The Connecticut Three-Point Laboratory Program as an Aid to Control of Pasteurized Milk*

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HISTORICALLY, the standard plate count has been responsible for advances in milk sanitation that have resulted in milk supplies of better grade than were once thought possible. However, many now realize that advance in milk control cannot progress further until a better laboratory yardstick is used to measure the quality of milk. The recent changes in the standard plate count have been designed to make it a more usable method under modern conditions but even these have fallen far short of the desired end. In other words, the maximum effect of an outmoded tool appears to have been realized and we must substitute for it a new program not subject to the limitations of the old standby which has served so faithfully. Methods for accomplishing this have been available for some time. It is the practice of some laboratories to supplement the standard plate count with the test for coliform organisms and the phosphatase test on pasteurized milk. While this is a step forward, it must be realized that no plate incubated at a given temperature will develop colonies of all bacteria present in milk, whether raw or pasteurized. In fact, the direct microscopic method of counting is considered by many to be superior to the plate count for accurate work.

A controversy exists over the merits of the direct microscopic count on pasteurized milk. The focal point of this controversy is the contention of some that dead bacteria are counted by the direct method. To support this contention it is argued that many organisms observed in smears from some pasteurized milks do not grow on plates. That this is a poor argument is emphasized when the Committee on Standard Methods for the Examination of Dairy Products [1] recognizes the inability of the standard method to grow all bacteria present and urges the use of temperatures other than the standard ones for incubation of supplementary plates. As a matter of fact, there is good evidence [2, 3, 4, 5] that the bacteria which are killed by pasteurization are usually rendered unstainable.

It is pointless to continue this controversy here for the reason that as proponents of the direct count we are not seeking to duplicate plate count figures. We are, rather, seeking to set new standards, entirely apart and distinct from those used for plate counts, so that a method offering many adventages over any plate count may be used by the control official to judge milk quality. These standards must be just, and they must also be sufficiently strict to bring about control at least comparable to, and preferably better than, that achieved by use of current plate count standards. If these objectives are fulfilled, it matters little if some dead bacteria may be counted, especially since direct counts will include psychrophilic, mesophilic, and thermophilic bacteria, and even types too fastidious to grow on standard agar, and will do this without resort to additional platings at a series of incubation temperatures.

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Prior to 1935, interest in the direct microscopic bacterial clump count in Connecticut had been confined to its use as a means of investigating outbreaks of milk-borne disease, particularly streptococcal infections. However, in October, 1935, a survey [6] of pasteurizing plants in cooperation with the Dairy and Food Commissioner was initiated which demonstrated the usefulness of the method in detecting in the finished product heat-resistant bacteria not found by standard plate counts.

During 1936, a study [7] was made of the reproducibility of results on replicate samples examined by the standard plate count in several laboratories. The study demonstrated that even when portions of the same sample kept under identical conditions were examined by several workers in conformance with Standard Methods, there were factors uncontrollable by any practicable means that caused wide discrepancies between any two laboratories. Direct microscopic counts on these samples were considerably higher than plate counts, and were supported by high plate counts only in those laboratories in which observed conditions of incubation were most favorable.

Throughout these studies we were impressed by the rapidity with which the direct microscopic count could be made, as compared with the standard plate count and by the added information concerning a milk sample, gained by observing the types of bacteria present. Accordingly, during September, 1937, arrangements were made with the Dairy and Food Commissioner to carry out a survey [8] of milk and cream processed by pasteurizing plants in Connecticut. During that study, 773 samples of pasteurized milk and cream from 249 plants were examined by the direct microscopic clump count, the test for coliform organisms, and the phosphatase test. That was the beginning of the present three-point program for laboratory work on pasteurized milk.

As a result of that survey the following tentative standards were set in 1937:

1. *Direct microscopic clump count limits:*
   Grade A pasteurized milk—200,000 per ml.
   Grade B pasteurized milk—500,000 per ml.
2. *Coliform organisms:* Absent in 0.1 ml. (later, 1.0 ml.).
3. *Phosphatase test:* Less than 0.05 mg. phenol.

That the use of these standards has worked no hardship on dealers in good quality milk is shown by the fact that the standards have been in operation for four years and the only change has been to make them more rigid by modifying the requirement for coliform organisms to a provision that these be absent in 1.0 ml. (January, 1941).

Perhaps a word of explanation is necessary concerning the application of results obtained. None of the three laboratory tests as yet enjoys official recognition in existing laws and regulations in Connecticut. Nevertheless, all are being used by the State Dairy and Food Commissioner and by local health officers to improve milk supplies. Perhaps this would not be possible under a program which is entirely punitive in nature, but the Connecticut 3-point plan has proved to be a workable and effective means whereby milk control officials can readily check the condition of a given supply, and either make definite recommendations for improvement or concentrate upon specific items for investigation. These are indispensable means of furthering a control program which is largely based upon the educational approach. For the persistent violator hearings have so far been an efficient corrective force without resort to court action.

With respect to direct microscopic clump counts on pasteurized milk, it has been our experience that the great majority of samples are easily classed either as good or as very bad. Only a comparatively small percentage (less than 10 percent) presents a borderline picture. Table 1 shows the distribution

TABLE 1

PERCENTAGE DISTRIBUTION OF DIRECT MICROSCOPIC CLUMP COUNTS ON PASTEURIZED MILK

Range of Counts	*Percent Grade A Samples (Total 836)*	*Percent Grade B Samples (Total 2,255)*	*Percent Both Grades (Total 3,091)*
Below 100,000	70.0	50.6	55.9
100,000–180,000	11.0	13.8	13.0
200,000–300,000	4.1	7.0	6.2
330,000–480,000	3.6	5.5	5.0
500,000–1,000,000	11.3	6.5	7.8
Over 1,000,000	0.0	16.6	12.1

of counts on samples for one year (1939) to illustrate this point.

It will be seen that only 4.1 percent of samples of Grade A pasteurized milk fell into the group, with counts in the borderline range just exceeding the standard of 200,000 clumps per ml. Furthermore, only 6.5 percent of Grade B samples fell into what might be termed a broad borderline range extending from the 500,000 limit to 1,000,000 per ml. It will also be seen that to have lowered the limit for Grade B milk from 500,000 to 200,000 per ml. would have affected only 12.5 percent more of the samples. To have done so would have resulted in a compliance of 68.9 percent for all 3,091 samples. This figure is comparable to the result [9] obtained in New York State.

The results of our experience with this program over a five-year period are shown in Table 2, which gives the summary including over 15,000 samples. It will be noted that there is good correlation between the coliform determination and the direct count. When samples were classed as satisfactory on the basis of absence of coliforms in 0.1 ml., the direct count was slightly the more stringent standard of the two. When absence of coliforms in 1.0 ml. was required (1941), the coliform test compliance fell below the direct clump count. It is apparent that a more strict direct count standard (200,000 per ml.) for Grade B milk would result in even closer correlation between these tests on a compliance basis.

There is possibly little need to point out the true significance of a test for coliform organisms, but there are still some who persist in the idea that it is meant to provide an index of barn contamination. The coliform group is so broad and so widely distributed that this is readily seen to be fallacious.

TABLE 2

PERCENT COMPLIANCE OF SAMPLES OF PASTEURIZED MILK WITH TENTATIVE STANDARDS

Year	*1937*	*1938*	*1939*	*1940*	*1941*	*5 Years, 1937–1941*
Direct microscopic count [1]	63.1	78.8	77.0	79.4	74.6	77.4 (15,300 samples)
Coliform organisms [2]	70.0	74.7	84.7	82.7	65.3*	75.6 (15,452 samples)
Phosphatase test [3]	86.7	92.1	91.3	93.7	94.7	92.7 (15,438 samples)

[1] Grade A, fewer than 200,000 clumps per ml.; Grade B, fewer than 500,000 clumps per ml.
[2] For years 1937–1940 inclusive, coliforms not present in 0.1 ml.; for 1941, coliforms not present in 0.1 ml.
[3] Phosphatase values below 0.05 mg. phenol.
* Standards for coliforms changed in 1941; see Note 2 above.

percentage of samples complying with the tentative standards for the three tests by years, together with a five-year

Assuming a milk to have been properly processed, the test is of value only to indicate improper handling somewhere

between the end of the heat treatment and delivery to the consumer. Coliform organisms are not particularly heat resistant, and so those present in the raw milk are reduced by pasteurization at least to a minimum not detectable by the method used. Their reappearance is evidence either of contamination during cooling, bottling, or capping, or of inadequate refrigeration permitting multiplication of the minimal number which may have survived pasteurization.

There seems little need to discuss the phosphatase test except to point out that it rounds out and completes the three-point program. It is the test most directly related to the safety rather than to the quality of the milk, and so is indispensable.

The advantages of the three-point program for pasteurized milk may be listed as follows:

1. The direct microscopic clump count presents a more nearly true picture of the bacteriological quality of a milk supply than does the plate count, and provides a much more rapid, more complete, and hence more effective laboratory service to the control official.
2. Tests for coliform organisms, furnishing evidence of improper handling after pasteurization, yield supplementary information correlating well with the direct count.
3. The phosphatase test yields the only positive information obtainable on the effectiveness of pasteurization, and hence is an indispensable index of probable safety of a supply.

The combination of these three laboratory tests is superior to any combination which includes the standard plate count as a means of estimating bacterial numbers; in pasteurized milk, no one of the three should be omitted from a well balanced control program. Standards for the direct clump count which are entirely separate and distinct from those for the standard plate count have been applied for four years in Connecticut without hardship to milk dealers. Application of the three-point program on a state-wide basis in Connecticut has resulted in a laboratory service giving essential information not otherwise obtainable to milk control officials with a minimum of delay.

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