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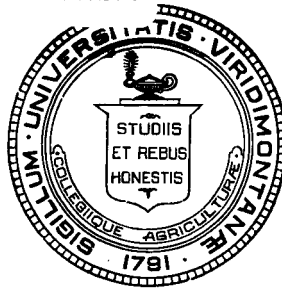
LIGHT AND TEMPERATURE AS FACTORS  
IN THE METHYLENE BLUE  
REDUCTION TEST

By J. M. FRAYER

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The relation of light and previous holding temperature to the proper conduct and understanding of the methylene blue reduction test has not heretofore been clearly apprehended. Samples were taken from miscellaneous herd milks at five periods. i.e. (a) when fresh, (b) at once after cooling to 40° F., (c) after holding for 24 hours at 40° F., (d) after holding for 48 hours at 40° F., and (e) after holding for 72 hours at 40° F. Three sets of duplicates were drawn at each sub-sampling period. Reduction tests were conducted simultaneously under three degrees of light, i.e., (a) complete darkness, (b) ordinary room light, and (c) artificial light. Plate counts were made of many of the samples for purposes of comparison.

It appears that both artificial and natural light exert a pronounced reducing action upon methylene blue in market milk as a substrate. This factor is quite within the control of the test operator, who should conduct the test in reduced light or in the dark.

Holding milk at a low temperature in some way retards the reduction of methylene blue, a fact which every test operator should appreciate.

## **BULLETIN 374: LIGHT AND TEMPERATURE AS FACTORS IN THE METHYLENE BLUE REDUCTION TEST**

By J. M. FRAYER

The methylene blue reduction test has come to be a very popular measure of the quality of milk. Its equipment is inexpensive, its results quickly secured and its conduct readily mastered by untrained workers. Perhaps the very fact that it is so simple and so easily used leads laymen to deem it infallible. For this reason attempts are continually being made to place interpretations on test results which seem to be considerably out of line with the facts and, furthermore and naturally, its technic has varied more or less with the individual idiosyncrasies of the operator.

It is probably true of this test, as it is of many others which have come rapidly into popular use, that its obvious advantages have obscured the importance of certain factors which are fundamental to its correct conduct. Even when faultily operated the results secured have not been without value. Even in its early days, when all its environmental and operative limitations were not clearly apprehended, much constructive work of lasting value was done and such is still being done. No one, least of all the writer, would seek to undermine public confidence in its usefulness or to advocate curtailment or abandonment. However, a clearer conception of its limitations and of the several factors qualifying its use should make it a more accurate and serviceable diagnostic agency.

Many attempts have been made to correlate bacterial counts with reduction times. Lay operators have clamored for tables whereby reduction times might be readily translated into supposedly equivalent bacterial counts. It is sometimes erroneously held that the latter, with all their inherent inaccuracies, should be made the yardstick whereby the efficiency of other quality tests must be measured in order properly to be understood. Many careful students of this problem have found an exact correlation to be impossible in individual cases because of the wide limits of variation. Indeed, so far as the methylene blue reduction test is concerned, it has been possible to classify milk into only about four classes.

It was early found that different types of organisms varied widely in their ability to reduce the dye. Hammer (1) says that "the variations in reducing powers of different species indicate that correlation between the reduction time and the number of organisms present originally can not be an exact one." It is said in "Fundamentals of Dairy Science"

(2) that, "the evident disagreement between counts and reduction times may arise from differences in counting technique, enumeration of non-reducing bacteria, or inability of reducing varieties to multiply on the media used. Far more important than these inevitable experimental discrepancies is the fact that in some species the reducing activity is much more intense than in others. The relation of reduction time to numbers present would then depend to some degree on the nature of the predominant flora." In other words, different organisms vary in their oxygen requirements for growth and it is assumed that the rate of oxygen consumption determines that of reduction. This dictum has been generally accepted as a plausible explanation of the lack of a more definite correlation between reduction times and bacterial counts. However, it has been held that this is not as much of a handicap in practical usage as might appear since most milk samples contain a mixed bacterial flora in which organisms which reduce the dye rapidly are usually in the majority. But this assumption would seem to be true only within limits, since previous holding temperatures largely govern the predominant type of flora actually in the ascendancy at the time of sampling. In view of results obtained at this Station (5), as well as of unpublished data in the writer's files, he is led to reason that many of the organisms which grow, perhaps somewhat slowly, at the lower temperatures are among those types which do not rapidly bring about dye reduction.

It is felt that the significance of the two factors, light and holding temperature of milk previous to sampling, should be understood by every test operator to assist him in properly using it and in interpreting the results. The one is within his control; the other, of course, is not.

Whitehead (3) has observed that methylene blue added to fresh milk is quickly reduced in the presence of sunlight at 37° C. (although he does not state whether the sunlight was direct or filtered through glass), and that such reduction is not due to enzymic action. He suggests that sunlight catalyses an oxidation-reduction reaction in which unsaturated fats are oxidized and methylene blue is reduced. Aikens and Fay (4) confirm his conclusions that reduction by light is a reaction distinct from that induced by bacteria and add that, "artificial light hastened the reduction of methylene blue in market milk. Light from a 75-watt electric lamp induced a potential drift in milk differing only in degree from that observed in the case of sunlight. The reduction of methylene blue in one sample of milk was hastened 2.5 hours by exposure to light from an electric bulb."

As previously noted by the writer (5, 6), reduction times when market milk is held at low temperatures increase up to between 48 and

72 hours of holding time (at 40° F. or below), the bacterial counts often increasing at the same time. In these earlier trials it was found that the reduction times were considerably shortened when the milk had been quickly cooled to and held at 40° F. for 24 hours and then warmed to and held at 60° F. for a few hours. Conceivably quite different types of organisms may have gained the ascendancy during the six to eight hours 60° F. holding period or else the previous holding temperature is a factor in the result irrespective of and apart from the bacterial content.

A series of trials were planned with the view of further checking the effect of the holding temperature of milk previous to sampling and including the factor of light exposure during the test.

#### PROCEDURE

Samples were taken from miscellaneous sources in order to secure widely divergent bacterial floras. The original samples (herd milks) were sub-sampled in duplicate at five periods:

- (a) When fresh (before cooling and within one-half hour after milking);
- (b) At once after cooling to 40° F. (a process which took on the average 20 minutes);
- (c) After holding for 24 hours at 40° F.;
- (d) After holding for 48 hours at 40° F.;
- (e) After holding for 72 hours at 40° F.

Each set was transferred with sterile 10 cc. pipettes into sterile test tubes into which had been previously measured 1 cc. of methylene blue dye solution of such strength that the final mixture had a concentration of one part dye to 200,000 parts of milk. At each sub-sampling period three sets of duplicates were drawn in order to make the reduction tests under three different conditions of light:

- (a) Complete darkness (water bath located in a photographic dark room);
- (b) Ordinary room daylight (water bath located in the center of laboratory, nine feet from the nearest window but not in direct sunlight);
- (c) Brilliant electric light (water bath located six inches under a 100-watt electric bulb; some daylight filtering in from the sides).

The water baths were of the usual open-top type and were held at 38° C. ( $\pm 1^\circ$ ). During 30 series, additional samples were drawn at each sub-sampling period for plate counts, "Standard Methods of Milk Analysis" technic being used.

The purpose of these trials was to observe the effects:

- (a) Of previous holding temperatures in relation to time held;
- (b) Of different degrees of light;
- (c) Of light in relation to holding temperatures and times and vice versa.

#### REDUCTION TIMES OF ALL SAMPLES

The data in table 1 and graphically displayed in figure 1 indicate that the degree of light to which milk samples are subjected during the test has an important bearing upon the result (for detailed data of individual samples, see appendix table 1, pages 22 to 25). Six and one-tenth percent of the samples held in absolute darkness reduced in less time than when held under the 100-watt light; 8.5 percent reduced in the same time; and 85.4 percent required more time, ranging from 15 minutes to over six hours, the average reduction time increment for all samples being about one and three-fourths hours. Furthermore, it

TABLE 1.—AVERAGE REDUCTION TIMES INVOLVING 1,230 TESTS OF RAW MILKS FROM 82 MISCELLANEOUS SOURCES

Light conditions under which tests were made		Conditions of time and temperature				
		Fresh	At once	After	After	After
			after cooling to 40° F.	being held 24 hours at 40° F.	being held 48 hours at 40° F.	being held 72 hours at 40° F.
		Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.
100-watt light	Average	6:03	6:22	6:47	7:04	7:02
	Maximum	12:45	12:15	13:30	14:45	16:30
	Minimum	0:30	0:30	0:30	0:15	0:15
Ordinary room light	Average	7:33	8:01	8:38	9:06	8:53
	Maximum	13:57	14:08	15:20	16:35	16:25
	Minimum	0:30	0:30	0:30	0:15	0:15
Absolute darkness	Average	7:47	8:05	8:51	9:22	9:16
	Maximum	14:00	15:15	17:20	16:55	15:32
	Minimum	0:30	0:45	0:45	0:15	0:15

should be noted that one of the four fresh samples that reduced the dye sooner when held in absolute darkness than when held under the 100-watt lamp, required eight and one-fourth hours to accomplish that end while the other three required over 10 hours to effect reduction. These samples represented milks of low bacterial content and their reduction times were consequently more or less erratic. The fresh samples, held under the 100-watt light, reduced the dye in times ranging from a maximum of twelve and three-fourths hours to a minimum of 30 minutes, while those held in absolute darkness varied from a maximum of 14 hours to a minimum of 30 minutes. It was observed that in practically all cases of artificial lighting dye reduction was

less positive than usual, the end point being somewhat difficult to determine. It was also noted that when the tubes were carefully "up-ended" once (especial care being taken to incorporate little or no oxygen-laden air) the color was restored for a variable length of time. Conceivably the intense light failed to penetrate through to the tube center because of the opalescence of its contents, thus causing the dye located near its circumference to be the first to reduce. It should be remembered that the bacterial counts were theoretically identical in each of the three sub-samples of each series. This fact would seem to bear out the theory that the shortening of the reduction time due to the influence of either artificial or natural light is not in any way involved in the complexities of bacterial activity but that it is a phenomenon which has not yet been fully interpreted.

It is impossible to explain, with the information at hand, why the effect of light as a reducing agent is not more uniform. It would seem not illogical to conjecture that the reduction rate by light should be fairly constant, but this does not appear to be the case. If it is true, as Whitehead (3) suggests, that the fats function, then the varying composition of the different samples of milk used may explain this lack of uniformity. Conceivably, given a medium of constant composition and a constant bacterial flora, reduction data through the influence of light might be more nearly consistent. However, this much is certain that by conducting the test in the presence of light, either artificial or natural, a factor is introduced which seems to be unpredictable in its exact consequence and which possibly is prejudicial to the accuracy of the test.

Thus far the influence of light unaffected by the temperature has been considered. This could well be done when comparing the reduction times of fresh, previously uncooled samples. When, however, the possible variant of previous holding temperature is introduced, the

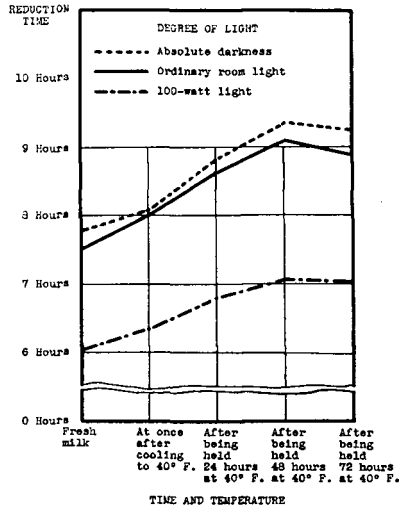


FIG. 1.—Comparison of the averages of 1,230 reduction tests involving raw milk from 82 miscellaneous sources.

results are of interest and seem significant. Referring again to the data in table 1 and figure 1, it will be noted that the maximum reduction times occurred under all three light conditions in those samples cooled to and held at 40° F. for 48 hours. Their average reduction times were greater than those of the fresh milk by about an hour (artificial light), by one hour and 33 minutes (diffused daylight) and by one hour and 35 minutes (absolute darkness). The average decreases in reduction times due to the light factor as compared to reduction times of samples tested in the dark appear in table 2.

TABLE 2.—AVERAGE DECREASE IN REDUCTION TIMES DUE TO THE LIGHT FACTOR AS COMPARED TO REDUCTION TIMES OF SAMPLES TESTED IN THE DARK.  
(TOTAL AVERAGES)

Conditions of time and temperature	Decrease due to	
	Room light	100-watt lamp
	Hrs. Min.	Hrs. Min.
Fresh milk .....	0:11	1:44
At once after cooling to 40° F. ....	0:04	1:43
After being held 24 hours at 40° F. ....	0:13	2:04
After being held 48 hours at 40° F. ....	0:14	2:18
After being held 72 hours at 40° F. ....	0:29	2:14

The individual sample data (appendix table 1, pp. 22-25) summarized in table 3, dealing with the reduction times of samples cooled to and held at 40° F. for 48 hours as contrasted with those secured with fresh samples, indicate that of those subjected to artificial light 26.8 percent reduced the dye more quickly when held than when fresh, 7.3 percent in the same time, while 63.3 percent exhibited increments of between 15 minutes and four hours and 2.5 percent of over four hours; that of those subjected to ordinary room light 17.1 percent reduced the dye

TABLE 3.—PERCENTAGES OF INDIVIDUAL REDUCTION TIMES OF SAMPLES HELD 48 HOURS AT 40° F., SHOWING INCREASE OVER REDUCTION TIMES OF SAME SAMPLES WHEN FRESH. (ALL SAMPLES)

Time distribution	Percentage distribution		
	Held under 100-watt lamp	Held at ordinary room light	Held in absolute darkness
	%	%	%
Less .....	26.8	17.1	12.2
Same .....	7.3	6.1	6.1
0-30 minutes .....	10.9	8.5	8.5
+30-1 hour .....	14.6	17.1	24.4
+ 1-2 hours .....	18.3	15.8	17.
+ 2-3 hours .....	13.4	14.6	14.6
+ 3-4 hours .....	6.1	10.9	7.3
Over 4 hours .....	2.4	9.7	9.7



more quickly when held than when fresh, 6.1 percent in the same time, while 66.9 percent exhibited increments of between 15 minutes and four hours and 9.7 percent of over four hours; and that of those kept in complete darkness 12.2 percent reduced the dye more quickly when held than when fresh, 6.1 percent in the same time, while 71.8 percent exhibited increments of between 15 minutes and four hours and 9.7 percent of over four hours. The influence of light counteracts to an appreciable degree the effect on dye reduction of previous holding milk at low temperatures.

The percentage increases over fresh samples attributable to previous holding temperature and the percentage decreases from reduction times obtained in absolute darkness which might be attributed to exposure to light appear in table 4. It is interesting to note that samples cooled to

TABLE 4.—AVERAGE REDUCTION TIMES OF ALL SAMPLES, SHOWING PERCENTAGE INCREASES OVER REDUCTION TIMES OF FRESH SAMPLES DUE TO HOLDING CONDITIONS AND PERCENTAGE DECREASES FROM REDUCTION TIMES OBTAINED IN ABSOLUTE DARKNESS, DUE TO LIGHT CONDITIONS

Conditions of time and temperature	Light conditions under which tests were made								
	100-watt light			Ordinary room light			Absolute darkness		
	Reduction times	Percentage increase over fresh	Percentage decrease from samples held in darkness	Reduction times	Percentage increase over fresh	Percentage decrease from samples held in darkness	Reduction times	Percentage increase over fresh	
	Hrs. Min.			Hrs. Min.			Hrs. Min.		
Fresh milk .....	6:03	0.	-22.2	7:33	0.	-2.9	7:47	0.	
At once after cooling*.....	6:22	+ 5.8	-21.2	8:01	+ 6.2	-0.8	8:05	+ 3.8	
After being held 24 hours†...	6:47	+12.1	-23.4	8:38	+14.3	-2.4	8:51	+13.2	
After being held 48 hours†...	7:04	+16.8	-25.1	9:06	+20.5	-3.5	9:22	+20.3	
After being held 72 hours†...	7:02	+16.2	-24.1	8:53	+17.6	-3.9	9:16	+19.	

\* To 40° F. † At 40° F.

and held at 40° F. for 48 hours when artificially lighted showed the maximum increase over the average reduction time of fresh samples and also the maximum decrease over the average reduction times of those held in darkness. As for the samples held in diffused daylight, it appears that the percentage increases over fresh samples due to holding at 40° F. are some larger and the percentage decreases over those held in absolute darkness are much smaller. The maximum decrease in samples thus held was attained in those which were held for 72 hours at 40° F. These figures are not deemed to be absolute in the sense of establishing a formula. However they do seem to show

an unmistakable trend. The averages include a wide range of reduction times varying from 15 minutes to 18 hours.

Did any difference exist in the effect of light and of previous holding temperatures on samples which reduced the dye slowly as compared with those which reduced it more quickly? And how should one separate the one from the other?

Differences of opinion exist as to the reduction time range within which the results are most likely consistently to check with actual keeping qualities. It is held by some investigators that reduction times of five hours or less are not as erratic as those that are beyond the point, while others hold that reduction times up to 10 hours are reasonably dependable. However, this study does not concern itself so much with the accuracy of the test as with the determination of possible differences existing between the effects of light and temperature on mixed milks falling in the high and in the low range of reduction times. Accordingly the 82 samples studied were divided into two groups, consisting respectively of those reducing in six hours or less and those taking more than six hours to reduce.

#### REDUCTION TIMES OF SIX HOURS OR LESS

For this purpose all reduction times have been taken of any sample which reduced the dye in six hours or less when fresh and when held in absolute darkness. The averages, involving 345 tests together with the maximum and minimum reduction times included therein, are shown in table 5 and are graphically displayed in figure 2.

TABLE 5.—AVERAGE, MAXIMUM AND MINIMUM REDUCTION TIMES OF MILK SAMPLES REDUCING IN SIX HOURS OR LESS WHEN FRESH AND TESTED IN ABSOLUTE DARKNESS

Light conditions under which tests were made		Conditions of time and temperature				
		Fresh	At once	After	After	After
			after cooling to 40° F.	being held 24 hours at 40° F.	being held 48 hours at 40° F.	being held 72 hours at 40° F.
		Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.
100-watt light	Average	3:09	3:40	3:45	3:32	2:57
	Maximum	5:30	6:45	6:30	7:30	8:22
	Minimum	0:30	0:30	0:30	0:15	0:15
Ordinary room light	Average	3:45	4:18	4:51	4:50	4:08
	Maximum	5:50	9:08	11:30	12:15	12:45
	Minimum	0:30	0:30	0:30	0:15	0:15
Absolute darkness	Average	3:46	4:20	4:51	5:09	4:30
	Maximum	6:00	9:15	12:00	12:15	12:45
	Minimum	0:30	0:45	0:45	0:15	0:15

The average reduction time of the fresh samples held under artificial light was 37 minutes less and those held in diffused daylight a minute less than the average for those held in absolute darkness. After being cooled to and held at 40° F. for 72 hours the average reduction time of samples held under artificial light was one hour and 33 minutes less and of those held in diffused daylight 22 minutes less than the average of those held in absolute darkness. Apparently the increased reduction times due to holding the milk for a long period at low temperatures, progresses up to a certain point faster than does the reducing effect of light. These data are exhibited in table 6.

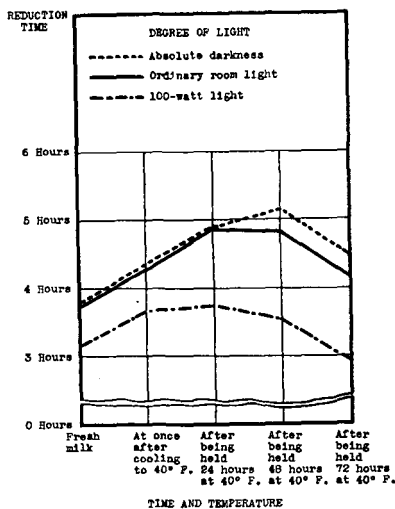


FIG. 2.—Average reduction times of milk samples reducing in six hours or less when fresh and tested in absolute darkness.

TABLE 6.—AVERAGE DECREASE IN REDUCTION TIMES DUE TO THE LIGHT FACTOR, AS COMPARED TO REDUCTION TIMES OF SAMPLES TESTED IN THE DARK. (SAMPLES REDUCING IN SIX HOURS OR LESS WHEN TESTED IN ABSOLUTE DARKNESS)

Conditions of time and temperature	Decrease due to	
	Room light	100-watt lamp
	Hrs. Min.	Hrs. Min.
Fresh milk .....	0:01	0:37
At once after cooling to 40° F. ....	0:02	0:40
After being held 24 hours at 40° F. ....	0:00	1:06
After being held 48 hours at 40° F. ....	0:19	1:37
After being held 72 hours at 40° F. ....	0:22	1:33

In not a single instance was the reduction time shown by the fresh milks shorter when held in darkness rather than in artificial light, in 26 percent the time elapsing was the same and in 74 percent it was greater, varying from 15 minutes to over two hours more. The trend seems to be positive enough, but the differences do not appear to follow any predictable pattern; indeed reduction times which originally were six hours or less were found to decrease from zero to two and one-half hours. Table 7 shows the reduction time percentage increases due to holding conditions over fresh samples and the reduction time percentage decreases due to light conditions from reduction times obtained in absolute darkness.

TABLE 7.—AVERAGE REDUCTION TIMES (INITIALLY SIX HOURS OR LESS) SHOWING INCREASES OVER REDUCTION TIMES OF FRESH SAMPLES DUE TO HOLDING CONDITIONS AND PERCENTAGE DECREASES FROM REDUCTION TIMES OBTAINED IN ABSOLUTE DARKNESS, DUE TO LIGHT CONDITIONS

Conditions of time and temperature	Light conditions under which tests were made							
	100-watt light			Ordinary room light			Absolute darkness	
	Reduction times	Percentage increase over fresh	Percentage decrease from samples held in darkness	Reduction times	Percentage increase over fresh	Percentage decrease from samples held in darkness	Reduction times	Percentage increase over fresh
	Hrs. Min.			Hrs. Min.			Hrs. Min.	
Fresh milk .....	3:09	0.	-16.3	3:45	0.	-0.4	3:46	0.
At once after cooling*.....	3:40	+16.4	-15.4	4:18	+14.6	-0.7	4:20	+15.
After being held 24 hours†...	3:45	+19.	-22.6	4:51	+29.3	-0.	4:51	+27.6
After being held 48 hours†...	3:32	+12.2	-29.4	4:50	+28.8	-6.1	5:09	+36.7
After being held 72 hours†...	2:57	-6.3	-34.4	4:08	+6.6	-8.1	4:30	+19.

\* To 40° F. † At 40° F.

It is possible that in cases where the degree of light did not seem to influence the results the samples were rapidly reducing, that, in other words, the effect due to natural bacterial increase was at least as strong as and concurrent with the reducing influence of light.

The maximum average reduction time prolongation which might be attributed to previous low holding temperatures was reached in samples held 24 hours when samples were subjected to room or artificial light and in samples held 48 hours when tests were made in the dark and were 36 minutes, an hour and six minutes, and an hour and 23 minutes respectively.

The trend indicated by the averages was not always manifested. Under the 100-watt lamp 39.1 percent of the samples tested after being held 48 hours reduced in less time than did the fresh samples, 21.7 per-

TABLE 8.—PERCENTAGES OF INDIVIDUAL REDUCTION TIMES OF SAMPLES HELD 48 HOURS AT 40° F., SHOWING INCREASE OVER REDUCTION TIME OF SAME SAMPLE WHEN FRESH. (REDUCTION TIMES INITIALLY SIX HOURS OR LESS)

Time distribution	Percentage distribution		
	Held under 100-watt lamp	Held in ordinary room light	Held in absolute darkness
	%	%	%
Less .....	39.1	26.1	17.4
Same .....	21.7	13.	0.
0-30 minutes .....	13.	13.	8.7
+30-1 hour .....	8.7	17.4	43.5
+ 1-2 hours .....	4.3	8.7	0.
+ 2-3 hours .....	8.7	8.7	17.4
+ 3-4 hours .....	4.3	8.7	0.
Over 4 hours .....	0.	4.3	13.

cent in the same time, 39.1 percent in 15 minutes to four hours more and 0 percent in over four hours more; in ordinary room light the corresponding percentages are 26.1, 13, 56.5 and 4.3; and in absolute darkness 17.4, 0, 69.6 and 13 (table 8). As has already been noted this grouping clearly indicates the fact that the reducing effect of light neutralizes to some extent the apparent retarding effect of previous holding at a low temperature.

TABLE 9.—AVERAGE, MAXIMUM AND MINIMUM REDUCTION TIMES OF MILK SAMPLES REDUCING IN MORE THAN SIX HOURS WHEN FRESH AND TESTED IN ABSOLUTE DARKNESS

Light conditions under which tests were made		Conditions of time and temperature				
		Fresh	At once	After	After	After
			after cooling to 40° F.	being held 24 hours at 40° F.	being held 48 hours at 40° F.	being held 72 hours at 40° F.
Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.		
100-watt light	Average	7:11	7:24	7:57	8:26	8:37
	Maximum	12:45	12:15	13:30	14:45	16:30
	Minimum	3:30	3:00	3:15	4:00	3:15
Ordinary room light	Average	9:02	9:28	10:06	10:45	10:43
	Maximum	13:57	14:08	15:20	16:35	16:25
	Minimum	6:15	6:15	6:15	5:45	5:15
Absolute darkness	Average	9:20	9:35	10:47	11:00	11:07
	Maximum	14:00	15:15	17:20	16:55	15:32
	Minimum	5:15	6:15	6:30	6:15	5:45

REDUCTION TIMES OVER SIX HOURS

All fresh samples which reduced the dye in more than six hours when held in absolute darkness were segregated as the basis for selection. The averages, together with the maximum and minimum reduction times included in the averages, are shown in table 9 and graphically displayed in figure 3. The average reduction times of fresh samples held in diffused daylight was 18 minutes less and of those held under the artificial light two hours and nine minutes less than those held in darkness. Further comparisons appear in table 10. As was to be expected, these figures are larger than those appearing in the pre-

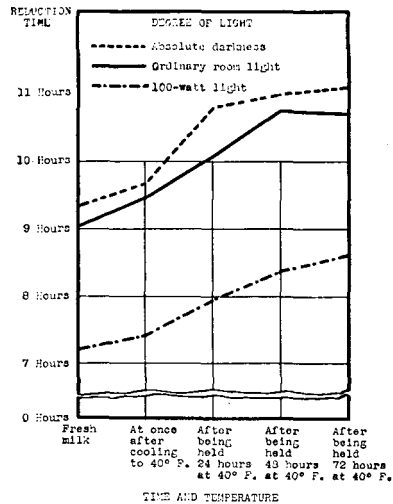


FIG. 3.—Average reduction times of milk samples reducing in more than six hours when fresh and tested in absolute darkness.

TABLE 10.—AVERAGE DECREASE IN REDUCTION TIMES DUE TO THE LIGHT FACTOR, AS COMPARED TO REDUCTION TIMES OF SAMPLES TESTED IN THE DARK. (SAMPLES REDUCING IN MORE THAN SIX HOURS WHEN TESTED IN ABSOLUTE DARKNESS)

Conditions of time and temperature	Decrease due to	
	Room light	100-watt lamp
	Hrs. Min.	Hrs. Min.
Fresh milk .....	0:18	2:09
At once after cooling to 40° F. ....	0:07	2:11
After being held 24 hours at 40° F. ....	0:44	2:53
After being held 48 hours at 40° F. ....	0:15	2:34
After being held 72 hours at 40° F. ....	0:24	2:30

ceding section and in tables 2 and 6, probably because the samples were subjected to light for a longer period of time.

Analyzing the original figures and comparing the reduction times of fresh samples held under the 100-watt lamp with the reduction times of those held in complete darkness, it will be noted that 8.4 percent reduced in less time in darkness than in the bright light, 1.7 percent in the same time, 74.6 percent in 15 minutes to four hours less time, and 15.3 percent in over four hours less time.

The maximum prolongation due to previous holding temperatures was reached in case of samples subjected to artificial light in the 72-hour series, being one hour and 26 minutes; in those kept in daylight, in the 48-hour series, one hour and 43 minutes; and in those kept dark in the 72-hour series, one hour and 41 minutes.

The percentage distribution of individual samples held 48 hours at 40° F. is set forth in table 11 wherein are shown variations from reduction times secured when the same samples were fresh. Among those that were held under the 100-watt lamp, 22 percent were less than when fresh, 1.7 percent the same, 73 percent from 15 minutes to four

TABLE 11.—PERCENTAGES OF INDIVIDUAL REDUCTION TIMES OF SAMPLES HELD 48 HOURS AT 40° F., SHOWING VARIATION FROM REDUCTION TIME OF SAME SAMPLE WHEN FRESH. (REDUCTION TIMES INITIALLY MORE THAN SIX HOURS)

Time distribution	Percentage distribution		
	Held under 100-watt lamp	Held in ordinary room light	Held in absolute darkness
	%	%	%
Less .....	22.	13.6	10.2
Same .....	1.7	5.1	8.3
0-30 minutes .....	10.2	5.1	6.8
+30-1 hour .....	17.	17.	18.6
+ 1-2 hours .....	23.7	18.6	23.7
+ 2-3 hours .....	15.3	17.	13.6
+ 3-4 hours .....	6.8	13.4	10.2
Over 4 hours .....	3.4	10.2	8.5

hours more and 3.4 percent over four hours more; among those held in ordinary room light, 13.6 percent were less, 5.1 percent the same, 71.1 percent from 15 minutes to four hours more and 10.2 percent over four hours more; and among those held in darkness 10.2 percent were less, 8.3 percent the same, 72.9 percent from 15 minutes to four hours more and 8.5 percent over four hours more.

The average reduction times (initially more than six hours) showing percentage increases over reduction times of fresh samples, due to holding conditions, and percentage decreases from reduction times obtained in darkness, due to conditions of light, are shown in table 12. As a

TABLE 12.—AVERAGE REDUCTION TIMES (INITIALLY MORE THAN SIX HOURS) SHOWING INCREASES OVER REDUCTION TIMES OF FRESH SAMPLES DUE TO HOLDING CONDITIONS AND PERCENTAGE DECREASES FROM REDUCTION TIMES OBTAINED IN ABSOLUTE DARKNESS, DUE TO LIGHT CONDITIONS

Conditions of time and temperature	Light conditions under which tests were made								
	100-watt light			Ordinary room light			Absolute darkness		
	Reduction times	Percentage increase over fresh	Percentage decrease from samples held in darkness	Reduction times	Percentage increase over fresh	Percentage decrease from samples held in darkness	Reduction times	Percentage increase over fresh	
	Hrs. Min.			Hrs. Min.			Hrs. Min.		
Fresh milk .....	7:11	0.	-23.	9:02	0.	-3.2	9:20	0.	
At once after cooling*.....	7:24	+ 3.	-22.8	9:28	+ 4.8	-1.2	9:35	+ 2.6	
After being held 24 hours†...	7:57	+10.6	-26.2	10:06	+11.8	-6.3	10:47	+15.5	
After being held 48 hours†...	8:26	+17.4	-21.8	10:46	+19.	-2.3	11:00	+17.8	
After being held 72 hours†...	8:37	+19.9	-22.5	10:43	+18.6	-3.6	11:07	+19.1	

\* To 40° F. † At 40° F.

rule, the better the milk the longer reduction is postponed by holding it at low temperatures previous to testing. This is as it should be, since in samples in which the initial bacterial population was small, and which were immediately cooled to and held at low temperatures, there were probably very few actively growing bacteria. Furthermore, those which were able to develop at all may have been of the psychrophilic types that are apt to reduce the dye slowly. On the other hand there probably was an initially large bacterial population in those samples which more promptly reduced the dye. It is usually held (7, 8) that these protoplasmic bodies, even though not actively growing, have some reducing power independent of the exhaustion of oxygen incident to and as a result of the growth process.

## BACTERIAL COUNTS

One hundred and fifty bacterial counts were made on 30 of these lots of milk, sub-samples being taken from the originals immediately prior to sub-sampling for reduction test work. The individual, average (geometric mean) of plate counts, maximum and minimum of the bacterial counts, and reduction times are set forth in detail in appendix, table 2, and summarized in table 13, averages being graphically displayed in figures 4 and 5. A great diversity of bacterial counts are

TABLE 13.—AVERAGES, MAXIMUM AND MINIMUM, OF 450 REDUCTION TIMES AND AVERAGES (GEOMETRIC MEAN), MAXIMUM AND MINIMUM BACTERIAL COUNTS OF 150 SAMPLES OF RAW MILK FROM 30 MISCELLANEOUS SOURCES, SHOWING RELATION OF TIME, TEMPERATURE AND LIGHT CONDITIONS

Time and temperature conditions	Bacterial counts		Light conditions under which tests were made		
			Reduction times		
	Colonies per cc.	Under 100-watt light	Ordinary room light	Absolute darkness	
			Hrs. Min.	Hrs. Min.	Hrs. Min.
Fresh milk	Minimum	1,000	0:30	0:30	0:30
	Maximum	730,000	12:45	12:45	12:45
	Average	29,200	6:21	7:27	7:47
At once after cooling to 40° F.	Minimum	600	0:30	0:30	0:45
	Maximum	560,000	12:15	12:45	14:00
	Average	26,500	6:42	7:48	7:48
After being held 24 hours at 40° F.	Minimum	1,100	0:30	0:30	0:45
	Maximum	1,260,000	14:00	14:22	14:45
	Average	41,000	7:06	8:11	8:22
After being held 48 hours at 40° F.	Minimum	800	0:15	0:15	0:15
	Maximum	2,500,000	14:45	15:15	14:45
	Average	51,000	7:06	8:24	8:47
After being held 72 hours at 40° F.	Minimum	600	0:15	0:15	0:15
	Maximum	2,600,000	16:30	16:00	15:30
	Average	69,000	6:51	7:59	8:32

involved in these averages. Some as low as 600 per cc. and some as high as 2,600,000 per cc. appear with reduction times ranging from 16 hours to 15 minutes. The number of cases entering into these averages is too meager to make them surely representative, yet the trend is marked enough to make it significant. In each series choice may be made of three reduction times for each plate count. They can not all be right.

The effects of both light and previous holding temperature are here traced in much the same fashion as in the preceding pages. Significantly, the average bacterial counts increase as the length of holding



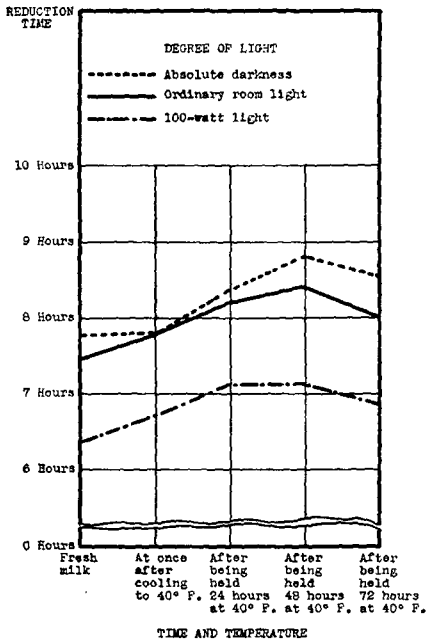


FIG. 4.—Comparison of the averages of 450 reduction times involving raw milk from 30 miscellaneous sources.

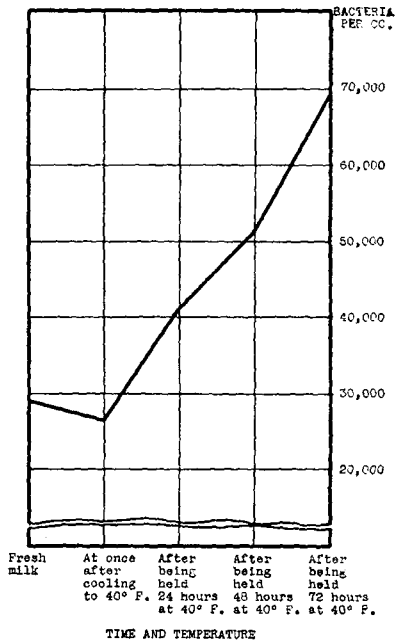


FIG. 5.—Comparison of the averages of 150 plate counts, involving raw milk from 30 miscellaneous sources.

time is extended, while at the same time the average reduction times increase up to 48 hours of holding time, the two tests showing opposite trends. For instance, exposed to diffused daylight fresh milk reduced

TABLE 14.—AVERAGES OF 120 REDUCTION TIMES (INITIALLY SIX HOURS OR LESS) AND 40 PLATE COUNTS INVOLVING RAW MILK FROM EIGHT MISCELLANEOUS SOURCES

Time and temperature conditions	Bacterial counts (geometric mean)	Average reduction times	Light conditions
		Hrs. Min.	
Fresh milk	108,000	3:04	Under 100-watt light
		3:36	Ordinary room light
		3:50	Absolute darkness
At once after cooling to 40° F.	89,300	4:04	Under 100-watt light
		4:14	Ordinary room light
		4:06	Absolute darkness
After being held 24 hours at 40° F.	181,000	3:17	Under 100-watt light
		4:22	Ordinary room light
		3:59	Absolute darkness
After being held 48 hours at 40° F.	293,000	2:55	Under 100-watt light
		3:45	Ordinary room light
		4:36	Absolute darkness
After being held 72 hours at 40° F.	581,000	2:15	Under 100-watt light
		2:36	Ordinary room light
		3:08	Absolute darkness

in seven and one-half hours, the geometric mean count being 29,000, while milk held 72 hours at 40° F. and similarly exposed reduced in eight hours, the geometric mean count being 69,000.

Here, as previously, are segregated all reduction times and plate counts of any sample which reduced the dye in six hours or less when fresh and held in darkness, the averages being shown in table 14 and graphically displayed in figures 6 and 7; and of all reduction times and plate counts of any sample which reduced the dye in more than six

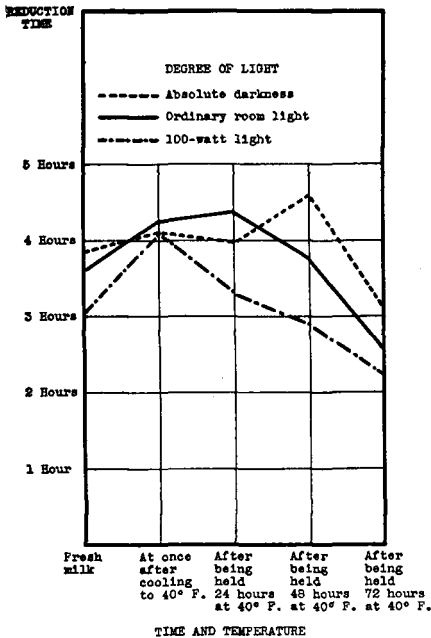


FIG. 6.—Comparison of the averages of 120 reduction times (initially six hours or less), involving raw milk from eight miscellaneous sources.

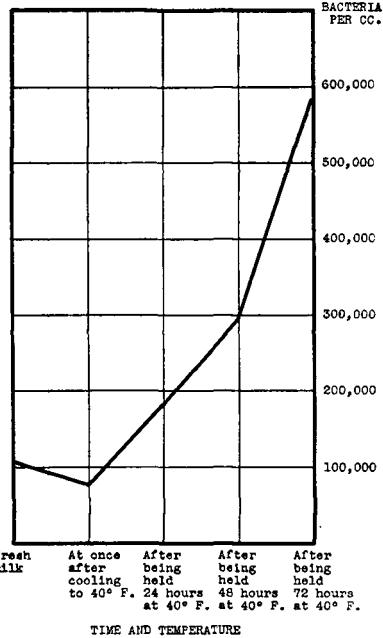


FIG. 7.—Comparison of the averages of 40 plate counts (reduction times initially six hours or less) involving the raw milk from eight miscellaneous sources.

hours when fresh and held in darkness, averages appearing in table 15 and graphically displayed in figures 8 and 9. The trends are much the same as have been previously discussed, differing only in degree. As might have been predicted the average reduction times of six hours or less increase for a shorter time due to the influence of previous holding at a low temperature and bow more quickly to the reducing effect of bright light.

LIGHT AND TEMPERATURE IN METHYLENE BLUE REDUCTION TEST 19

TABLE 15.—AVERAGES OF 330 REDUCTION TIMES (INITIALLY GREATER THAN SIX HOURS) AND 110 PLATE COUNTS INVOLVING RAW MILK FROM 22 MISCELLANEOUS SOURCES

Time and temperature conditions	Bacterial counts (geometric mean)	Average reduction times		Light conditions
		Hrs.	Min.	
Fresh milk	19,700	7:21		Under 100-watt light
		8:36		Ordinary room light
		9:12		Absolute darkness
At once after cooling to 40° F.	18,300	7:29		Under 100-watt light
		8:53		Ordinary room light
		8:55		Absolute darkness
After being held 24 hours at 40° F.	26,100	8:15		Under 100-watt light
		9:21		Ordinary room light
		9:41		Absolute darkness
After being held 48 hours at 40° F.	30,500	8:22		Under 100-watt light
		9:49		Ordinary room light
		10:04		Absolute darkness
After being held 72 hours at 40° F.	36,500	8:15		Under 100-watt light
		9:37		Ordinary room light
		9:44		Absolute darkness

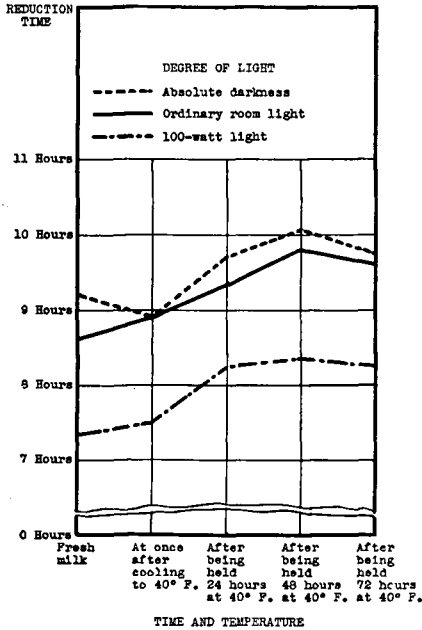


FIG. 8.—Comparison of the averages of 330 reduction times (initially more than six hours), involving raw milk from 22 miscellaneous sources.

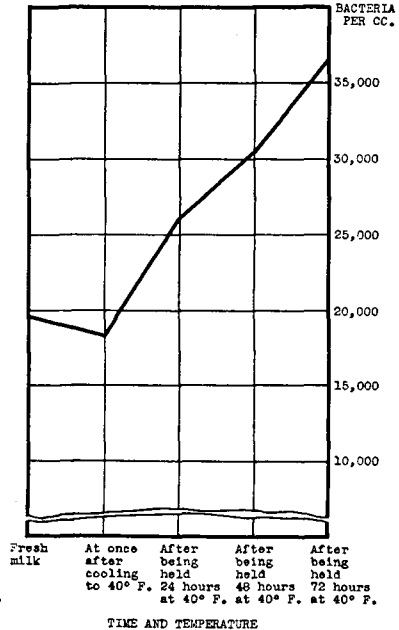


FIG. 9.—Comparison of the averages of 110 plate counts (reduction times more than six hours), involving raw milk from 22 miscellaneous sources.

## GENERAL DISCUSSION

The methylene blue reduction test is ordinarily conducted at a creamery or receiving station under varying degrees of light. Water baths with glass tops and sides are sometimes used in which the samples are subjected to the maximum of ordinary light which at times may be intense. Constant temperature incubators, containing one or two electric light bulbs as a part of their heating mechanism, are sometimes used in which at least a part of the samples are subjected to a flood of light and all to more than ordinary light. Frequently tests are conducted in all-metal baths open only at the top, which may occupy almost any conceivable place in the laboratory, before a window, under a bright artificial light or in a dark corner. Under such conditions the light would hardly ever be twice of the same intensity. This factor affecting the test has not received the attention that its importance merits.

The test as applied to market milk is predicated upon the time taken to reduce the methylene blue dye. This process is tacitly assumed to be accomplished solely through bacterial activity. It is this function which lends itself to its use as a quality test and obviously reduction of the dye by any agency unrelated to quality should be eliminated. The test should be conducted in the dark or at least in greatly reduced light. The general adoption of this procedure would seem to be a forward step in the removal of a variable within the control of the operator. Of course the tubes must be brought into the light to determine the end point.

The important consideration in connection with the effect upon reduction time of previously cooling milk to and holding at a low temperature is that a sample with a comparatively high bacterial content thus treated may require a longer reduction time than another sample of lower bacterial content which represents milk which has not been so cooled and held. Of course this is not invariably true but it is usually the case. Only one temperature has been used in these studies, that of 40° F., but the principle is amply indicated.

No certain explanation of this phenomenon can be advanced at present, but it is quite possible that bacterial reproduction is slowed down at low temperatures to a minimum, that only those bacteria which find life bearable at such temperatures reproduce at all and that these types may be slow oxygen consumers. In this connection a theory previously advanced by the writer (5) may be cited.

“Conceivably the balance between bacterial action and dissolved oxygen may be a controlling factor. It may happen that the proportion

of oxygen to active bacteria in milk, handled under certain conditions with a varying bacterial flora, will be less than in the case of milk that has been cooled to and is held at a low temperature. Under these later conditions the psychrophiles are likely to be passing through a period of readjustment or lag phase of their growth. Under such conditions the spread between the amount of dissolved oxygen and the actively growing bacteria is likely to increase for a time and thereby retard the reduction of the methylene blue dye, at the very time when the numbers of bacteria are slowly increasing. Oxygen—other than that consumed in bacterial growth—may be evolved to a greater or less degree, depending upon the temperature of the milk. Furthermore, during this period of readjustment the ratio of bacteria using relatively large amounts of oxygen may be narrowing. Hence it is that the reduction time may actually increase until the stage has been reached where normal balance is restored between oxygen which is being freed from solution, oxygen which is dissolved in the milk and oxygen which is used by growing bacteria." Although this factor is not within the control of the operator of the test, it should be understood in any appraisal of quality.

#### CONCLUSIONS

1. Light, either artificial or natural, exerts a pronounced reducing action upon the dye methylene blue in market milk as a substrate. This is within control of the operator. The test should be conducted in reduced light or in the dark.

2. Holding milk at a low temperature in some way retards the reduction of methylene blue, a fact which every operator of the test should appreciate.

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APPENDIX TABLE 1.—REDUCTION TIMES OF 82 SAMPLES OF RAW MILK WITH 410  
MADE UNDER THE FOLLOWING

Sample number	Held under 100-watt light + reflector + room light					Held in	
	Fresh	At once after being cooled to 40° F.	After being held 24 hours at 40° F.	After being held 48 hours at 40° F.	After being held 72 hours at 40° F.	Fresh	At once after being cooled to 40° F.
		Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.		Hrs. Min.
11	7:30	7:20	7:25	8:20	8:30	8:15	10:05
12	7:00	7:05	7:10	9:05	8:30	8:30	10:05
13	6:50	7:35	8:05	8:45	10:10	7:50	9:50
14	6:50	5:55	6:35	8:30	9:47	8:50	10:05
15	6:35	6:45	7:20	10:05	7:55	9:50	11:00
16	7:05	7:30	7:05	8:50	7:25	7:28	8:15
17	3:20	3:45	3:35	3:05	1:55	4:05	4:15
18	10:35	10:22	10:50	10:50	14:25	13:57	14:08
19	1:50	2:05	2:05	1:35	1:25	2:20	2:30
20	6:12	6:30	7:35	6:05	8:10	13:05	13:22
21	6:20	5:45	7:40	8:30	10:00	8:50	10:15
22	4:50	5:45	6:10	5:15	4:45	5:50	6:30
23	3:05	3:45	4:10	3:00	1:00	3:35	4:00
24	5:20	5:45	6:25	5:15	4:00	5:20	5:15
25	12:05	11:30	10:10	12:45	11:00	13:12	13:30
26	5:50	6:00	8:25	8:10	8:45	13:05	15:15
27	6:15	6:15	6:30	7:45	7:30	8:52	9:15
28	6:00	6:00	6:15	8:00	7:30	7:30	8:45
29	1:15	1:00	0:30	0:30	0:15	1:15	1:00
30	1:30	1:30	1:15	0:45	1:00	2:00	1:45
31	7:00	8:30	11:00	10:15	10:30	8:30	8:38
32	6:00	6:30	6:30	6:30	3:38	10:52	12:00
33	8:30	7:38	9:00	9:30	10:30	9:22	9:15
34	6:30	7:00	7:30	7:45	8:45	8:30	8:30
35	2:45	3:00	3:15	2:45	1:00	3:45	3:45
36	7:38	9:00	8:00	9:30	8:15	8:30	7:45
37	7:45	9:45	11:00	10:15	11:00	11:15	12:00
38	3:45	5:38	6:30	8:00	9:08	11:15	12:15
39	8:30	8:45	7:45	7:00	10:00	8:45	9:45
40	6:30	6:15	6:15	7:00	8:45	7:00	7:30
41	5:00	5:00	5:15	6:00	5:30	5:00	5:45
42	5:30	5:15	6:30	7:30	8:22	5:30	6:15
43	10:45	11:00	11:00	10:00	10:00	11:30	10:15
44	5:00	4:45	6:30	8:45	8:52	9:30	11:45
45	7:15	8:45	7:15	7:45	7:00	9:00	7:15
46	6:15	6:45	6:45	7:15	7:00	7:22	7:45
47	3:45	4:45	5:45	6:00	4:00	5:00	6:45
48	7:00	7:15	6:45	6:45	6:30	6:45	6:45
49	11:00	10:30	10:45	11:45	13:08	10:15	10:00
50	3:45	4:38	5:45	7:15	7:08	5:30	9:08
51	6:45	6:30	8:00	6:15	9:00	9:45	8:45
52	3:30	3:45	6:00	5:45	7:00	4:45	5:15
53	3:30	3:30	3:30	3:30	1:15	3:30	3:45
54	4:00	3:52	4:30	5:45	6:15	4:45	5:30
55	11:15	10:30	10:15	10:45	11:30	9:45	10:30
56	3:52	3:00	3:15	5:45	5:52	8:15	9:45
57	6:45	6:45	7:00	7:30	8:15	11:00	10:15
58	3:30	5:45	5:45	6:15	7:00	7:15	7:15
59	3:30	4:15	5:15	6:15	4:00	6:15	6:45
60	2:30	2:45	3:15	2:30	3:00	3:00	2:45
61	9:00	10:00	10:08	11:00	10:30	9:30	9:45
62	1:00	1:15	2:00	2:00	3:00	1:30	1:30
63	7:15	6:45	7:45	8:00	6:45	9:15	9:45
64	7:15	7:30	7:30	7:00	6:45	7:30	8:30

LIGHT AND TEMPERATURE IN METHYLENE BLUE REDUCTION TEST 23

OBSERVATIONS—THE TESTS OF MILK HELD FOR VARIOUS PERIODS AS INDICATED BEING TEMPERATURE AND LIGHT CONDITIONS

ordinary room light			Complete darkness				
After being held 24 hours at 40° F.	After being held 48 hours at 40° F.	After being held 72 hours at 40° F.	Fresh	At once after being cooled to 40° F.	After being held 24 hours at 40° F.	After being held 48 hours at 40° F.	After being held 72 hours at 40° F.
Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.
11:55	13:50	13:50	9:30	9:20	11:55	14:20	14:15
11:00	13:20	13:00	10:15	10:35	9:40	13:35	14:30
10:35	11:30	11:40	7:42	9:50	10:35	11:30	12:40
9:05	13:00	14:55	8:35	9:50	10:58	14:30	14:55
11:35	14:50	15:40	10:35	12:08	12:12	13:50	15:32
8:35	9:20	9:10	8:15	8:42	9:20	9:25	8:55
4:20	4:35	2:55	3:50	4:15	4:05	4:35	3:25
15:20	16:35	16:25	14:00	14:05	17:20	16:55	15:25
2:20	2:05	1:25	1:50	2:15	2:20	2:28	1:40
9:20	9:20	8:25	10:50	11:45	10:20	10:05	9:25
12:10	12:22	14:00	9:05	11:00	13:10	13:00	14:15
7:25	7:30	6:45	5:05	5:15	8:25	8:00	7:30
4:40	4:15	2:00	3:35	4:30	5:25	4:30	2:45
6:10	6:00	5:45	5:20	5:15	6:25	6:00	6:45
12:40	15:15	13:45	12:35	14:00	13:25	14:30	14:15
15:48	15:45	15:45	13:35	15:15	15:55	15:45	15:00
10:00	9:30	9:00	9:15	9:30	11:00	9:15	9:30
10:15	9:45	9:45	7:22	8:00	10:00	10:15	11:15
1:00	0:30	0:15	1:15	1:00	1:00	0:30	0:15
1:45	1:45	1:15	1:30	1:45	2:00	2:15	1:45
12:15	13:15	13:52	9:08	8:52	12:15	13:00	13:45
12:15	13:45	11:30	11:52	11:38	12:30	11:45	11:45
12:30	12:30	13:00	10:30	11:15	11:15	12:30	10:52
10:00	9:22	9:22	8:30	8:15	9:00	9:30	11:30
4:15	4:00	2:15	3:45	4:15	4:00	4:00	2:00
8:00	8:15	7:22	8:30	8:00	8:45	8:30	8:00
10:52	12:08	14:00	11:30	11:45	11:15	13:15	14:00
13:00	13:30	14:15	12:30	12:45	13:45	13:30	13:30
12:00	12:15	12:22	10:15	11:00	12:00	13:00	13:00
7:30	9:00	9:00	7:15	8:00	8:22	10:00	9:52
5:45	8:45	8:00	5:15	5:45	7:15	7:45	9:00
6:30	8:15	7:53	6:30	6:15	6:15	8:30	8:15
10:45	11:00	11:15	9:30	10:15	10:22	11:15	10:22
12:15	10:45	12:08	10:30	10:00	11:08	11:30	12:22
9:08	10:45	11:15	9:30	9:00	8:45	11:08	10:15
8:30	9:45	9:45	7:45	7:45	8:38	9:15	10:38
5:30	6:00	5:00	5:15	6:15	7:30	6:00	5:30
7:15	7:30	7:30	7:15	6:45	7:30	7:15	7:15
10:52	12:00	13:22	10:30	10:15	11:00	12:38	13:30
11:30	12:15	12:45	6:00	9:15	12:00	12:15	12:45
9:30	10:45	9:00	10:30	9:45	9:30	10:45	11:00
7:45	8:15	8:45	4:45	5:00	8:00	9:00	8:30
4:30	4:30	4:30	3:30	3:45	4:00	4:00	2:30
8:00	7:30	8:52	4:45	5:00	6:30	7:15	10:00
10:15	11:00	11:00	9:45	10:00	10:08	10:45	11:15
10:00	9:15	9:30	7:45	9:30	10:45	9:45	8:45
11:15	11:00	11:45	11:00	10:30	11:15	11:15	11:45
9:15	9:30	9:45	6:45	7:45	9:30	11:00	11:00
8:30	8:30	6:00	5:15	6:45	9:00	9:45	8:00
3:30	3:30	2:45	2:45	2:45	3:45	3:45	3:00
11:00	11:00	11:30	10:15	10:45	11:15	11:15	11:45
2:30	3:30	5:30	1:30	1:30	2:30	4:15	4:15
10:30	10:45	11:30	9:15	9:15	11:15	11:00	11:15
7:30	8:30	5:30	8:00	7:30	7:45	8:00	7:15

APPENDIX TABLE 1.—REDUCTION TIMES OF 82 SAMPLES OF RAW MILK WITH 410  
MADE UNDER THE FOLLOWING TEMPERATURE

Sample number	Held under 100-watt light + reflector + room light					Held in	
	Fresh	At once after being cooled to 40° F.	After being held 24 hours at 40° F.	After being held 48 hours at 40° F.	After being held 72 hours at 40° F.	Fresh	At once after being cooled to 40° F.
	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.
65	11:45	11:15	13:30	14:45	16:30	12:45	12:45
66	8:00	6:45	10:45	11:45	10:15	10:15	10:00
67	8:00	6:45	7:15	7:15	7:45	12:00	12:45
68	8:15	7:52	7:15	7:15	7:15	7:15	7:37
69	12:45	12:15	14:00	11:22	9:45	8:00	11:30
70	6:45	7:00	6:22	6:15	6:22	7:00	7:30
71	8:07	7:45	10:15	13:45	8:30	11:30	11:15
72	7:15	7:45	6:45	7:15	10:15	7:15	7:30
73	3:45	6:45	3:30	2:45	1:30	4:30	6:22
74	6:22	7:00	8:00	8:07	12:45	10:00	10:15
75	4:45	5:45	6:45	6:15	9:15	6:15	6:45
76	5:00	6:30	7:30	7:45	6:45	10:15	10:00
77	9:15	10:15	12:00	11:30	8:45	8:45	9:15
78	4:45	6:15	5:15	4:52	5:00	5:00	5:00
79	2:30	3:00	3:30	2:45	0:45	3:00	4:30
80	8:45	8:00	7:15	9:22	9:15	11:15	10:30
81	6:30	7:15	6:15	6:52	6:15	6:30	6:45
82	5:15	6:00	6:15	6:52	6:00	6:30	7:00
83	0:30	0:30	0:30	0:15	0:15	0:30	0:30
84	4:00	4:00	3:45	4:00	4:00	5:00	5:00
85	4:15	5:00	5:15	6:00	4:22	7:30	7:30
86	9:15	9:15	12:15	9:45	12:30	10:07	9:15
87	6:45	6:45	6:15	6:30	5:15	7:00	7:00
88	3:45	5:00	5:15	4:30	6:45	5:30	6:00
89	8:30	8:45	9:07	8:15	8:45	9:00	8:45
90	5:30	5:15	6:30	6:15	3:15	6:45	6:15
91	2:15	3:45	2:15	2:00	0:30	3:00	4:00
92	3:45	4:15	4:15	3:45	3:45	4:15	4:15
Average	6:03	6:22	6:47	7:04	7:02	7:33	8:01



LIGHT AND TEMPERATURE IN METHYLENE BLUE REDUCTION TEST 25

OBSERVATIONS—THE TESTS OF MILK HELD FOR VARIOUS PERIODS AS INDICATED BEING AND LIGHT CONDITIONS—*concluded*

ordinary room light			Complete darkness				
After being held 24 hours at 40° F.	After being held 48 hours at 40° F.	After being held 72 hours at 40° F.	Fresh	At once after being cooled to 40° F.	After being held 24 hours at 40° F.	After being held 48 hours at 40° F.	After being held 72 hours at 40° F.
Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.	Hrs. Min.
14:22	14:07	14:15	12:45	11:30	13:30	13:15	14:15
7:15	8:45	9:45	9:30	9:15	9:30	9:45	9:15
12:00	12:37	16:00	12:30	13:00	14:45	13:22	15:30
6:45	6:30	7:30	6:22	6:45	6:45	7:15	6:30
12:52	15:15	13:45	12:30	11:15	14:37	13:45	14:30
7:45	8:30	8:52	7:30	7:45	7:00	7:15	8:22
12:45	14:45	12:15	12:30	14:00	11:52	14:45	13:45
7:30	7:15	7:00	7:45	8:00	7:15	7:15	7:30
4:45	3:45	2:00	5:00	5:00	5:15	4:30	4:00
9:07	9:22	10:45	9:45	9:15	9:22	9:45	13:00
7:00	7:15	7:07	6:15	6:30	7:00	7:15	12:00
8:45	11:00	11:45	9:45	10:15	10:45	10:45	7:15
8:45	10:15	8:22	9:15	9:07	10:07	10:52	9:45
5:30	5:00	4:45	5:00	5:00	4:45	6:00	5:00
7:00	5:45	0:45	3:15	4:00	4:52	7:30	1:15
10:30	11:45	11:00	10:45	10:30	11:15	12:22	11:37
6:15	7:00	6:30	7:15	7:15	7:00	7:07	6:45
10:07	10:30	10:30	6:30	7:15	10:00	11:30	10:30
0:30	0:15	0:15	0:30	0:45	0:45	0:15	0:15
5:00	5:00	4:30	5:07	5:15	4:30	4:45	5:00
10:15	10:45	7:30	7:30	7:45	9:45	9:45	10:52
12:00	10:15	11:15	10:15	10:00	12:07	11:30	12:15
7:00	6:30	6:45	7:15	7:15	6:30	6:15	7:30
8:00	9:15	9:00	7:30	7:00	8:30	10:45	9:30
10:15	9:22	9:22	9:45	8:52	10:00	10:45	9:00
7:45	5:45	5:15	6:15	6:15	6:30	7:15	5:45
3:15	2:15	1:15	3:15	3:45	3:15	4:00	1:45
4:30	4:15	4:45	4:45	5:00	4:30	5:15	4:45
8:38	9:06	8:53	7:47	8:05	8:51	9:22	9:16

APPENDIX TABLE 2.—REDUCTION TIMES AND PLATE COUNTS FROM SAMPLES OF RAW MILK TAKEN FROM 30 MISCELLANEOUS SOURCES WITH TIME, TEMPERATURE AND LIGHT CONDITIONS AS NOTED

Sample number	Time conditions	Reduction times			Plate count	
		100-watt	Room	Dark	Number	Logarithm
		Hrs. Min.	Hrs. Min.	Hrs. Min.		
63	Fresh milk .....	7:15	9:15	9:15	32,000	4.50
	At once after cooling to 40° F.....	6:45	9:45	9:15	31,000	4.49
	After being held 24 hours at 40° F...	7:45	10:30	11:15	29,000	4.46
	After being held 48 hours at 40° F...	8:00	10:45	11:00	31,500	4.50
	After being held 72 hours at 40° F...	6:45	11:30	11:15	30,600	4.48
64	Fresh milk .....	7:15	7:30	8:00	14,000	4.15
	At once after cooling to 40° F.....	7:30	8:30	7:30	18,700	4.27
	After being held 24 hours at 40° F...	7:30	7:30	7:45	21,000	4.32
	After being held 48 hours at 40° F...	7:00	8:30	8:00	24,000	4.38
	After being held 72 hours at 40° F...	6:45	5:30	7:15	26,000	4.42
65	Fresh milk .....	11:45	12:45	12:45	3,300	3.52
	At once after cooling to 40° F.....	11:15	12:45	11:30	3,500	3.54
	After being held 24 hours at 40° F...	13:30	14:22	13:30	4,700	3.66
	After being held 48 hours at 40° F...	14:45	14:07	13:15	4,500	3.65
	After being held 72 hours at 40° F...	16:30	14:15	14:15	5,200	3.72
66	Fresh milk .....	8:00	10:15	9:30	52,000	4.72
	At once after cooling to 40° F.....	6:45	10:00	9:15	54,000	4.73
	After being held 24 hours at 40° F...	10:45	7:15	9:30	80,000	4.90
	After being held 48 hours at 40° F...	11:45	8:45	9:45	90,000	4.95
	After being held 72 hours at 40° F...	10:15	9:45	9:15	96,000	4.98
67	Fresh milk .....	8:00	12:00	12:30	16,000	4.20
	At once after cooling to 40° F.....	6:45	12:45	13:00	21,000	4.32
	After being held 24 hours at 40° F...	7:15	12:00	14:45	24,000	4.38
	After being held 48 hours at 40° F...	7:15	12:37	13:22	21,000	4.32
	After being held 72 hours at 40° F...	7:45	16:00	15:30	22,500	4.35
68	Fresh milk .....	8:15	7:15	6:22	80,000	4.90
	At once after cooling to 40° F.....	7:52	7:37	6:45	78,000	4.89
	After being held 24 hours at 40° F...	7:15	6:45	6:45	108,000	5.03
	After being held 48 hours at 40° F...	7:15	6:30	7:15	150,000	5.18
	After being held 72 hours at 40° F...	7:15	7:30	6:30	180,000	5.26
69	Fresh milk .....	12:45	8:00	12:30	2,700	3.43
	At once after cooling to 40° F.....	12:15	11:30	11:15	3,600	3.56
	After being held 24 hours at 40° F...	14:00	12:52	14:37	3,600	3.56
	After being held 48 hours at 40° F...	11:22	15:15	13:45	4,900	3.69
	After being held 72 hours at 40° F...	9:45	13:45	14:30	1,600	3.20
70	Fresh milk .....	6:45	7:00	7:30	17,500	4.24
	At once after cooling to 40° F.....	7:00	7:30	7:45	15,500	4.19
	After being held 24 hours at 40° F...	6:22	7:45	7:00	23,700	4.37
	After being held 48 hours at 40° F...	6:15	8:30	7:15	20,000	4.30
	After being held 72 hours at 40° F...	6:22	8:52	8:22	13,000	4.11
71	Fresh milk .....	8:07	11:30	12:30	23,000	4.36
	At once after cooling to 40° F.....	7:45	11:15	14:00	19,800	4.30
	After being held 24 hours at 40° F...	10:15	12:45	11:52	25,700	4.41
	After being held 48 hours at 40° F...	13:45	14:45	14:45	27,500	4.44
	After being held 72 hours at 40° F...	8:30	12:15	13:45	17,000	4.23
72	Fresh milk .....	7:15	7:15	7:45	4,200	3.63
	At once after cooling to 40° F.....	7:45	7:30	8:00	4,700	3.67
	After being held 24 hours at 40° F...	6:45	7:30	7:15	6,800	3.84
	After being held 48 hours at 40° F...	7:15	7:15	7:15	7,600	3.88
	After being held 72 hours at 40° F...	10:15	7:00	7:30	3,700	3.57

LIGHT AND TEMPERATURE IN METHYLENE BLUE REDUCTION TEST 27

APPENDIX TABLE 2.—REDUCTION TIMES AND PLATE COUNTS FROM SAMPLES OF RAW MILK TAKEN FROM 30 MISCELLANEOUS SOURCES WITH TIME, TEMPERATURE AND LIGHT CONDITIONS AS NOTED—*continued*

Sample number	Time conditions	Reduction times			Plate count	
		100-watt	Room	Dark	Number	Logarithm
		Hrs. Min.	Hrs. Min.	Hrs. Min.		
73	Fresh milk .....	3:45	4:30	5:00	200,000	5.30
	At once after cooling to 40° F.....	6:45	6:22	5:00	190,000	5.28
	After being held 24 hours at 40° F...	3:30	4:45	5:15	320,000	5.50
	After being held 48 hours at 40° F...	2:45	3:45	4:30	360,000	5.56
	After being held 72 hours at 40° F...	1:30	2:00	4:00	450,000	5.65
74	Fresh milk .....	6:22	10:00	9:45	23,400	4.37
	At once after cooling to 40° F.....	7:00	10:15	9:15	19,000	4.28
	After being held 24 hours at 40° F...	8:00	9:07	9:22	25,700	4.41
	After being held 48 hours at 40° F...	8:07	9:22	9:45	28,000	4.45
	After being held 72 hours at 40° F...	12:45	10:45	13:00	23,000	4.36
75	Fresh milk .....	4:45	6:15	6:15	5,600	3.75
	At once after cooling to 40° F.....	5:45	6:45	6:30	3,000	3.48
	After being held 24 hours at 40° F...	6:45	7:00	7:00	5,900	3.77
	After being held 48 hours at 40° F....	6:15	7:15	7:15	7,400	3.87
	After being held 72 hours at 40° F...	9:15	7:07	12:00	14,500	4.16
76	Fresh milk .....	5:00	10:15	9:45	40,000	4.60
	At once after cooling to 40° F.....	6:30	10:00	10:15	42,000	4.62
	After being held 24 hours at 40° F...	7:30	8:45	10:45	44,000	4.64
	After being held 48 hours at 40° F...	7:45	11:00	10:45	52,000	4.72
	After being held 72 hours at 40° F...	6:45	11:45	7:15	36,000	4.56
77	Fresh milk .....	9:15	8:45	9:15	1,000	3.00
	At once after cooling to 40° F.....	10:15	9:15	9:07	600	2.78
	After being held 24 hours at 40° F...	12:00	8:45	10:07	1,100	3.04
	After being held 48 hours at 40° F...	11:30	10:15	10:52	800	2.90
	After being held 72 hours at 40° F...	8:45	8:22	9:45	600	2.78
78	Fresh milk .....	4:45	5:00	5:00	24,000	4.38
	At once after cooling to 40° F.....	6:15	5:00	5:00	20,000	4.30
	After being held 24 hours at 40° F...	5:15	5:30	4:45	52,000	4.72
	After being held 48 hours at 40° F...	4:52	5:00	6:00	120,000	5.08
	After being held 72 hours at 40° F...	5:00	4:45	5:00	132,000	5.12
79	Fresh milk .....	2:30	3:00	3:15	315,000	5.50
	At once after cooling to 40° F.....	3:00	4:30	4:00	275,000	5.44
	After being held 24 hours at 40° F...	3:30	7:00	4:52	490,000	5.69
	After being held 48 hours at 40° F...	2:45	5:45	7:30	600,000	5.78
	After being held 72 hours at 40° F...	0:45	0:45	1:15	1,600,000	6.20
80	Fresh milk .....	8:45	11:15	10:45	12,500	4.10
	At once after cooling to 40° F.....	8:00	10:30	10:30	13,200	4.12
	After being held 24 hours at 40° F...	7:15	10:30	11:15	14,700	4.17
	After being held 48 hours at 40° F...	9:22	11:45	12:22	32,000	4.50
	After being held 72 hours at 40° F...	9:15	11:00	11:37	52,000	4.72
81	Fresh milk .....	6:30	6:30	7:15	25,000	4.40
	At once after cooling to 40° F.....	7:15	6:45	7:15	30,000	4.48
	After being held 24 hours at 40° F...	6:15	6:15	7:00	37,000	4.57
	After being held 48 hours at 40° F...	6:52	7:00	7:07	48,000	4.68
	After being held 72 hours at 40° F...	6:15	6:30	6:45	53,000	4.72
82	Fresh milk .....	5:15	6:30	6:30	93,000	4.97
	At once after cooling to 40° F.....	6:00	7:00	7:15	70,000	4.84
	After being held 24 hours at 40° F...	6:15	10:07	10:00	102,000	5.01
	After being held 48 hours at 40° F...	6:52	10:30	11:30	110,000	5.04
	After being held 72 hours at 40° F...	6:00	10:30	10:30	120,000	5.08

APPENDIX TABLE 2.—REDUCTION TIMES AND PLATE COUNTS FROM SAMPLES OF RAW MILK TAKEN FROM 30 MISCELLANEOUS SOURCES WITH TIME, TEMPERATURE AND LIGHT CONDITIONS AS NOTED—*concluded*

Sample number	Time conditions	Reduction times			Plate count	
		100-watt	Room	Dark	Number	Logarithm
		Hrs. Min.	Hrs. Min.	Hrs. Min.		
83	Fresh milk .....	0:30	0:30	0:30	252,000	5.40
	At once after cooling to 40° F.....	0:30	0:30	0:45	230,000	5.36
	After being held 24 hours at 40° F..	0:30	0:30	0:45	1,260,000	6.10
	After being held 48 hours at 40° F...	0:15	0:15	0:15	2,500,000	6.40
	After being held 72 hours at 40° F...	0:15	0:15	0:15	2,600,000	6.42
84	Fresh milk .....	4:00	5:00	5:07	15,000	4.18
	At once after cooling to 40° F.....	4:00	5:00	5:15	13,500	4.13
	After being held 24 hours at 40° F..	3:45	5:00	4:30	22,000	4.34
	After being held 48 hours at 40° F...	4:00	5:00	4:45	35,000	4.54
	After being held 72 hours at 40° F...	4:00	4:30	5:00	46,000	5.66
85	Fresh milk .....	4:15	7:30	7:30	35,500	4.55
	At once after cooling to 40° F.....	5:00	7:30	7:45	34,000	4.53
	After being held 24 hours at 40° F..	5:15	10:15	9:45	40,000	4.60
	After being held 48 hours at 40° F...	6:00	10:45	9:45	50,000	4.70
	After being held 72 hours at 40° F...	4:22	7:30	10:52	200,000	5.30
86	Fresh milk .....	9:15	10:07	10:15	56,000	4.75
	At once after cooling to 40° F.....	9:15	9:15	10:00	40,000	4.60
	After being held 24 hours at 40° F..	12:15	12:00	12:07	60,000	4.78
	After being held 48 hours at 40° F...	9:45	10:15	11:30	62,000	4.79
	After being held 72 hours at 40° F...	12:30	11:15	12:15	70,000	4.84
87	Fresh milk .....	6:45	7:00	7:15	33,000	4.52
	At once after cooling to 40° F.....	6:45	7:00	7:15	30,000	4.48
	After being held 24 hours at 40° F..	6:15	7:00	6:30	50,000	4.70
	After being held 48 hours at 40° F...	6:30	6:30	6:15	56,000	4.75
	After being held 72 hours at 40° F...	5:15	6:45	7:30	180,000	5.26
88	Fresh milk .....	3:45	5:30	7:30	40,000	4.60
	At once after cooling to 40° F.....	5:00	6:00	7:00	36,000	4.56
	After being held 24 hours at 40° F..	5:15	8:00	8:30	47,000	4.67
	After being held 48 hours at 40° F...	4:30	9:15	10:45	48,000	4.68
	After being held 72 hours at 40° F...	6:45	9:00	9:30	64,000	4.81
89	Fresh milk .....	8:30	9:00	9:45	60,000	4.78
	At once after cooling to 40° F.....	8:45	8:45	8:52	45,000	4.65
	After being held 24 hours at 40° F..	9:07	10:15	10:00	120,000	5.08
	After being held 48 hours at 40° F...	8:15	9:22	10:45	148,000	5.17
	After being held 72 hours at 40° F...	8:45	9:22	9:00	208,000	5.32
90	Fresh milk .....	5:30	6:45	6:15	52,000	4.72
	At once after cooling to 40° F.....	5:15	6:15	6:15	45,000	4.65
	After being held 24 hours at 40° F..	6:30	7:45	6:30	160,000	5.20
	After being held 48 hours at 40° F...	6:15	5:45	7:15	420,000	5.62
	After being held 72 hours at 40° F...	3:15	5:15	5:45	508,000	5.71
91	Fresh milk .....	2:15	3:00	3:15	730,000	5.86
	At once after cooling to 40° F.....	3:45	4:00	3:45	560,000	5.75
	After being held 24 hours at 40° F..	2:15	3:15	3:15	780,000	5.89
	After being held 48 hours at 40° F...	2:00	2:15	4:00	2,160,000	6.33
	After being held 72 hours at 40° F...	0:30	1:15	1:45	4,200,000	6.62
92	Fresh milk .....	3:45	4:15	4:45	40,000	4.60
	At once after cooling to 40° F.....	4:15	4:15	5:00	25,000	4.40
	After being held 24 hours at 40° F..	4:15	4:30	4:30	36,000	4.56
	After being held 48 hours at 40° F...	3:45	4:15	5:15	38,000	4.58
	After being held 72 hours at 40° F...	3:45	4:45	4:45	47,500	4.68
Averages						
	Fresh milk .....	6:21	7:27	7:47	29,200	4.46
	At once after cooling to 40° F.....	6:42	7:48	7:48	26,500	4.42
	After being held 24 hours at 40° F..	7:06	8:11	8:22	41,000	4.61
	After being held 48 hours at 40° F...	7:06	8:24	8:47	51,000	4.71
	After being held 72 hours at 40° F...	6:51	7:59	8:32	69,000	4.84