Part III

LABORATORY CONTROL

CHAPTER 10

WHY THE CONTROL LABORATORY IS NECESSARY [g]

Careful supervision at every stage in milk handling is essential to ensure that the consumer receives a safe supply of pasteurized milk of good keeping quality. In this, laboratory control plays an all-important part. By the use of suitable laboratory tests, detailed information may be obtained about the bacteriological quality of the raw milk and its suitability for pasteurization, about the efficiency of the heat-treatment process itself, and about the care taken to clean and sterilize all milk-handling plant and so to prevent re-contamination of the milk after pasteurization.

It is obviously desirable to institute some system of official supervision, to lay down standards of quality for the raw milk received at the processing plant and for the pasteurized milk distributed to the consumer, and to carry out tests frequently to ascertain whether producers, processing plants, and products comply with the standards imposed.

To ensure at all times that the finished milk will comply with official standards, it is necessary to make regular tests on samples taken at different points in the handling. To carry out such control effectively, a laboratory at the processing dairy provides the most satisfactory arrangement. Methods of testing have been evolved which are relatively simple and cheap to carry out, and this makes it possible for all but the smallest pasteurizing units to undertake their own laboratory control.

Supervision is likely to prove most effective where a close and friendly relationship exists between the producers, the processing dairy, and the official supervising authority. This will be fostered by frank discussion between the parties concerned of the methods of testing, the results obtained, and the possible causes of failure to comply with the standards. It is well to remember that in control work of this kind the techniques of testing have an important bearing on the results, and that it is essential to standardize, as far as possible, the techniques in use in the different laboratories.

It is generally more convenient, especially where the official control laboratory is some distance from the processing plant, for the plant laboratory itself to carry out most of the tests on the incoming raw milk, only occasional check tests being made officially.

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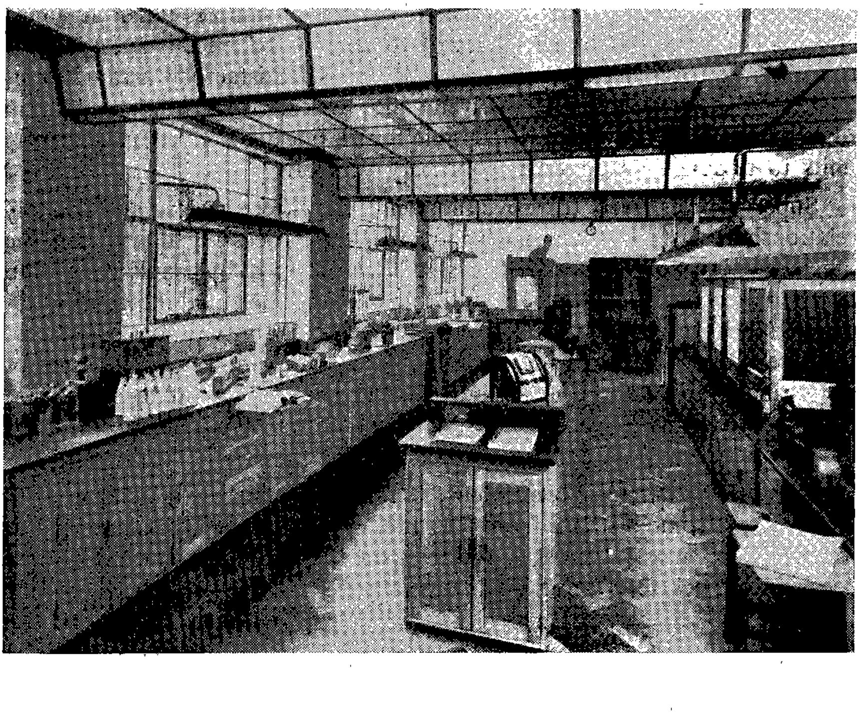
g Details of most of the tests mentioned in chapters 10-16 may be found in Davis.[8]

Whatever system is followed, it is highly desirable to report all test results to individual farmers so as to maintain their interest and to encourage the production of milk of the highest possible standard of quality. A differential system of payment to producers based on the results of tests may be well worth considering as an incentive to improvement. Alternatively, it may be possible to encourage competition by sending the results, arranged in order of merit under code letter, to each individual producer.

Laboratory control at the processing centre should be planned to yield the maximum information about technical efficiency at every stage. To forestall any possibility of defect in the processed milk reaching the consumer requires constant laboratory control at all operational stages and a detailed study of the results in relation to the actual conditions prevailing in the plant at the time of sampling. Fig. 88 shows the control laboratory of a large distributing dairy. The laboratory must indeed be regarded as an essential part of a modern milk-processing centre. By taking an active and sympathetic interest in its work, the official controlling authority can contribute materially to the effectiveness of such a laboratory.

FIG. 88. CONTROL LABORATORY OF A LARGE DISTRIBUTING DAIRY



Duties of the Control Laboratory

In planning the control laboratory, either that of the processing unit or of the official supervising authority, due consideration should be given to the number and types of test to be carried out, having regard to the quantity of milk to be handled. Such a laboratory need not be lavishly equipped, but it must be staffed by persons competent to carry out the necessary tests and to interpret the results in practical terms.

In general, the laboratory at the processing unit will be called upon to undertake the following work:

(1) Control of the incoming raw milk. This will involve regular testing of the milk from every producer by both bacteriological and other methods, and, where necessary, testing of samples taken during visits to farms where results indicate unsatisfactory production methods. In many pasteurization centres, the findings of this laboratory provide the basis for advisory work to the milk producer.

(2) Tests to determine the efficiency of the pasteurization process.

(3) Bacteriological and other tests on samples of milk taken at different points in processing, as well as on samples of the finished milk distributed to the consumer. This in turn will involve frequent checks of the efficiency of cleaning and sterilization of plant, including checks on the sterility of milk bottles and milk cans.

(4) Investigation (where considered necessary) of complaints from consumers concerning the keeping quality or other technical characteristics of the pasteurized milk distributed to them.

(5) Tests to determine the chemical or compositional quality of the incoming milk (particularly when payment is made on a quality basis (fat or non-fatty solids) or where the addition of water is suspected), and occasionally of the pasteurized milk. The routine determination of percentages of fat and non-fatty solids will not be dealt with here. (See Davis [8] or any modern dairy-chemistry manual.)

(6) Tests to determine whether preservatives have been added to the incoming milk. (See Davis [8] or any modern dairy-chemistry manual.)

CHAPTER 11

CONTROL OF RAW MILK

Pasteurization cannot eliminate the need for care in the production and handling of raw milk, and careless methods on the farm give rise to stubborn difficulties in the pasteurizing unit. As a result of poor farm technique, inadequate cooling of the milk, or delays in transport, off-flavours or even frank souring of the milk may occur before it reaches the pasteurizing unit. Quite apart from the question of souring, carelessly produced raw milk frequently contains large numbers of bacteria which are not killed by pasteurization. These heat-resistant, or thermoduric, bacteria are added to milk from the surfaces of improperly cleaned and sterilized milk equipment on the farm. Indeed, a determination of the number of thermoduric bacteria serves as a useful guide to the efficiency of cleaning and sterilization of the milk equipment by the milk producer.

Fortunately, these thermoduric bacteria are harmless to human health. In general, too, they have little effect on the keeping quality of the pasteurized milk. Nevertheless, some types may cause rapid spoilage of pasteurized milk in the house of the consumer, especially where facilities for keeping the milk cool are lacking. Moreover, thermoduric bacteria in the raw milk are frequently responsible for high bacteria counts in the bottled pasteurized milk, and may lead to failure of the latter to comply with the official standards imposed.

Careful scrutiny of every individual consignment of raw milk to determine its suitability for inclusion in the bulk for pasteurization is the first link in any system of effective control. Owing to the large number of individual consignments which has to be rapidly checked at the receiving platform, this link in the chain of control is, in many respects, the most difficult to establish effectively. In general, insufficient time is available to permit a detailed laboratory examination of samples before the milk is tipped. For this reason it is the usual practice to rely mainly on the initial examination of every can of milk by a person with a " trained nose ", experienced in the detection of off-flavours or other abnormalities, followed by a more-detailed laboratory examination of suspected consignments (which are kept on one side in the meanwhile) before finally reaching a decision as to whether or not to accept them for pasteurization.

This method of dealing with the problem falls short of the ideal, for unsatisfactory milk may escape detection even by an experienced platform examiner. Its shortcomings in practice largely disappear if it is supplemented by a system of routine control with detailed laboratory examination of raw milk from each supplying farm once or twice a month. The results so obtained will indicate those producers most likely to supply unsatisfactory milk. Future consignments of milk from such producers may then be more carefully scrutinized and, where time permits, examined daily by a rapid laboratory test before tipping.

Results of routine tests, if communicated regularly to producers, will stimulate interest in the bacteriological quality of the milk they supply. Such tests also form an objective basis for advisory work among producers and may, with advantage, be used in relation to differential or bonus payments, a system which has proved invaluable in many countries in encouraging farmers to improve methods of milk handling on the farm.

Added Water in Milk

Although the question is not directly concerned with pasteurization, the control laboratory of a milk-receiving depot or of a pasteurizing dairy may from time to time be confronted with the need to establish whether or not water has been added—deliberately or inadvertently—to raw or even to pasteurized milk. By far the most dependable method of ascertaining this is to determine the freezing-point of the milk, since added water brings the freezing-point from its normal figure (for both raw and pasteurized milk) of about —0.545°C towards that of pure water (0.0°C) to an extent depending on the proportion of water added. Details of the modern method are to be found in Davis [8] or in almost any up-to-date textbook of dairy chemistry. The freezing-point determination, even with the Temple modification of the Hortvet method, is rather slow, and only a limited number of tests could be performed per day by the laboratory of a pasteurizing dairy.

Rapid Platform-Tests

As previously stated, it is essential to examine each can of milk before it is accepted for pasteurization. Speed is vital and the usual method is to examine the milk in each can by smell immediately the lid is removed. Where the milk is frankly sour there should be no need to examine it further, but numerous cases will occur where doubt exists whether to accept or reject the milk. In such cases, the opinion of the checker should be reinforced by some objective laboratory test. Cases will also occur where it is desirable to apply a laboratory test to milk from known unsatisfactory producers, even though the checker fails to detect any abnormality by physical inspection.

The time factor limits the choice of tests to be applied to milk at the receiving platform. Three possible methods deserve consideration: the acidity test, the alcohol (ethanol) test, and the ten-minute resazurin test.

Acidity test

In the acidity test, milk is titrated with standard alkali, using phenolphthalein as indicator, and the result is calculated as "per cent lactic acid". The test really measures the buffering power of the milk (which changes with the percentage of non-fatty solids in the milk) as well as any lactic acid produced by souring bacteria. If fresh milk has a pH of 6.6, the calculated percentage of lactic acid will be the amount of alkali required to change the pH from this value to about 8.4, at which point the phenolphthalein changes colour. If, by the action of milk-souring bacteria, the pH drops to 6.3, the additional amount of alkali required to bring the pH to 8.4 would measure the degree of souring. But, since the acidity of milk samples from individual cows or from individual herds immediately after production varies, the calculated acidity figure for any one producer's milk on arrival at the pasteurizing dairy is an unreliable measure of the amount of acidity produced by bacterial action, which is the factor of real interest as a measure of souring.

Despite its convenience, this method cannot therefore be recommended as a test of the suitability for pasteurization of milk from individual herds. Nevertheless, where milk from a large number of herds is received in bulk, the acidity test may, with advantage, be used as one of the quick tests to be applied before the milk is accepted for pasteurization. For such milk any value in excess of 0.15% may reasonably be assumed to measure the amount of developed acidity.

Ethanol test

The ethanol test is the most rapid of the three tests mentioned above and is carried out simply by mixing equal volumes of milk and 68% ethanol; milk which curdles is regarded as unsatisfactory.

Recent research has shown that a positive result with this test occurs, on the average, only three to four hours before the milk curdles when it is boiled. It follows that milk which is negative by this test, and therefore accepted for pasteurization, may have undergone marked deterioration. Experience of the test has, in fact, shown that many milks giving a negative reaction by this test should be rejected as unsuitable for inclusion in the bulk for pasteurization.

There is also the further possibility that occasional samples of milk from individual herds which are in every respect satisfactory for pasteurization may react positively with 68% ethanol. The factor or factors responsible for this ethanol-instability of some milks, when quite fresh, are obscure.

The increased incidence of positive reactions with normal milk precludes the use of higher concentrations of ethanol in the test. Were it not for this, the use of, for example, 72% or 74% instead of 68% ethanol would improve the value of the test for the segregation of milks unsuitable for pasteurization.

Despite its convenience, therefore, the ethanol test cannot be considered seriously as a standard method for the rejection of milk on the receiving platform.

Ten-minute resazurin test

In Great Britain, the ten-minute resazurin test has been fairly recently accepted by most pasteurizing dairies as a quick objective means of determining the suitability of milk for acceptance at the dairy platform. Any cans of milk suspected by the platform examiner, as well as milk from known unsatisfactory producers, are now placed on one side and examined by this test before tipping.

In this test, 1 ml of 0.005% resazurin solution is added to 10 ml of milk in a stoppered sterile tube. After incubation at 37°-38°C for ten minutes, the stage of reduction of the dye is measured by comparison with a standard colour disc. The colours range from 6 initially to 0 when the dye is completely reduced.

The results after ten minutes are interpreted as follows:

Resazurin disc reading	*Action to be taken*
4-6	Accept for pasteurization
1-3½ (both inclusive)	Advisable to reject as unsuitable for pasteurization
0 and ½	Must be rejected

With the above interpretation, the ten-minute resazurin test sets a higher standard for milk for pasteurization than either the acidity or the relatively unreliable ethanol test. Moreover, experience has shown that it is risky—and the warmer the weather the riskier it is—to accept milk giving a disc reading of 3½ or less.

One of the main weaknesses of all rejection tests lies in the fact that some unsatisfactory milks are not detectable by sensory means on the platform. Unfortunately, it is rarely possible to apply the ten-minute resazurin test to every can of milk before tipping. For this reason it is imperative that full use should be made of routine tests to determine beforehand those producers whose milk should at all times be carefully scrutinized and, preferably, examined by the ten-minute resazurin test before tipping.

Routine Control Tests

In the selection of a test or tests for the routine control of raw milk, it should be borne in mind that the results should give as much information as possible not only about the suitability of the milk for pasteurization, but also about the quality of the work done on the farm. Further, in countries or districts where mastitis is prevalent or suspected, routine tests on each producer's milk for the presence of organisms associated with mastitis, or for the changes in the chemical quality of the milk which are usually brought about by such infections, are of obvious value both to the pasteurizing dairy and to the producer himself, who may not be aware of the presence of udder disease in his herd. Even the centrifuging of 10 ml of milk in a pointed centrifuge-tube often gives useful information. Tests for mastitis will not be referred to again in this monograph, but details of various laboratory procedures are to be found in Chalmers [1] and Davis. [8]

The methods available for routine control purposes may broadly be considered under three headings : bacteria counts, dye-reduction tests, and sediment tests.

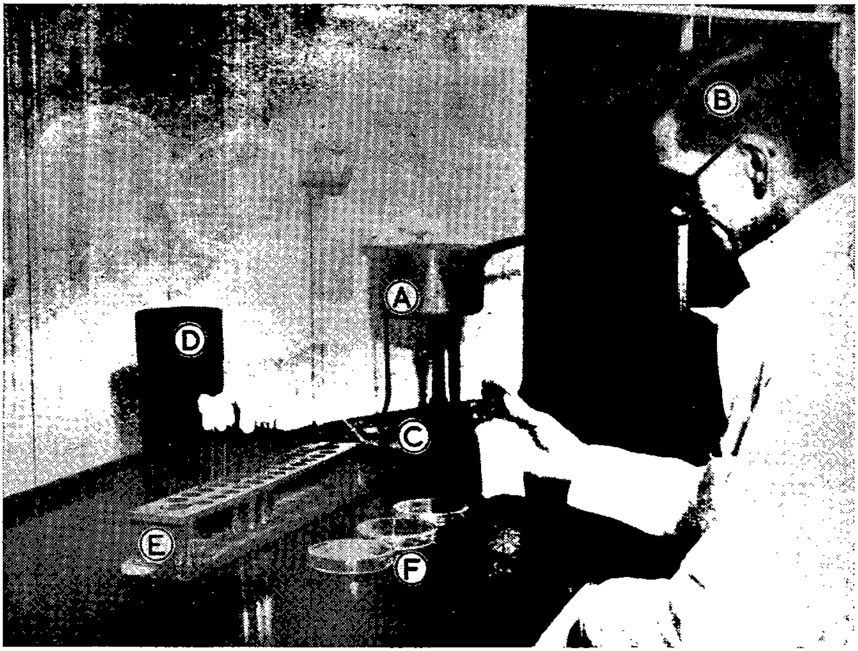
Bacteria counts

Because the results are readily understood by most producers, the bacteria-count method has, deservedly, enjoyed great popularity for routine control purposes (see fig. 89, 90, and 91). The well-known method using the conventional Petri-dish technique is rather expensive, cumbersome, and time-consuming; were it not for these disadvantages, the method would, undoubtedly, enjoy even greater popularity. In the newer roll-tube technique, the whole process is simplified, speeded up, and made cheaper by semi-mechanization (see fig. 92 and 93). The Breed smear, the Frost " little-plate ", the Burri smear, and the Van Oijen methods have, for the same reasons of economy and rapidity, found popular support for routine bacteria counts on raw milk.

It can be stated categorically that the colony-count method has no equal for routine control purposes where the majority of the raw-milk supplies reaching a dairy are of a high standard of bacteriological quality. Indeed, in these circumstances, there is no other test that can adequately distinguish the better from the less good producers. Where, however, the majority of producers are supplying milk of poor or mediocre bacteriological quality, there is little purpose in attempting differentiation between them by the colony-count method, since the counts are all likely to be too high.

For obtaining estimates of the number of thermoduric bacteria in raw milk, there is no satisfactory alternative to the colony-count method. The

FIG. 89. ESSENTIAL APPARATUS FOR PLATE COUNT



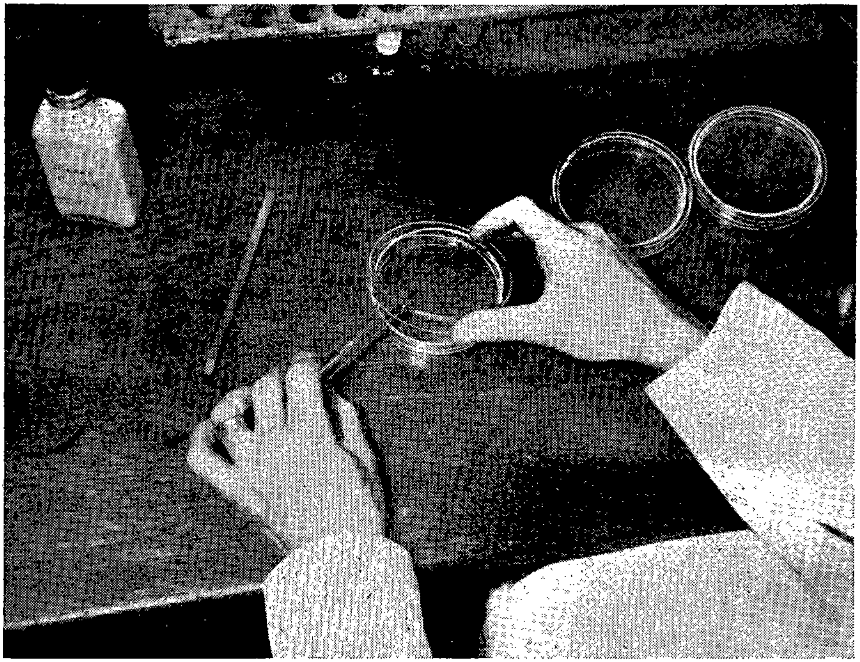
A = saucepan for melting agar medium
B = operator
C = metal tube containing sterile pipettes
D = metal box containing sterile Petri dishes
E = rack with tubes of sterile diluent
F = Petri dishes

routine examination of raw-milk samples for thermoduric bacteria gives valuable information about the efficiency of cleaning and sterilization of milk-handling equipment at the farm.

For the enumeration of thermoduric organisms in raw milk, it is necessary first to laboratory-pasteurize samples at 63°-64°C for 30 minutes, and then to count the surviving bacteria by either the Petri-dish or the roll-tube method. Counting by the Breed smear method is not satisfactory because of the difficulty of differentiation between dead and living bacteria in stained preparations.

From what has been said, it may be seen that the decision as to whether or not to use colony counts for routine control purposes must depend, in the main, on the general level of bacteriological quality of the raw-milk supplies under consideration. Where the majority of supplies is of a high standard, the colony-count method is indispensable. Equally where the majority of supplies is of a low standard, counts of thermoduric bacteria—after laboratory pasteurization—reveal information of great value.

FIG. 90. PLATE COUNT — I



The melted agar is poured on to the plate for mixing with the dilution of milk under test.

In some countries, particularly in America, the Breed smear method is in widespread use for routine control purposes. In this method the number of bacteria in stained preparations of the milk is counted under the microscope. The method is quick and, apart from the microscope, requires little equipment. The count, which includes the number of both the dead and the living bacteria originally present in the milk, is usually about four to five times that of the colony count. The method is not suitable for the examination of samples of low bacteria counts (under 500,000 per ml), but is adequate for differentiation with samples having high bacteria counts.

The Breed smear method may be used to obtain a rapid estimate of the number of stainable bacteria in milk. It is also claimed that experienced workers can differentiate between milks in which the majority of the organisms are derived from unsterilized utensils and those in which the high counts are the result of defective cooling. The method may also afford some indication of the existence of mastitis in the herd.

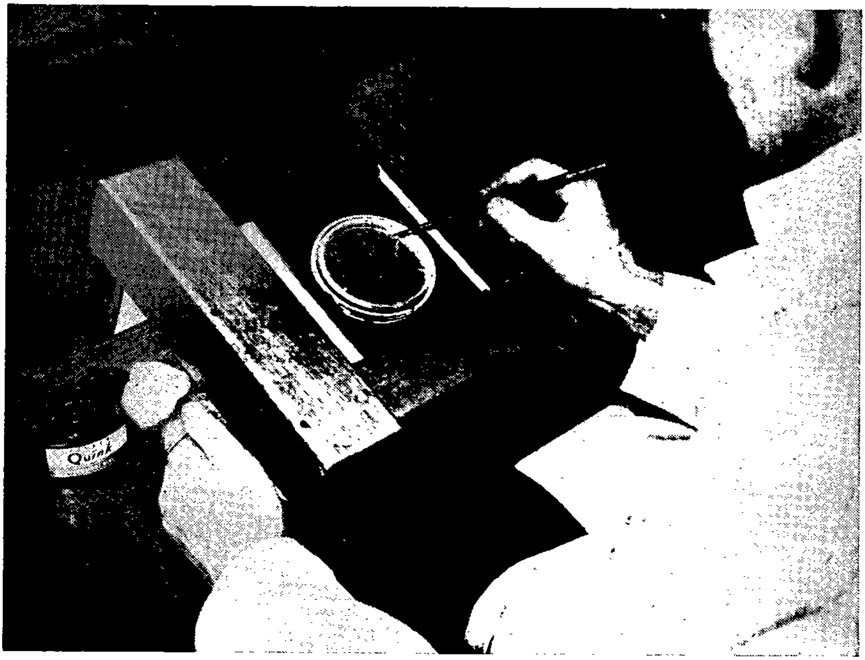
The Burri smear method, in which a standard platinum loopful of milk is smeared successively over two agar slopes and, after suitable incubation of the slopes, the colonies are counted directly by means of a lens, gives results less accurate than the plate (Petri dish) method. However, the test can be started in the field with the simplest of easily portable equipment. It is a method suitable for routine control when what is required is a simple and cheap means of giving an answer to the question as to whether a given milk is of good, bad, or indifferent bacteriological quality. As with the ordinary plate colony-count, the results are not available for some 48 hours.[8]

Dye-reduction tests

The methylene-blue and resazurin tests are in widespread use for the routine control of raw-milk supplies.

With methylene blue, there is only one end-point, represented by a change in colour of the dye-milk mixture from blue to white. With resazurin, the dye-milk mixture changes in colour from blue, through various shades of mauve, to pink and finally to white. Because of these

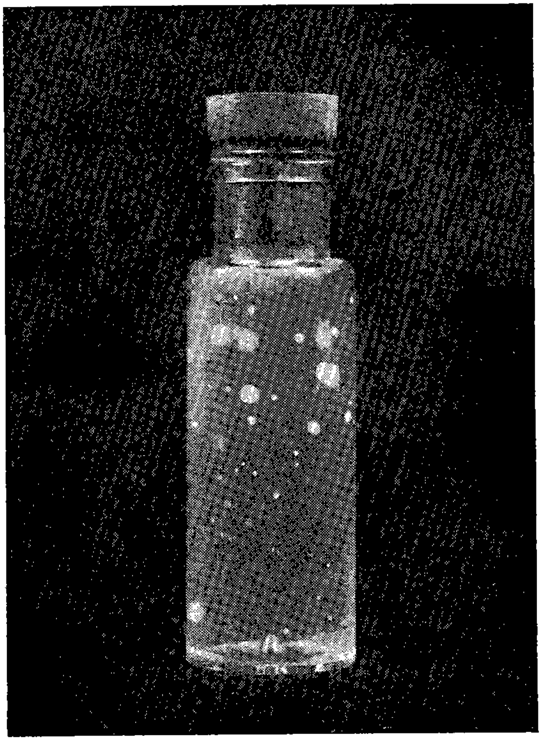
FIG. 91. PLATE COUNT — II



After a period of incubation, the colonies of bacteria are counted in a specially illuminated counting chamber. The operator is holding a tally-counter in his left hand.

intermediate stages of reduction with resazurin, which can be measured accurately by comparison with glass colour-standards (e.g., Lovibond disc or Munsell colour-standards), attempts have been made to shorten the test by recording the stage of reduction reached after incubation for a fixed period of time. Thus, the actual colour of the dye-milk mixture may be measured after incubation at 37°-38°C for, for example, one hour, instead of waiting until the dye is completely reduced from blue to white, as must be done with methylene blue.

FIG. 92. ROLL-TUBE METHOD OF COUNTING BACTERIA IN MILK

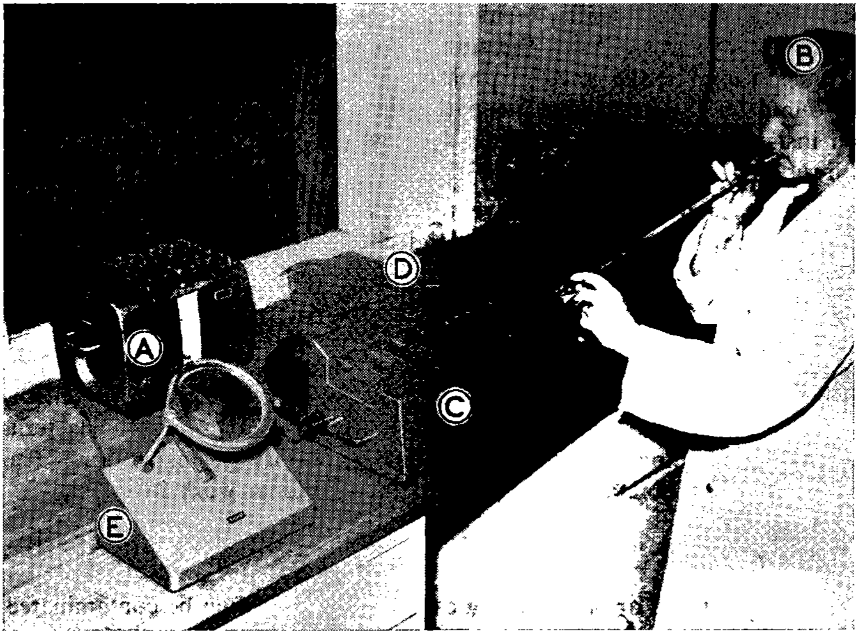


Bacterial colonies ready for counting are shown in the roll-tube.

Recent research has shown that it is not possible to forecast the time needed to complete the reduction of resazurin from the stage of partial reduction represented by the colour after a fixed period of incubation. Thus, two samples requiring the same length of time for complete reduction—for example, five hours—may show quite a different colour stage from one another after incubation for one hour. For this reason, the advantages claimed for the resazurin test as compared with the methylene-blue test are not as great as was at one time thought. Nevertheless, where a large number of samples has to be dealt with and time is an important factor limiting the number of routine tests that can be done, the use of the resazurin test is justified in practice.

In Great Britain, for example, the one-hour resazurin test is frequently used for routine control purposes. The test is carried out exactly as described earlier for the ten-minute test (page 171), but the disc number is

FIG. 93. APPARATUS FOR ROLL-TUBE METHOD OF COUNTING BACTERIA IN MILK



A = electrically-heated bath for keeping melted agar at 44°C
B = operator pipetting milk-dilution into roll-tube
C = apparatus for spinning tubes to obtain an even, thin layer of medium
D = wire basket for test-tubes
E = counting equipment, with lens

recorded after incubation at 37°-38°C for one hour. The results are interpreted as follows :

Resazurin disc reading	*Interpretation*
4-6	Satisfactory
1-3½	Doubtful
0 or ½	Unsatisfactory

The dye tests measure indirectly the activity of bacteria in milk. Thus, the greater the number of active bacteria present, the more rapidly will they reduce the dye added. It follows that samples with short reduction times, for example, one or two hours, are of poorer bacteriological quality than those which take four or five hours to reduce the dyes.

Not all the bacteria in milk participate in the reduction of dyes at 37°-38°C. Many of the thermoduric bacteria are particularly inert towards dyes, and other common milk bacteria are unable to multiply in milk at this temperature. Experience has shown that, for samples with reduction times in excess of four to five hours, erroneous conclusions regarding the true bacteriological quality of individual samples may be drawn from the results of dye tests. With the rather prolonged incubation at 37°-38°C, reduction may be the result of proliferation of types of bacteria which are favoured by this particular incubation temperature, but which play only an insignificant rôle in the actual spoilage of the milk at lower temperatures.

Where many producers have to be tested regularly, the dye tests, because they are relatively simple and cheap to carry out, may be used with advantage. This is especially true where the majority of supplies are of poor or mediocre quality. Because of the unreliability of results where reduction times are long (over five hours), the dye tests cannot adequately replace the colony-count method for the differentiation of samples of a high bacteriological quality. Where dye tests are used for routine control, periodic checks should be made of the number of thermoduric bacteria by using the colony-count method after laboratory pasteurization. The information so obtained fully justifies the additional work involved.

Sediment tests

Milk produced or handled in a careless way will often be contaminated by " visible dirt ", that is, dirt that is easily seen when the milk is filtered through a suitable filter disc or pad.

In general, it has been found that there is rather poor correlation between the amount of sediment as shown on such a filter pad and the keeping quality of the milk, which depends on the number and quality of the milk-souring organisms. Nevertheless, the sediment test, particularly when producers are of mediocre quality, or are still learning the elements of clean milk production, has considerable educative value. If the pads from tests with his own milk are shown to the dirty producer or cowman alongside of pads derived from properly produced milk, the object lesson is striking and may assist in eliminating some of the grosser types of carelessness.

The test is usually carried out by filtering or centrifuging a measured quantity of milk (obtained after shaking or stirring thoroughly the bulk to be tested) in a simple apparatus of which there are two or three types on the market. Satisfactorily produced milk gives no " visible dirt " ; poor milk gives a dark brown or blackish deposit on the pad. The amount of sediment is determined by comparison with an arbitrary set of standards prepared beforehand, and the usual method of assessment is by classifying in a few grades only, such as excellent, good, fair, bad, and very bad.

One advantage of this test is that the filter pads may be sterilized by drying and treating with formalin and passed back to the producer as visible evidence of at least one aspect of his production methods. With many milk producers, such evidence is more easily understood than a mere statement of the numerical result of a resazurin test, though the latter gives a far better assessment of probable keeping quality. (Details of sediment tests and apparatus may be found in Davis [8] and Harvey & Hill.[12])

CHAPTER 12

CONTROL OF PASTEURIZATION EFFICIENCY

In pasteurization, it is imperative that the time and temperature of heat-treatment shall be such as to ensure the destruction of all pathogenic organisms in the raw milk. Moreover, the greatest possible care must be taken to avoid admixture of the pasteurized milk with raw or partially treated milk containing living pathogens. Constant checking of the efficiency of the pasteurization process must, therefore, be regarded as one of the major functions of laboratory control.

Of the pathogens commonly found in raw milk, the tubercle bacillus is the most resistant to heat. Accordingly, in the pasteurization of milk the times and temperature of heat-treatment must be arranged so as to ensure complete destruction of this organism. In the commercial process, provision is made to ensure an ample margin of safety in its destruction.

Laboratory methods for the detection of tubercle bacilli and other pathogenic organisms in milk are too cumbersome and time-consuming to serve as a satisfactory routine check of the efficiency of pasteurization. Moreover, other routine bacteriological methods provide no information of real value about the pasteurization process in terms of its efficiency in the destruction of pathogens.

Phosphatase Test

A new approach to the problem of routine laboratory control of pasteurization was made in 1935 when Kay & Graham introduced their phosphatase test. Following Kay's observation that all raw milk contained an "alkaline" phosphatase, these authors established that this phosphatase was slightly more resistant to heat than the tubercle bacillus. They evolved a routine laboratory method for the detection of minute amounts of phosphatase in milk. It has now been established by experiment and by long experience of commercial pasteurization that milk which gives a negative phosphatase test may be assumed to have been subjected to heat-treatment, which ensures the complete destruction of tubercle bacilli and other less heat-resistant pathogens.

This test, with or without minor modifications, is now in widespread use as a routine method of laboratory control (see Davis [8] for further details). In the test, a measured volume of milk is added to disodium phenylphosphate buffered with sodium diethyl barbiturate and incubated at 37°-38°C for 24 ± 2 hours. The free phenol liberated by the action of any residual phosphatase is measured in terms of blue units using a standard Tintometer disc. The results are interpreted as in the following tabulation :

Lovibond blue units	*Interpretation*
Less than 2.3	Pasteurization satisfactory
2.3-6.0	Slight errors in pasteurization or slight addition of raw milk
Over 6.0	Major errors in pasteurization or addition of raw milk

The test will detect slight errors in pasteurization or the addition of as little as 0.2% raw milk to properly pasteurized milk.

Since the introduction of this test, various modifications have been suggested, but the principle of the test has remained unaltered. It has proved of the greatest value. With its introduction, faults in the running as well as in the design of some commercial plants then in use were revealed. Plant manufacturers were among the first to recognize the significance of the test and to make modifications in the design of plant to rectify the inherent faults that then existed. The test is now in use in several countries for the official control of pasteurized milk.

The laboratory of the processing dairy, also, will find the test of great service as a check on the efficiency of the pasteurization process. In routine control, the test should be applied to samples of the bottled milk at frequent intervals throughout each day's run. In this way, any errors in processing will be detected and the measures necessary to eliminate faults can quickly be undertaken.

It is desirable to emphasize the need for skill and precision in carrying out the phosphatase test in the laboratory. Carelessness will inevitably lead to false-positive results which will involve the management and laboratory staff in a tedious and fruitless search for non-existent faults in processing. This happened in one pasteurizing unit because a laboratory assistant, in rather carelessly pipetting milk samples during a test, contaminated some of them with saliva (which contains phosphatase).

As a check on the laboratory work, all reagents used must be tested for purity, and even an occasional sample of the filter-papers for phenols, using prescribed methods. Control tests, designed to detect the presence of phenols, must also be carried out on all milk samples which give positive results. For the latter purpose, all samples should be stored in a refrigerator until the result is available ; the control test may then be applied to those samples which give positive results.

Bacteriological Control of Pasteurized Milk

Three groups of bacteria are normally found in samples of bottled pasteurized milk, namely: thermoduric bacteria, thermophilic bacteria, and contaminants which enter the milk during handling after heat-treatment.

Most of the thermoduric bacteria are very resistant to heat and a high proportion survives the normal times and temperatures used commercially for the pasteurization of milk. Many are derived from carelessly produced raw milk; others may be added from the surfaces of improperly cleaned and sterilized plant at the pasteurizing unit itself. Fortunately, as already stated, these thermoduric bacteria are not pathogenic. They do not normally contribute materially to spoilage of pasteurized milk except where the milk is stored by the consumer at temperatures in excess of 18°-20°C—a not infrequent happening in the warmer countries.

The main source of thermophiles is the surfaces of hot sections of the pasteurization plant; relatively few of these bacteria are normally found in samples of the incoming raw milk. The majority form spores and their elimination from contaminated plant surfaces is, therefore, difficult. The remedy lies in scrupulous care at all times in the cleaning and sterilization of the hot-milk surfaces where thermophiles would otherwise thrive.

Temperatures in the range 50°-70°C are the most suited to the rapid multiplication of thermophiles. Thus, at the temperatures normally used for the holder process of pasteurization, the addition of a few thousand per ml to milk from plant surfaces at the beginning of a run may lead to counts of several million in the pasteurized milk after a continuous run of four or five hours. Indeed, experience has shown that, with the holder process, it is necessary to curtail the run to a period of four or five hours in view of the risk of spoilage by thermophiles where the run is further prolonged. Where continuous operation for longer periods is unavoidable, a break in the run to allow for flushing the hot sections first with cold and then with hot water at 83°-85°C is the only sure method of obviating trouble with thermophiles. If this precaution is not taken, thermophiles may lead to off-flavours or, in extreme cases, even to curdling of the hot milk.

At temperatures in excess of 70°C, the thermophiles normally do not multiply rapidly. Accordingly, they do not present the same serious problem in the HTST as in the holder method of pasteurization.

Once the pasteurized milk has been cooled, multiplication of the thermophiles ceases, so that, if precautions are taken to restrict their activities during pasteurization, this group of organisms presents no serious problem.

Bacteria of the third group are added from the surfaces of plant with which milk comes in contact after its heat-treatment. Their numbers are a direct reflection of the care taken to clean and sterilize these sections of plant. Of even greater importance is the part they play in the subsequent spoilage of pasteurized milk after its delivery to the consumer. Indeed, it is organisms belonging to this third group that are the main cause of poor keeping quality in pasteurized milk.

From what has been said, it will be seen that laboratory control should be planned to give information about the proportion of each of the three groups of bacteria mentioned and, in addition, to locate the sources of contamination with organisms of the third group, which have such an important influence on the keeping quality of the pasteurized milk.

Detection of thermophiles

Two methods may be used for the detection of thermophiles. A count of thermophiles may be made using Petri dishes or roll tubes which are incubated at 62°-63°C. These organisms also reduce dyes actively, and an estimate of the extent of infection may be obtained using either the resazurin or the methylene-blue test with incubation of the tubes at 62°-63°C. Either of these tests may be applied to samples of the pasteurized milk taken at intervals during the run. A reduction time of one hour or less indicates that the number of thermophiles has reached a dangerously high level. Processing should then be stopped and the hot sections flushed as indicated earlier.

Detection of thermoduric bacteria

In chapter 11, which deals with the laboratory control of raw milk, reference was made to the use of colony counts after laboratory pasteurization for the detection of thermoduric bacteria (page 172). Similarly, an estimate of the number of thermoduric organisms in pasteurized milk may be obtained by making a colony count after re-pasteurization of the sample in the laboratory.

If a colony-count standard is imposed officially for pasteurized milk, it is important to recognize that failure to comply with the standard may result from the use of raw milk with a high content of thermoduric bacteria and not necessarily from errors in handling at the pasteurizing dairy. Moreover, it almost invariably happens that the colony count of samples of the bottled pasteurized milk taken at intervals during the run fluctuates according to the thermoduric content of the raw-milk supply passing through the plant. Table II, an example from an actual run at a commercial dairy, illustrates the type of result that may be expected.

TABLE II. BACTERIA COUNT (PER ML) AT 30°C OF MILK SAMPLES FROM A COMMERCIAL DAIRY

Time (hours)	Raw milk	Raw milk — laboratory pasteurized	Bottled pasteurized milk	Bottled milk — laboratory pasteurized
8.00	5,400,000	41,000	39,000	34,000
10.00	3,200,000	7,500	10,500	14,300
11.00	6,400,000	112,000	125,000	102,000
12.00	970,000	28,500	24,300	21,400
13.00	1,200,000	51,000	58,000	46,000

Obviously, colony-count results for bottled pasteurized milk need careful interpretation. High counts may be due either to thermoduric organisms added from the raw milk, as in the example given in table II, or to contamination from unsterile plant surfaces after pasteurization. A colony count after re-pasteurization of the sample is the only satisfactory method of distinguishing these two possible causes of high colony counts.

CHAPTER 13

DETECTION OF POST-PASTEURIZATION CONTAMINATION

Because of the important role in the spoilage of milk played by bacteria which enter after pasteurization, the detection of these organisms is one of the major objects of laboratory control. The test for the presence of coliform organisms and the dye-reduction tests have proved valuable for this purpose. For the reasons already given, colony-count determinations are not particularly suitable.

Presumptive Coliform Test

Except on very rare occasions, members of the *coli-aerogenes* group of bacteria are killed by pasteurization. Accordingly, a test for the presence of this group of organisms is a valuable guide to the re-contamination of milk after pasteurization.

These organisms produce acid and gas from lactose and their detection in milk is a relatively simple matter. Bottled milk immediately after pasteurization, or after a period of refrigeration pending delivery to the consumer, should not contain coliform organisms in 1-ml or even 10-ml quantities if care has been taken to prevent re-contamination during cooling and bottling.

Methylene-Blue and Resazurin Tests for the Detection of Post-Pasteurization Contamination

The dye tests applied to samples of bottled milk immediately after pasteurization or after a period of refrigeration are not sufficiently sensitive for the detection of post-pasteurization contamination. However, they are eminently suited for this purpose if the tests are applied to milk samples after a period of incubation to permit multiplication of the contaminating bacteria.

For routine control purposes, the most satisfactory method is to incubate samples of the pasteurized milk at 18°-20°C for 24 hours and then to test with methylene blue or resazurin with incubation at 37°-38°C.

Under these conditions post-pasteurization contaminants will multiply rapidly; the types of thermoduric bacteria which multiply at this temperature are relatively inert in their action on methylene blue and resazurin. Indirectly, therefore, the time of reduction of these dyes at 37°-38°C reflects the extent of contamination of the milk, after pasteurization, with bacteria added from unsterile plant surfaces or containers.

Where samples are stored at temperatures much in excess of 20°C before the dye tests are applied, proliferation of thermoduric types, particularly streptococci, may occur, and these types may play an active role in the reduction of the dyes at 37°-38°C. In these circumstances, the dye tests are not specific for the detection of post-pasteurization contamination.

When applying a dye test after storage at 18°C, observations should be made at half-hourly intervals after the beginning of incubation at 37°-38°C. Heavily contaminated samples may be expected to reduce the dyes immediately or in less than half an hour. Observations should not be made beyond three hours for, with longer incubation times, it has been found that the dye tests, as indicators of post-pasteurization contamination, lose their sensitivity.

CHAPTER 14

CONTROL OF CLEANING AND STERILIZATION EFFICIENCY

The Plant

Sampling the first milk during its passage through the various sections of the plant provides the simplest and most reliable check on the efficiency of cleaning and sterilization. The samples are then examined using the methods already outlined. It is desirable that sampling cocks should be provided at suitable points in the plant; pipe-lines must not be disconnected for sampling.

Great care must be taken to exclude contamination in sampling. All sampling equipment must be sterilized in the laboratory and protected from contamination until required for sampling. Care must also be taken to ensure thorough cleaning and sterilization of sampling cocks on the plant before the samples are taken.

The exact points of sampling can be decided only in relation to the layout of each individual plant. In general, however, the aim should be to sample the milk at the following points:

(1) the raw-milk supply-tank;
(2) milk immediately after pasteurization and cooling;
(3) milk at the point of entry into the pasteurized-milk storage-tank;
(4) milk from the pasteurized-milk storage-tank;
(5) milk from the bottle filler;
(6) filled bottles.

To enable a strict comparison to be made between the results for such a series of samples, the same bulk of raw milk must be followed in its passage through the plant. The results given in table III serve to illustrate the method of interpretation.

The colony count after pasteurization of sample (1) and re-pasteurization of samples (2) and (6) shows that an appreciable proportion of the bacteria in the raw milk was thermoduric, and that few bacteria of this type were added to the milk from the surfaces of plant at the dairy. The absence of coliform organisms from 10 ml of milk and a methylene-blue reduction-time in excess of three hours shows further that virtually no contamination of the pasteurized milk occurred before its entry into the storage tank. The presence of coliform organisms in 1 ml and the drop in the methylene-blue reduction-time from three to one-and-a-half hours in sample (4) indicates appreciable contamination of the milk from the surfaces of the storage tank. Further contamination of the milk occurred either from pipe-lines leading to the bottle filler or from the bottle filler itself. Since sample (6) showed no further deterioration, it may be assumed that no contamination occurred from the milk bottle.

Such a series of samples of the first milk to be processed, taken daily, and accompanied by tests on bottles of milk sampled at intervals throughout the day, should provide all the information necessary to establish the efficiency of cleaning and sterilization of plant at the pasteurization unit.

To establish the exact source of contamination, suspected sections of plant may be swabbed. Swab samples must, of course, be taken some time before the pasteurization run begins so as not to interfere with the normal schedule of plant operation.

An estimate, using the colony-count method, is made of the number of bacteria recovered by swabbing a known surface area, e.g., 30 cm × 30 cm (one square foot), of different sections of plant. It has been found that counts per 900 cm^2 of less than 5,000 may be expected where milk-plant surfaces are properly washed and sterilized; counts in excess of 25,000 indicate negligence.

Bottle Washing

Bottle washing is a separate process requiring its own system of laboratory control.

The washed bottles may be tested for sterility using a standard rinse technique. Briefly, the method is to add 25 ml of sterile $^1/_4$-strength Ringer solution to the bottle to be tested, and to estimate the number of bacteria in the bottle using a colony-count method. Properly washed bottles should not contain more than one organism per ml of capacity.

Frequent checks, using the above method, should be made, at least one bottle being taken off each of, for example, six lines of the bottle-washer.

Maintenance of the correct strength and temperature of the detergent solution in bottle washing is all-important. Accordingly, the laboratory must test the alkalinity of these solutions as a regular routine. The alkalinity of the detergent solutions may be determined by simple titration with standard acid to the phenolphthalein end-point. From the results of tests carried out at intervals throughout the run, the laboratory will be in a

TABLE III. RESULTS OF TESTS ON MILK SAMPLES FROM A COMMERCIAL PLANT.

Sampling point	Colony count (per ml) * at 30°C	Colony count (per ml) * at 30°C after pasteurization or re-pasteurization	Coliform organisms (per ml) * at 37°C	Methylene-blue reduction time at 37°–38°C (hours) **
(1)	6,400,000	45,000	+0.001	not tested
(2)	53,000	42,000	—10	3+
(3)	49,000	not tested	—10	3+
(4)	58,000	not tested	+1	1½
(5)	56,000	not tested	+0.1	½
(6)	59,000	49,000	+0.1	½

* Tests were applied immediately after sampling.
** Before testing, samples were kept at 18°–20°C until 9.00–10.00 hours on the day after sampling.

position to indicate to the management the quantity of detergent (in solution) which must be added to maintain the desired concentration, and the frequency of such additions.

Bottles leaving the detergent section are for practical purposes sterile, provided the detergent solution is maintained at the correct concentration and temperature. However, re-contamination of the bottles may occur from the rinse waters. Accordingly, samples must be checked at intervals using the colony-count method with incubation at 30°C.

CHAPTER 15

BACTERIOLOGICAL CONTROL OF WATER-SUPPLY

When a new pasteurization plant is being planned, one of the most important requirements is an adequate and bacteriologically satisfactory water-supply. Grave risk to human health is involved if water of doubtful purity is used. This is no academic statement; serious epidemics have occurred in the past through the use of contaminated water for cleaning bottling machines or rinsing bottles. Accordingly, not only must there be careful scrutiny when planning the unit, but constant supervision of the bacteriological quality of the water-supply must be looked upon as an important function of laboratory control.

The standards to apply must be considered in relation to the local conditions which prevail. In general, colony counts will be made, as well as tests, for the presence of coliform organisms. The methods to be followed in the examination of the water-supply for a pasteurizing dairy should be based on the methods in general use in the country concerned for the examination of potable waters.

It is usual to attach greater significance to the colony count at 37°C than to that at 22°C, and to the presence of faecal than to that of non-faecal coliform organisms in the interpretation of results on potable waters. It may be emphasized, however, that bacteria growing at 22°C and the non-faecal coliform group of bacteria play an important part in the spoilage of milk. These facts should be borne in mind when dealing with water-supplies for any type of dairy plant.

CHAPTER 16

TRAINING OF STAFF FOR THE CONTROL LABORATORY

From the foregoing chapters it will be clear that, for effective laboratory control, a well-trained, competent, and responsible staff is needed. It cannot be too strongly urged that a control laboratory with a sufficient complement of reliable staff is an essential part of a modern pasteurizing dairy working on any but the smallest scale. Even in the small processing dairy which may not possess a laboratory, some form of regular laboratory inspection of the pasteurized product should be arranged with an outside laboratory competent to carry out all the necessary tests.

The training of staff for the milk-control laboratory is no easy task. In the larger dairies, the head of the laboratory is, preferably, a graduate in dairy science, chemistry, or bacteriology, or if not a graduate at least a person with high qualifications in one or other of these subjects. He may also act as scientific adviser to the organization owning or operating the dairy.

In countries where pasteurizing plants or other milk-processing plants are being installed for the first time, it is quite likely that neither skilled plant operatives nor trained laboratory personnel will at first be available. In such cases, it is highly desirable either to send abroad for further training a sufficient number of intelligent and trustworthy students, having, for example, a degree or diploma in chemistry, bacteriology, or engineering, to obtain a sound theoretical and practical knowledge of pasteurization and other forms of milk processing such as sterilization and dried-milk manufacture and the relevant laboratory-control methods, or, alternatively, to engage foreign experts for an agreed period to train sufficient future technical operatives, managers, and laboratory-control staff on the plant itself.

The former method, if time permits, is, from the long-range standpoint, much to be preferred. In many, if not most, countries in which pasteurization, sterilization, etc., have been, for years, established methods of milk processing, regular courses of instruction, specially designed for the training of those who are to be dairy technologists and technicians, take place. Students from overseas with a reasonable scientific background are usually accepted for such courses without difficulty, and have among other advantages the opportunity of seeing different types of plant in operation and observing at first hand how a wide variety of milk-processing problems and control questions are handled.

Once a competent nucleus of technical staff has been established in a country hitherto unused to milk processing, and plants of suitable size and performance are in action, the training of such further personnel as are needed for operation and control of any additional installations presents few difficulties. But even in these circumstances, it is obviously advantageous to continue the training abroad of a limited number of picked men from whom the technical leadership of the industry can be reinforced. The same applies not only to countries in the early stages of a milk-pasteurization programme, but also to countries where pasteurization and processing of milk are well established.