

# HYGIENIC CONTROL OF FLUID MILK

FRANKLIN W. BARBER, B.S., M.S., Ph.D. \*

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The hygienic control of fluid milk is necessary to provide the public with a safe, wholesome, high-quality beverage. It involves many carefully controlled steps, regardless of whether the end result is fluid milk and cream or any other of the numerous milk products or by-products. It is concerned with such matters as the health of the cow, the health of the farmer, the condition of the farm, the milking operation and equipment, delivery of the milk to the dairy, processing of the milk and eventual delivery to the home.

Other chapters of this monograph have dealt with various aspects of milk hygiene up to and including pasteurization; the hygienic control of fluid milk can be said to embrace all these aspects, because only if each step in milk production and processing is carefully controlled can high-quality fluid milk be obtained. The final evaluation of the finished product is determined by how well the product meets the standards of quality set by controlling agencies, whether they be federal, state or municipal.

For the most part controlling agencies have set up specifications or conditions relating to the operation of the dairy industry. These specifications or conditions, which should cover milk on the farm, milk in transit and milk in the plant, have already been discussed. However, it is when the finished product is obtained that the efficiency of these operations is evaluated. Although finished-product standards may vary from one country to another, or even from one municipality to another within a given area, in general, the same standards of quality are involved. These are:

1. A pasteurized product must give a negative test for the enzyme phosphatase.
2. A pasteurized product must contain less than a specified number of micro-organisms per ml.
3. A pasteurized product must contain less than a specified number of coliform organisms per ml.

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\* Assistant Research Manager, Research and Development Division, National Dairy Products Corporation, Glenview, Ill., USA.

By meeting these standards, plus the many processing specifications, the product is regarded as safe and wholesome for the public.

For many years the hygienic quality of fluid milk has been regulated and judged by laboratory control tests. It has been the practice in the dairy industry to evaluate the effectiveness of processing and the quality of the milk by the results of laboratory tests carried out on the product immediately after pasteurization and packaging. The most common laboratory tests are (a) the phosphatase test, (b) the standard plate count, and (c) the coliform count (Johns, 1953, 1955). In more recent years, in some areas, a psychrophile count and some kind of keeping-quality test have also been added to the other routine tests conducted by the laboratory on the finished product. In the USA and Canada the various laboratory procedures used to evaluate the hygienic quality of dairy products have been standardized and presented in *Standard Methods for the Examination of Dairy Products* (American Public Health Association, 1960).

### The Phosphatase Test for Pasteurization

The phosphatase test is a chemical method for determining the efficiency of processing and has a direct relationship to the safety of pasteurized products. The test is based on the fact that phosphatase, an enzyme occurring normally in raw milk, is progressively inactivated by increasing heat treatment. Complete inactivation occurs at a thermal exposure just barely below the temperature and time required for pasteurization, and above the temperatures required for the destruction of disease-producing organisms. The unusual sensitivity of the enzyme to slight variations in the processing operations required for proper pasteurization makes the phosphatase test exceptionally valuable for discovering recontamination with raw milk and for locating causes of under-pasteurization.

There are a number of tests for phosphatase activity reported in the literature (Gilcreas & Davis, 1940; Kay & Graham, 1935; Sanders & Sager, 1948; Sharer, 1953), but all of them are based upon the ability of alkaline milk phosphatase to liberate phenol from phosphoric-phenyl esters. The activity or amount of enzyme present in a milk sample can be detected by colorimetric determination of the liberated phenol. In most areas in the USA and Canada the Scharer one-hour test has been widely used as a screening method (Burgwald, 1942). Suspect samples are generally confirmed by one of the two official methods described in the 11th edition of *Standard Methods*. In European countries the Kay & Graham phosphatase test is used more frequently (Kästli, 1957).

The phosphatase test is regarded as a valuable tool in the hygienic control of pasteurized milk. Fay (1939), in an early paper on the value of the phosphatase test, lists a number of defects in pasteurization which were revealed by this test:

“ A. *Short holding*

1. Fast recording thermometers
2. Leaky outlet valves
3. Deliberate short holding
4. Confusion of valves in a multiple valve system

“ B. *Low temperature : shown on recorder*

1. Vats only partially filled—milk does not hold temperature
2. Holders cold at start of run
3. Holders in a draught
4. Poorly insulated holders
5. Cold layer on the bottom of vats—poor agitation.

“ C. *Low temperature : not shown on the recorder*

1. Thermometer bulb passing through heated jacket
2. Improperly pasteurized foam contaminating the last milk out of the vat
3. Long thermometer bulbs touching the glass or metal lining of a jacketed vat

“ D. *Added raw milk*

1. Defective valves or valves improperly closed
2. Standardizing during or after heating with raw or improperly pasteurized milk or cream
3. Leaks in regenerator section
4. Passing milk through equipment previously used for raw milk.”

Any of the foregoing conditions might lead to an improperly pasteurized product. The use of the phosphatase test not only on the finished product but also on samples taken during the processing operation enables the plant operator to spot the faulty equipment or operation and then apply the proper corrective measures. The phosphatase test is reported to be able to detect (a) a 1.5-degree drop in the temperature of pasteurization, (b) a 5-minute shortening of the holding time, or (c) the addition of 0.1 % raw milk.

Valuable as the phosphatase test may be in the control of pasteurized milk, there are conditions which can invalidate the test. It is essential that the factors affecting the accuracy of the test be recognized and that directions for making the test be followed carefully (Barber, 1942). The precautions to be taken are clearly stated in the general instructions for conducting the test (see, for example, *Standard Methods*).

A number of workers (Hammer & Olson, 1941; Barber & Frazier, 1943) have shown that under some conditions bacterial phosphatase can interfere with the test. Certain micro-organisms are capable of producing sufficient amounts of phosphatase to cause a positive phosphatase reaction on properly pasteurized products. Barber & Frazier showed that bacterial phosphatase was more resistant to heat than milk phosphatase and required a heat treatment of 76.7°C (169°F) for 30 minutes for inactivation, while milk phosphatase was inactivated at 62.8°C (144°F) for 30 minutes. This difference in heat resistance was found to be a convenient method for distinguishing between the two types of phosphatase.

More recently, several workers have reported obtaining positive phosphatase tests on products subjected to high-temperature, short-time (HTST) pasteurization (Fram, 1957a, b; Wright & Tramer, 1953a, b, 1954). It appears that there is a reactivation of phosphatase when products are pasteurized at high temperatures and subsequently held at temperatures above refrigeration temperature.

Fram (1957a) has reported the reactivation of phosphatase in dairy products of various fat content (from skim milk to 38 % fat cream) after heating at various times and temperatures. Immediately after pasteurization and when stored at 4.4°C (40°F), all products gave negative phosphatase tests. When the products were held at 31.1°C (88°F), however, phosphatase reactivation was observed in HTST-treated products but not in products pasteurized by the long-hold method. The minimum short-time temperature above which reactivation occurred, and the minimum period of storage at 31.1°C before reactivation, appeared to be a function of the fat content—the higher the fat content of the product, the lower the temperature of heat treatment and the shorter the period of storage at 31.1°C before reactivation of the enzyme.

It is suggested that with products subjected to HTST pasteurization, phosphatase tests should be conducted either immediately after pasteurization or on products which have been quickly cooled after pasteurization and held at temperatures below 4.4°C, to avoid positive phosphatase test results due to reactivation of the enzyme.

### Bacterial Flora in Milk

Before considering the bacteriological tests that are of importance in the hygienic control of fluid milk, mention should be made of the various types of organisms that may be encountered in milk. Because milk is an ideal medium for the growth of micro-organisms, strict control over its production, processing and distribution is necessary. For the purpose of this discussion only the minimum amount of detail will be presented regarding the various types of bacteria than can be found in milk. For more complete information, the reader is referred to Foster et al. (1957) and Elliker (1949). With respect to pathogenic bacteria, present information is ably summarized in a recent paper by Kästli (1957).

Milk aseptically drawn from the cow is not completely free from bacteria, as there are always organisms present in the teat canal and the udder. The health of the cow determines the types and extent of the bacterial flora of the milk as it is produced. Milk from a normal healthy udder may contain varying numbers of micrococci and small rod forms. Mastitis infection naturally results in increased bacterial numbers. In addition to the usual mastitic streptococci, micrococci (staphylococci) and *Pseudomonas*

have been reported as being implicated in mastitis. If the animal is not healthy, pathogenic bacteria such as mycobacteria, brucellae and rickettsiae (Q fever) may be present in the milk.

Further contamination of milk can occur during milking from the coat of the cow, milking utensils, air, feed, dust, manure, etc., if good milking practices are not followed. Many types of organisms are represented in this kind of contamination: lactic-acid bacteria, spore-forming rods, coliforms and *Pseudomonas* in particular. If the milk is not cooled rapidly and properly after milking, growth of these organisms may take place and markedly increase the count of the raw milk. A satisfactorily produced milk should have a bacterial count of less than 200 000 per ml when it is delivered to the processing plant.

At the plant the sanitary condition of the equipment and the temperature and time of raw-milk holding also have a marked effect on the bacterial population of the milk. Proper pasteurization destroys all possible pathogens and reduces the number of viable organisms in the milk. Here again, if proper cleaning and sanitizing procedures are not followed for pasteurizing equipment, an increase in certain types of organisms is possible, owing to a build-up of milk solids in the equipment. This is particularly true in the long-hold pasteurization method, where one vat may be used several times a day for pasteurizing different batches of milk without being cleaned between batches. If the original raw milk contains types of organisms that are resistant to heat or capable of growth at pasteurization temperatures, the chances of this type of build-up in the equipment are increased. These organisms are known as thermoduric (heat-resistant) and thermophilic (heat-loving) bacteria.

The thermoduric and thermophilic organisms, for the most part, are the bacteria responsible for high counts immediately after pasteurization. This is because the thermodurics are not destroyed by pasteurization and the thermophilics are capable of growing at the temperatures of the long-hold process and even, though infrequently, at the temperatures of the short-time process. The thermoduric organisms are generally micrococci or microbacteria, which originally get into the milk on the farm from improperly cleaned and sanitized utensils or equipment. The thermophiles also may gain access to milk on the farm through soil, bedding, feeds or other sources. Raw milk generally contains relatively few thermophiles and it is primarily through mishandling of the milk by exposure to high temperatures for comparatively long periods that the thermophilic flora builds up. As has been mentioned, this may be the result of continued use of vat pasteurizers without cleaning between batches. The undesirable practice of re-pasteurization of returned milk, and operating without foam heaters on the pasteurizing vats or with improperly designed ones also may cause a build-up of these organisms and contamination of the equipment and the product.

Assuming that the raw milk is of good quality, and that proper handling and pasteurizing practices have been followed, there is still the possibility of contamination of the product after pasteurization. The source of this contamination is usually improperly cleaned and sanitized pipelines, milk coolers, bottle-filling equipment and the bottles themselves. Condensate in the filler room frequently can be a serious cause of contamination if proper precautions are not taken. The principal post-pasteurization contaminants are reported to be the coliform organisms and psychrophiles in general.

The coliform organisms, because of their prevalence in nature (in manure, in soil, in the intestinal tract of man and animals, and on dirty utensils), are practically always present in raw milk. However, their heat resistance is such that they are usually completely destroyed by pasteurization and therefore their presence in pasteurized milk is considered to be an indication either of improper pasteurization or of post-pasteurization contamination.

The term psychrophile, as commonly used in the dairy industry, refers to those bacterial species which are capable of relatively rapid growth at low temperatures, generally within the range of 1.7°-10°C (35°-50°F). These organisms are largely Gram-negative, non-spore-forming rods with varying degrees of proteolytic activity. They are invariably present in all raw milk supplies and, like the coliforms, thermodurics and thermophilics, a part of the normal flora of raw milk. Their numbers depend upon the sanitary conditions under which the milk is produced, the temperature of holding and the time that elapses before processing. They are not regarded as being able to survive proper pasteurization treatments and therefore their presence in pasteurized milk is considered an indication of post-pasteurization contamination.

The psychrophilic bacteria have received increased attention by investigators during recent years because modern developments in the handling and transportation of milk have resulted in milk being held for longer periods at low temperatures before processing, manufacture or consumption. The literature on psychrophilic micro-organisms in milk has been completely reviewed recently by Thomas (1958).

### Standardized Bacteriological Tests

#### *Standard plate count*

The bacteriological control of fluid milk is dependent upon the determination of the numbers and kinds of bacteria present in the product. The oldest and most frequently used method for the enumeration of bacteria in milk is the standard plate count. Although this method was originally intended to count all the bacteria contained in the sample plated, actually the counts obtained are only estimates. This is because the conditions

of the test (incubation temperature, medium, oxidation-reduction potential, existence of clumps of bacteria, etc.) impose limitations that may prevent the growth of some of the bacteria in the sample. Therefore if bacterial standards are to fulfil their purpose as an indication of the quality of the product, every detail pertaining to apparatus, technique of plating, incubation, counting, and reporting of counts must be uniform, so that counts obtained by one laboratory can be accepted as authoritative by all concerned. These details for the standard plate count are set forth in the 11th edition of *Standard Methods for the Examination of Dairy Products*.

Although it has long been recognized by many investigators that there are many limitations to the standard plate count, the method is of value in that it provides a comparative index of the care used in the processing of the milk and is a means of ascertaining whether or not the product meets predetermined count standards. Controlling agencies may vary in their standards, but for the most part a standard plate count of 30 000 per ml is common, although in many areas a standard of 10 000 per ml is required for pasteurized milk. With present-day equipment and controls it is not difficult to produce a pasteurized product with a standard plate count of less than 10 000 or even as low as 1000 per ml.

### *Coliform count*

The coliform test has also been standardized so that counts obtained by different laboratories will have meaning. Coliform bacteria can be enumerated by plating the sample, as is done in the standard plate count, but using a selective nutrient agar that will permit the distinctive colonies of coliform bacteria to develop while suppressing the growth of other bacteria. An alternative procedure, in which liquid media are employed, can also be used to estimate the coliform content of the sample. Both of these methods are clearly and specifically recorded in *Standard Methods for the Examination of Dairy Products*.

Both tests are based on the fact that coliform organisms are Gram-negative, non-spore-forming rods that ferment lactose with the production of acid and gas. Both methods utilize media inhibitory to most other species, but since some other organisms may grow, positive results on the primary media are considered to be positive presumptive tests. To confirm a presumptive positive result, further tests with additional selective media have to be carried out.

It is recognized by most investigators that present-day pasteurized products should not contain any coliform organisms. However, in many areas, coliform standards are not quite so strict and usually specify that coliforms be absent in 1.0-ml or 0.1-ml samples (Kästli, 1957). A survey of coliform standards in the USA carried out in 1955 by the Committee on Applied Laboratory Methods of the International Association

of Milk and Food Sanitarians (Committee on Applied Laboratory Methods, 1956), showed the following:

“ *Milk* : Of the 46 replies, 19 States have legal standards; 19 have unofficial standards; 8 have no standards. Legal standards were as follows: one State with 1 per ml., one with 3 per ml., one with 5 per ml., 15 with 10 or <10 per ml., and one State did not give a standard. Unofficial standards include one State with 0 per ml., one with 5 per ml; and 17 with 10 or <10 per ml. The majority of replies (36) indicated routine testing with good compliance.”

### Other Bacteriological Tests

The standard plate count and the coliform test are bacteriological methods required in many areas by controlling agencies for evaluating the hygienic quality of fluid milk. However, many other methods are often used to obtain more detailed information regarding the quality of the milk or to aid in locating and correcting conditions which have caused a high count to be obtained on the pasteurized product. One procedure frequently used to locate trouble spots is the “line run”. In this procedure samples are taken at various locations along the processing route or at different times during the processing operation, and the source of high counts is thus determined. The samples are usually plated by standard procedures, but sometimes special methods of examination are of value.

#### *Methods for total counts*

*Oval-tube technique.* Because of the high costs of media, glassware and labour involved in routine standard plate counts, some investigators have been interested in other methods for making total counts. The oval-tube technique proposed by Myers & Pence (1941) can be used on pasteurized milk or on raw milk pasteurized in the laboratory. Oval tubes containing melted and tempered agar medium are inoculated with a 0.01-ml or 0.001-ml loopful of milk and mixed by gentle shaking. The tubes are slanted so that the medium forms a 2-3 inch (5-8 cm) surface along the tube, the agar is allowed to harden, and the tubes are then incubated horizontally, agar side up, in racks at 32°C (90°F) for 48 hours, after which time the colonies are counted. This method has shown close agreement with counts made by the regular plating method, and permits the examination of a large number of samples at one time quite economically.

*Roll-tube technique.* A similar technique is the roll-tube method, which is reported as being used quite extensively in countries other than the USA. As described by Thomas et al. (1952), this method utilizes a cylindrical tube which, after the melted medium has been inoculated with the sample, is placed on a spinner which causes the agar to harden as a thin film on the inside walls of the tube. After incubation, the colonies are counted as in the oval-tube method. This procedure has the advantages of the oval-

tube method, but requires special tubes, stoppers, a spinner and a counting device.

*Membrane-filter technique.* The membrane-filter technique (Goetz & Tsuneishi, 1951), although better adapted to and approved for water analysis, has also been used for obtaining total counts on milk samples. Cole (1955) has used this method extensively in the evaluation of the pasteurizability of producers' milk. A 0.01-ml loopful of milk is introduced into 20 ml of sterile water and passed through a Millipore filter. In this procedure all of the organisms present in the sample are collected on the surfaces of a membrane, and the membrane is transferred to a small Petri dish containing an absorbent pad with added nutrient medium. The complete unit is incubated overnight at the desired temperature and the following morning the colonies are counted. The greatest advantage of this method is that counts can be obtained in a relatively short length of time: 18 hours as compared to 48 hours for plate counts. The direct addition of small volumes of milk without dilution has been tried out, but a recent comparative study indicated that additional research is necessary before this modification can be regarded as a standard procedure for the examination of milk.

*Bacto-strip technique.* The Bacto-strip technique was developed in Switzerland (Forg, 1956) as a means of making microbiological determinations when laboratory facilities are not available. Bacto-strips are obtainable for determining total counts, yeast and mould counts, and coliform counts, and special strips have been designed for checking surface contamination (Johns & Berzins, 1957). The strips are lengths of sterile heavy filter-paper impregnated with a specific culture medium depending upon the type of strip and the desired count. The size of the strip controls the amount of sample absorbed (1 or 0.1 ml).

To make the test, a strip is removed from its sealed plastic envelope, dipped into the milk being tested, withdrawn, drained, and returned to the envelope for incubation. The upper portion, which has been contaminated during handling, is torn off at the perforation and discarded. The open end of the plastic envelope is placed between two glass slides and heat-sealed by passing the exposed edges through a flame. After incubation at 37°C (98.6°F) for 8-10 hours the colonies which have developed on the strip are counted. The method is considered to be a good screening procedure but not a control method at the present time. Its cost is also a disadvantage for most laboratories.

#### *Other special methods*

The foregoing test procedures can be used to obtain total counts or, by slight modifications in the medium (addition of inhibitory substances) or in the time and temperature of incubation, to obtain information regarding specific types of organism. As has already been indicated, the quality

of the final product is dependent upon the quality of the raw product, the effectiveness of processing and the conditions of packaging and distribution. Hence the bacteriological condition of the milk must be known throughout the entire operation.

*Microscopic examination.* Microscopic examination of pasteurized milk is generally not considered a suitable method for determining the quality of the milk because of the poor staining ability of bacteria after heat treatment and the difficulties encountered in making accurate microscopic counts on low-count samples. However, it is possible to obtain very rapidly an indication of the general quality of the milk and to detect grossly mistreated samples. The application of the microscopic method to pasteurized milk may detect milk processed from raw milk with an extremely high count; may show the presence of excessive numbers of leukocytes, indicating the possibility of mastitis in the producing animal; may indicate faulty plant practices by detecting the presence of large numbers of bacteria; and may demonstrate the growth of bacteria after pasteurization. In carrying out a microscopic examination of pasteurized milk it should be remembered that the accuracy of the method is greatest with high-count milk and progressively decreases as the count decreases.

*Examination for thermodurics.* If during the routine standard-plate-count evaluation of pasteurized products high counts are obtained, the investigator must systematically determine the cause of these counts. Usually the first step is a check on the bacteriological condition of the raw milk supply. Samples of raw milk are subjected to pasteurization in the laboratory at the times and temperatures used in the plant. These samples are plated by one of the procedures already described (standard plate, oval-tube, roll-tube or membrane-filter technique). If the laboratory-pasteurized samples also give a high count, contamination of the raw milk supply with thermoduric organisms is suspected. This condition requires a check on each producer to determine the source of the organisms. When the source is located it is usually found that poor sanitary conditions exist on the farm or that improper cleaning and sanitizing practices are being followed. A thorough clean-up of the farm and instruction as to the proper procedures for cleaning and sanitizing milking equipment and utensils usually will produce the desired results. Sometimes it has been found that the contamination is caused by improperly cleaned and sanitized bulk tank trucks. Therefore these trucks must be inspected and checked for satisfactory bacteriological condition.

At times it will be found that raw milk samples pasteurized in the laboratory will show a satisfactory reduction in count. In this case a build-up of thermoduric or thermophilic organisms within the pasteurizing plant is indicated. Here the line-run technique is used to determine just where and when the build-up of organisms occurs. Usually the cause can be

traced to improper cleaning and sanitizing of equipment, faulty installation of pipelines allowing dead ends, excessively long periods of pasteurization operation without clean-up of the equipment, use of returned or previously pasteurized products, accumulation of foam in pasteurizers, etc. Again proper cleaning and sanitizing practices will usually eliminate the problem.

*Examination for thermophilics.* Thermophilic bacteria are detected by the agar-plate, the oval-tube or the roll-tube method, with incubation at 55°C (131°F). These organisms are capable of growing at this temperature and a count of the number present can be made. A microscopic examination of films prepared from pasteurized milk also can be employed; the presence of large rod-shaped bacteria, which retain the blue stain, strongly suggests that thermophilic bacteria are present.

*Examination for spore-formers.* Generally when one speaks of thermoduric organisms in milk the organisms referred to are the heat-resistant micrococci and microbacteria. However, it is the spore-forming bacteria that are the truly heat-resistant organisms, and on rare occasions these have been the cause of high counts in pasteurized products. These spore-forming organisms can also cause spoilage of pasteurized milk. When this type of spoilage does occur the milk usually becomes putrid. The spore-forming bacteria are present in soil, dust, grain, etc. and easily contaminate the raw milk if proper sanitary and milking practices are not followed on the farm.

Spore-forming organisms are detected easily in milk by the simple procedure of heating the milk to 80°C (176°F) for 10 minutes to destroy all vegetative cells and then incubating it at about 35°C (95°F) for 72 hours. The presence of spore-forming bacteria is indicated by the formation of a gassy curd in the sample. Anaerobic spore-formers can be detected in a similar manner if anaerobic conditions are provided by the addition of Vaspar (a combination of petroleum jelly and paraffin) to sterile test tubes before the milk sample is introduced and heated. The presence of these organisms in milk is undesirable and is indicative of the presence of dirt and dust on the producing farm.

*Examination for coliforms.* When coliform counts are obtained on products which have been properly pasteurized, as evidenced by a negative phosphatase test, the line-run technique is again of value. In this instance the source of contamination is clearly indicated by the numbers of coliform colonies appearing on the plates. Frequently the contamination occurs during the bottle-filling operation and may be caused by condensate from the filler bowl dripping into the milk. Drip deflectors and frequent chlorine spraying will eliminate this problem. Often improperly cleaned and sanitized equipment also will be found to be the cause of contamination, and improperly cleaned and sanitized bottles can likewise contribute to the coliform problem. It cannot be stressed too strongly that the cleaning and

sanitizing of all utensils and equipment is essential for the production of high-quality products (Glenn & Olson, 1959).

*Examination for "ropy milk" bacteria.* One of the less frequently occurring defects in pasteurized milk is a type of spoilage known as "ropy milk". The causative organisms in this case are capable of synthesizing a viscous, capsular material which may be pulled out into threads or strands giving the appearance of rope. This type of spoilage is considered to be harmless but is undesirable from the aesthetic point of view. The most common causative organism (*Alcaligenes viscosus*) is found in water, feed, soil, and manure or on utensils and can contaminate the milk supply on the farm. Unless the extent of contamination is great these organisms are destroyed by pasteurization. Therefore occurrences of ropy milk are usually traceable to post-pasteurization contamination.

Burke et al. (1955) have reported that very low numbers of ropy milk organisms (less than 1 per ml) can eventually bring about the ropy milk defect. These workers developed a simple test that detects between 2 and 20 ropy milk organisms per 10 ml of milk in 48 hours. Briefly the test consists in adding 20  $\gamma$  of penicillin per ml to a milk sample; incubating this sample at 21°C (70°F) for 24 hours; streaking a 0.01-ml loopful of the incubated sample on to standard tryptone-glucose-extract agar containing 20  $\gamma$  of penicillin per ml; incubating the plate for 24 hours at 21°C; and finally, with a needle, checking for the presence of stringy or ropy colonies on the plate. This test has been found to be satisfactory for several types of ropy milk organisms.

*Preliminary incubation test.* The practice of prompt and adequate cooling of milk for pasteurization has been advocated for many years as a means of obtaining low bacterial counts. However, the advent of bulk handling of farm milk and the use of farm bulk-cooling tanks have made it so much easier to obtain low bacterial counts that good production practices may be neglected. This is particularly true in areas where dye-reduction tests are used for grading milk, because the bacteria are in a dormant state following refrigeration and dye reduction may be unduly delayed. With bacterial growth inhibited by efficient cooling, there is need for a procedure for the detection of milk produced under poor sanitary conditions.

Johns (1958) has reported on a preliminary incubation test by which poorly produced milk can be detected even though a satisfactory dye-reduction test may be obtained on the milk. In this test the milk sample is subjected to 18 hours' pre-incubation at 12.8°C (55°F) and is then tested by standard-plate-count or dye-reduction procedures. When samples show a marked deterioration in bacteriological quality, unsatisfactory sanitary conditions of production are indicated. Johns strongly recommends the adoption of this preliminary incubation test as an aid to quality control.

### Keeping Quality of Final Product

The advent of bulk handling of raw milk, mechanical cooling of milk on the farm, new and improved cleaning and sanitizing programmes on the farm, and the use of more severe pasteurization treatments than are needed for the destruction of pathogenic organisms, have presented the dairy industry with a product that has an increased shelf-life and a very low bacterial count. In many cases the pasteurized milk is practically free from lactic-acid-producing organisms, with the result that on storage at usual refrigerator temperatures the milk no longer turns sour; it does, however, undergo certain changes which give rise to putrid flavours and odours. In the absence of the lactic-acid bacteria, spore-forming bacteria and Gram-negative psychrophiles are able to multiply comparatively rapidly and cause the milk to become putrid and unfit for consumption.

The problem of laboratory tests for predicting the keeping quality or shelf-life of pasteurized products is a serious one. Although many tests have been proposed by numerous investigators, a completely satisfactory test that will predict the shelf-life of the product has yet to be found. As has been mentioned, the type of spoilage encountered most frequently today is due to bacteria which grow at low temperatures and is generally not evident until the milk has been held at refrigeration temperatures for several days. The problem is further complicated by the fact that at the time the product is pasteurized and packaged, these low-temperature organisms are present in such small numbers that they cannot be detected by the usual plating techniques. Also, the *Standard Methods* procedure for the enumeration of psychrophiles requires 7-10 days' incubation at 5°C (41°F), and by the time counts are obtained the product may already be unsaleable. Therefore the goal of investigators is a simple laboratory test, the results of which will be available quickly and will provide a reliable estimate of the probable shelf-life of the product.

#### *Dye-reduction test*

Numerous chemical and physical tests—for example, tests for protein stability, variations in nitrogen distribution, changes in pH, protein-degradation products, and lactose reduction—have been suggested and tried out, but the changes were too slight, too inconsistent and occurred too near the time of flavour spoilage to be of value. Day & Doan (1956) proposed a dye-reduction test which they claimed to be a simple method for detecting poor keeping quality of refrigerated pasteurized milk. The test is based on the reduction of the dye, neotetrazolium, by the growth of bacteria under specified conditions. Reduction of the dye to a definitely discernible pink colour at the end of 4 hours' incubation at 37°C is the criterion for a positive

test. Such a result obtained on bottled milk after a minimum of 3 days' refrigerated storage would predict spoilage within a period of 3-4 days.

In discussing their test Day & Doan state:

" Perhaps the simplest way for a milk plant laboratory to use the test would be first to determine the maximum number of days their product might be held before being consumed. This interval would then be employed as the period of time a sample would be held before applying the test. A negative test at this point might well be considered satisfactory, for it would indicate a keeping period of at least 3 days more than that absolutely required. "

This keeping-quality test has merit, but it should be pointed out that the test requires a 3-day incubation period prior to testing, as well as some special glassware and equipment.

Another recently proposed dye-reduction test is that of Broitman, Mallmann & Trout (1958). This test utilizes the colour change of 2,3,5-triphenyltetrazolium chloride when added to pasteurized milk and incubated at 20°C (68°F). The special dye solution used in the test consists of:

|   |        |
|---|--------|
| 2,3,5-triphenyltetrazolium chloride (TTC) . . . . . | 0.1 g  |
| Nacconol NR ST . . . . .                            | 1.0 g  |
| K <sub>2</sub> HPO <sub>4</sub> . . . . .           | 5.0 g  |
| KH <sub>2</sub> PO <sub>4</sub> . . . . .           | 0.1 g  |
| Distilled water . . . . .                           | 100 ml |

The dye solution is placed in a dark bottle, autoclaved at 121°C (250°F) for 15 minutes and stored in the dark at room temperature. One millilitre of the dye solution is aseptically placed in a sterile test-tube, 10 ml of milk sample are added, and the contents are mixed well and incubated. Observations are made after 12, 24, 36 and 48 hours' incubation. A pale pink to rose colour is considered a positive result.

Broitman and his colleagues claim satisfactory results with the test and have suggested the following interpretation of results:

Negative after 24 hours: good keeping quality

Positive after 12 hours: very poor quality; average shelf-life at 4.5°C, 4 days

Positive after 24 hours: poor keeping quality; average shelf-life at 4.5°C, 9 days

Positive after 36 hours: good keeping quality; average shelf-life at 4.5°C, 12 days

Positive after 48 hours: good keeping quality average shelf-life at 4.5°C, 15 days

#### *Plate counts*

It is recognized that the value of a psychrophilic count (7 days at 5°C—41°F) in freshly pasteurized milk is limited because of the possibility that very small numbers, less than 1 per ml, may be present initially and yet may

multiply within a few days to such an extent that spoilage occurs. Several techniques have been suggested to overcome this problem of quickly detecting small numbers of organisms in the sample.

*Incubation of sample prior to plating.* Although a psychrophilic count is recognized as that count obtained when samples are plated and incubated for 7 days at 5°C (41°F), it should be noted that incubation temperatures of 21°-25°C (70°-77°F) are also in the temperature range where essentially all psychrophilic types of importance in milk supplies will grow rapidly. Therefore it is possible to use incubation temperatures of 21°-25°C (70°-77°F) to obtain information regarding these particular organisms. Frequently it has been advantageous, with samples where the psychrophilic count is known to be so low that no organisms could be detected in a 1-ml sample, to subject the milk to 18-20 hours' incubation at 21°-25°C (70°-77°F) before plating. This procedure will not result in an accurate count, but it will indicate whether or not these organisms are present in the sample. It also should be noted that the additional criteria of typical colony formation and characteristic odour development should be met in the plates for the organisms to be considered psychrophilic types of importance in spoilage problems.

Galesloot (1955) has commented that incubation of samples prior to plating is a satisfactory procedure for the detection of post-pasteurization contamination of milk. He suggests incubation at 15°C (59°F) for 24 hours for the detection of the psychrophilic types and incubation at 20°C (68°F) for 48 hours or 27°C (81°F) for 24 hours for the detection of non-thermoduric streptococci.

The problem of the keeping quality of pasteurized milk varies in different parts of the world: in some areas milk is kept refrigerated from the time of processing to the time of consumption; in other areas home refrigeration facilities do not exist. In areas where there is no refrigerated storage after the milk leaves the processing plant, it is even more essential that the plant operator should know how long his product will remain in a saleable condition.

A review of available reports shows that most investigators agree that total bacterial counts, coliform counts or reduction tests made on the product immediately after processing have little or no value in predicting the shelf-life of the milk under the conditions prevalent in the home of the consumer. All investigators recommend that quality control tests, if they are to have any value, should be run on samples of the product that have been held at 17°C (63°F) for 24 hours.

Provan & Rowlands (1949) suggested that reduction tests would provide a direct measure of keeping quality if the samples were incubated at 18°C (65°F) for a period of 18-24 hours before the test was run. Samples not reducing methylene blue or resazurin in half an hour at 37°C have a keeping quality in excess of one day at 18°C.

Bertelsen, Mattsson & Dufeu (1956) recommended that coliform counts should be made on the product immediately after processing and again after 24 hours' incubation at 17°C. For good keeping quality, pasteurized milk should not contain over 100 coliform bacteria per ml after the incubation treatment. These authors also suggest that the reduction time for milk incubated for 24 hours at 17°C should exceed 5 hours if a shelf-life of 48 hours at 17°C is to be assured.

Berger & Anderson (1949) suggested that the incubation test (24 hours at 17°C) could be applied to line-run samples as a means of detecting "after-infection". Total counts, coliform counts and dye-reduction tests are carried out on various samples from the line as well as on the finished product.

*Membrane-filter method.* Modifications in the membrane-filter technique have been proposed that would permit the passage of 5-10 ml of a milk sample through the filter and thus collect all of the organisms present in the sample on the membrane. Incubation of the membrane on suitable media at 21°-25°C might give a reasonably accurate psychrophilic count. This modification is aided by the development of the DA membranes reported by Fifield et al. (1957) in their studies on the enumeration of coliforms by the membrane-filter technique.

*Special media.* Another proposal has been to add low concentrations of penicillin to the plating medium and incubate at 21°-25°C for 2-3 days.

This method has proved to be quite satisfactory for the detection of psychrophiles in cottage cheese.

Although the above-mentioned methods would permit the enumeration of psychrophiles more quickly than the procedures described in *Standard Methods*, no data are available to indicate the correlation between the early detection of small numbers of organisms and the subsequent spoilage of the product.

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