Babel *(13)* reported that acidity and amino nitrogen increase slowly in milk held at 40 F (4.4 C). Van der Zant and Moore *(48)* found no direct relationship between bacterial population level and proteolytic activity but increases in soluble nitrogen, tyrosine and tryptophan were noticeable when cell counts reached $10^5$-$10^9$ organisms per ml.

### OTHER ENUMERATION PROCEDURES

Punch and Olson *(39)* proposed a surface plate method for the enumeration of psychrophiles. Samples were spread over the surface of agar, and the plates were incubated at 42.8 $\pm$ 0.9 F (6 $\pm$ 0.5 C). After 5 days incubation, counts were equal to or greater than those obtained by incubating at 41-44.6 F (5-7 C) for 7-10 days. Advantages claimed in addition to the reduced incubation time were that colonies were more easily counted and that spreading colonies were avoided.

A membrane filter technique for enumeration of Gram-negative bacteria was suggested by Nutting *et al. (32)*. Filters containing Yeast Extract Glucose Medium were inoculated and incubated at 69.8 and 50 F (21 and 10 C). Colonies were stained with methylene blue and observed at a magnification of 30 times. It was found that maximum colony numbers developed in 12 hours at 69.8 F (21 C) and 72 hours at 50 F (10 C). Counts were in reasonably good agreement with agar plate counts obtained at corresponding incubation temperatures.

Broitman *et al. (16)* proposed a keeping quality

test based upon the activity of psychrophilic microorganisms. The test used Nacconol-tri-phenyltetrazolium chloride in milk which was incubated at 68 F (20 C) and observed at 12-hour intervals for 48 hours. A positive test after 12 hours incubation indicated a shelf life of 4 days for milk held at 40.1 F (4.5 C). Positive tests at 24, 36 and 48 hours indicated a shelf life of 9, 12 and 15 days, respectively. These workers stated that initial counts could not be used to predict keeping quality except that milks with populations of 10 or fewer organisms per ml would have long shelf lives.

## SUMMARY

The many methods which have been proposed for enumerating psychrophilic microorganisms indicate a lack of agreement on the subject. The problem seems to stem from the fact that each worker has his own definition of psychrophilic bacteria and uses a procedure which is dependent on the definition. These differences of opinion are clearly reflected in the changes in recommended time and temperature of plate incubation in successive editions of "Standard Methods for the Examination of Dairy Products." The adoption of one time and temperature combination by all workers would be desirable from the standpoint of providing a basis for comparison of work done in different laboratories. To accomplish this, we will have to resolve the difficult task of agreeing on a standard definition of psychrophiles.

## ADDENDUM

Since this review was prepared the following came to our attention: Leesment and Dufeu (16th Int'l. Dairy Congr. C:392-398. 1962) accelerated the development of colonies of psychrophilic bacteria by preincubating plates for 16 hr at 62.6 F (17 C) before further incubation at 44.6 F (7 C) or 39.2-42.8 F (4-6 C). Psychrophilic plate counts after 4 or 5 days coincided well with counts obtained after incubation for 10 or 20 days using the standard method.

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# COTTAGE CHEESE PROBLEMS IN PRODUCTION AND SANITATION

## QUALITY CONTROL IN COTTAGE CHEESE[1]

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Most of the spoilage occurring in cottage cheese is microbial rather than chemical. The organisms involved are usually psychrophiles that cause surface slime, or yeasts and molds. In general psychrophilic organisms are restricted to the genera *Pseudomonas, Alcaligenes, Flavobacterium* and *Achromobacter,* with the first two being the most frequent causes of spoilage. These organisms produce pigmented slime or gelatinous film along with objectionable flavors and odors (5).

Spoilage by molds occurs less frequently but is extremely objectionable. The molds most commonly encountered are *Geotrichum candidum* which produces an off-white or light tan to yellow color,

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