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SURVEYS OF MILK LABORATORIES IN WAR AREAS IN THE UNITED STATES [1]

II. PRACTICES OBSERVED IN MAKING DIRECT MICROSCOPIC EXAMINATIONS AND METHYLENE BLUE REDUCTION TESTS

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In a preceding paper (*1*) a summary was presented of the milk laboratory surveys made in the entire 48 States, with an analysis of the deviations observed in the 399 of the 408 laboratories surveyed which used the agar plate method. The general plan of procedure, forms used, and extent of the survey were discussed there. In this paper observations made of the direct microscopic method (Breed's method) and the methylene blue reduction test will be discussed. Relatively little use was made of these tests, even for analysis of samples of raw milk for pasteurization, as was shown in table 1 of the preceding paper. Only 38 laboratories employed the direct microscopic method, and only 61 used the methylene blue reduction test. This is considered fortunate inasmuch as these tests are considerably less accurate than bacterial plate counts, particularly when the sanitary quality of the local milk supply has been improved to the extent now attained in many localities.

Table 1 also showed that none of the 33 official laboratories surveyed in the four West South Central States or the 58 laboratories in the three Pacific States used the direct microscopic method for the examination of milk. On the other hand, none of the 30 official laboratories surveyed in the six New England States or the 20 laboratories in the three Middle Atlantic States used the methylene blue reduction test.

It should not be necessary to point out the greater accuracy of the agar plate method as this is generally agreed upon by competent and experienced bacteriologists. The vast majority of official laboratories using this procedure do not feel called upon to defend their choice. Those attending recent meetings and reading recent papers in certain journals, however, might be led to believe that the agar plate count has been superseded in accuracy by other rapid methods. This flurry

[1] From the Sanitation Section, States Relations Division.

apparently is due to a few individuals and is not even representative of any given group of States. It would seem to be aimed at displacing the more accurate agar plate method now in use in most laboratories by rougher methods, the result of which might well be that milk not now meeting Grade A bacterial standards would then pass the rougher standards and purportedly be of the highest grade.

While the proponents of these changes may be unprejudiced, such changes in applications of methods should be made upon their own merits and not under the pretext of a shortage of agar for bacteriological purposes.[2] Furthermore it is incumbent upon the advocates of such changes to present unbiased and convincing proof of their views—something which has not yet been done. Accordingly, while it is not necessary to defend the relative accuracy of the agar plate method, since the preceding paper (*1*) has dwelt at length upon the common failure to comply fully with the requirements of Standard Methods for the Examination of Dairy Products pertaining to the agar plate count, some may have construed the findings to mean that the method is not satisfactory. It should be pointed out that the deviations reported were those found at the time of the survey, and reports and observations indicate that many of the errors observed were corrected shortly thereafter.

A similar detailed analysis of the deviations observed has been prepared for the direct microscopic method and the methylene blue reduction test. These results are not as comprehensive as those reported for the agar plate count, partly because of the smaller number using such procedures, and partly because controls such as are required for the agar plate count were never included in these rougher tests, as, indeed, they need not have been when used for the purpose for which intended, namely, as estimates of bacteria present in raw milk, particularly that of lower quality. However, when individuals advocate the use of such rough tests as the sole bacteriological control not only of raw milk but even of pasteurized milk, it seems desirable not only to discuss observations of the tests as performed in the laboratories surveyed but also to summarize the investigations of others in comparative studies of these procedures.

Of the 408 official bacteriological milk laboratories surveyed in the 48 States and the District of Columbia, 399 used the agar plate method. Of these, 33 also used the direct microscopic examination and 57 used the methylene blue reduction method for samples of producers' milk. In addition, 4 laboratories surveyed used the direct microscopic method and 2 laboratories used the methylene blue

[2] Information available in 1942 was that while agar could be used only by bacteriological laboratories, under General Preference Order M-96 (Feb. 9, 1942), the supply was adequate for this purpose, and the War Production Board stated no further restrictions were contemplated. Present information (May 1943) is that no further restrictions have been made or are contemplated at this time.

reduction test (with one additional place purportedly using both procedures) as the sole procedure in the control of retail pasteurized and raw milk. Compilations were made of the number of laboratories conforming to, and deviating from, each subitem of equipment, preparation, technique, and reporting required by Standard Methods.

In the interest of clarity, the figures presented in the following tables list only deviations, items undetermined because of local conditions at the time of the survey, or items not used in the particular laboratory. Thus, at a glance one may single out common deviations, and some of these will be discussed briefly. The entire survey form has been divided into portions, and the material arranged so the results could be tabulated. The last 59 of the 92 laboratories reported upon (7 of which used both tests) were recorded on the revised forms and, in preparing the tables, the results of the first 33 laboratories recorded on the earlier form were also tabulated upon the present forms. This resulted, in some instances, in unduly large figures for items marked *undetermined*, where such an item was not included on the original forms. Likewise, the earlier surveys were based on the seventh edition of Standard Methods while the revised forms, based on the eighth edition, included a few additional items. Consequently, these additional items were marked *not used* when the results of the earlier surveys were transferred to the revised forms.

DIRECT MICROSCOPIC COUNT

The requirements of Standard Methods pertaining to apparatus and preparation under the direct microscopic method, and a summary of the survey results showing the deviations from Standard Methods, are presented in table 1 by geographic divisions with the totals for all States.

Of the 38 laboratories using this method, 29 used the correct pipettes, most of the others using loops, usually a standard loop but sometimes an ordinary bacteriological loop. In general, the apparatus (guides, slides, microscope, and microscope lamp) were purchased for this work and were suitable. There were 25 laboratories in which ocular discs were used; however, 11 laboratories did not use mechanical stages. The item pertaining to the binocular was not present on the original form, thus accounting for the larger number recorded in the *undetermined* column.

With regard to preparation, the stains generally met the requirements, sometimes being purchased ready for use. It was evident in more instances than shown that the microscopic factors in use may have been incorrect but no facilities were available for checking their accuracy. It was generally true that the laboratories did not fill

TABLE 1.—*Summary of items pertaining to apparatus and preparation used in direct microscopic count, indicating lack of conformity with Standard Methods for the Examination of Dairy Products (eighth edition)*

	Total			New England			Middle Atlantic			East North Central			West North Central			South Atlantic			East South Central			Mountain		
Number of laboratories surveyed	38			6			6			7			7			8			1			3		
	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No
4. Pipettes—0.01 ml. A. P. H. A. specifications or accurately calibrated, tips unbroken*	8	1	----	1	----	----	1	----	----	5	----	----	1	----	----	----	1	----	0	----	----	0	----	----
5. Guides—Glass or cardboard, square or round 1 sq. cm. areas	3	----	----	0	----	----	0	----	----	2	----	----	1	----	----	0	----	----	0	----	----	0	----	----
6. Slides—Permit legible and indelible labeling	1	1	----	0	----	----	0	----	----	0	----	----	1	1	----	0	----	----	0	----	----	0	----	----
7. Microscope—Equipped 1.8 mm. oil immersion objective	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
Rack and pinion substage	----	1	----	0	----	----	0	----	----	0	----	----	0	----	----	----	1	----	0	----	----	0	----	----
Condenser with iris diaphragm	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
Ocular or oculars permitting adjustment of draw tube to field of desired diameter	----	----	1	0	----	----	0	----	----	0	----	----	----	----	1	0	----	----	0	----	----	0	----	----
When accurate counts are desired, use ocular diaphragm or micrometer disc to limit field examined to desired size	2	2	9	----	1	----	0	----	----	----	1	----	----	----	6	1	----	1	0	----	----	1	----	2
Use special mechanical stage with 2 x 4½-inch slides	4	1	6	0	----	----	0	----	----	1	1	----	1	----	5	0	----	----	0	----	----	2	----	1
Binocular if many samples	----	12	7	0	----	----	----	4	----	----	7	----	----	----	5	----	----	1	----	1	----	----	----	1
And oculars adjusted or counts corrected	----	14	7	0	----	----	----	4	----	----	7	----	----	----	5	----	2	1	----	1	----	----	----	1
8. Illumination—Standard microscope lamp or equivalent artificial light source	----	1	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	----	1	----
9. Stains—Certified stains used	1	1	----	0	----	----	0	----	----	0	----	----	----	1	----	1	----	----	0	----	----	0	----	----
Chemicals of highest purity	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
Correct formula	1	2	----	0	----	----	0	----	----	0	----	----	1	----	----	----	2	----	0	----	----	0	----	----
Avoid use of old stains or those containing suspended matter	----	1	----	0	----	----	0	----	----	0	----	----	----	1	----	0	----	----	0	----	----	0	----	----
Protect solvents, fixatives, and stains not in use by covering or stoppering containers	----	1	----	0	----	----	0	----	----	0	----	----	----	1	----	0	----	----	0	----	----	0	----	----
Use fresh solutions whenever old ones are unfit	----	1	----	0	----	----	0	----	----	0	----	----	----	1	----	0	----	----	0	----	----	0	----	----
Do not use as fat solvents those used previously to remove immersion oil	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
10. Microscope—Illumination: tube length adjusted to permit maximum optical resolution	----	1	1	0	----	----	0	----	----	0	----	----	----	1	----	----	----	1	0	----	----	0	----	----
Used in such position	----	2	----	0	----	----	0	----	----	0	----	----	----	1	----	----	1	----	0	----	----	0	----	----
Light source adjusted for maximum optical resolution	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
Field areas: diameter of field determined with stage micrometer	2	1	----	----	1	----	0	----	----	0	----	----	1	----	----	1	----	----	0	----	----	0	----	----
Area determined and microscopic factor computed	2	1	----	0	----	----	0	----	----	1	1	----	0	----	----	1	----	----	0	----	----	0	----	----
11. Pipettes—Filled with a cleaning solution after use	11	9	1	3	1	----	1	3	----	2	4	----	1	1	----	3	----	1	1	----	----	0	----	----
Stored in a cleaning solution	20	8	1	3	1	----	1	3	----	2	4	----	6	----	----	5	----	1	1	----	----	2	----	----
12. Slides—Physically clean	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
13. Films—Guide plate used	2	----	----	0	----	----	0	----	----	1	----	----	1	----	----	0	----	----	0	----	----	0	----	----
Each space identified with number or symbol corresponding to sample	----	----	2	0	----	----	0	----	----	0	----	----	----	----	2	0	----	----	0	----	----	0	----	----

*Calibrated loop permitted but not for official control.

De=Deviations. Un=Undetermined. No=Not used.

the pipettes with cleaning solution following use, and only one laboratory is recalled in which pipettes were regularly stored in such solution as prescribed by Standard Methods.

The requirements pertaining to procedure used in the direct microscopic method, and a summary of the deviations observed, are shown in table 2. The usual deviations in shaking samples already reported for the agar plate count were also observed in laboratories using the direct microscopic method. Frequent deviations were noted in measurement of the sample, in failure to rinse pipettes in the milk sample prior to measurement and in carelessness in manipulation of milk in or on the capillary pipette. Laboratories using loops for measurement naturally deviated from the 0.01 ml. portion prescribed for official counts. There was common failure to spread the amount carefully, and other deviations in handling and drying the films.

Most laboratories did not use this procedure for cream samples at the time of the surveys, and the procedure listed on the revised form appeared in the eighth edition only, thus accounting for larger numbers in the *not used* column for those details.

Staining of the preparations seemed well done, the majority of the laboratories using a one-solution technique. However, there was common failure to count the number of fields prescribed by Standard Methods, some errors in multiplication were noted, and frequently there was failure to preserve the microscopic preparations as required by Standard Methods. In most laboratories there was no second technician to check results; this probably accounted for some of the long-continued more serious errors observed in certain instances. Reports sometimes failed to show whether individual bacteria or clumps were recorded, and the results were frequently reported with several significant figures implying fictitious accuracy of the results.

METHYLENE BLUE REDUCTION TEST

The requirements of Standard Methods pertaining to the methylene blue reduction method, and a summary of the deviations observed, are presented in table 3.

In general the apparatus used by the laboratories employing this test had been purchased for this method, but some was improvised and much of it older types, one-third of the laboratories not having a thermostatically controlled water-bath or incubator. About 40 percent did not use solid stoppers or leakproof closures, but instead used cotton plugs or more frequently no closure at all.

With regard to preparation, over 25 percent of the laboratories were not using the prescribed methylene blue thiocyanate tablets, standard now through the last two editions of Standard Methods. Such solutions themselves were usually not stored as prescribed, as noted

TABLE 2.—*Summary of items pertaining to procedure used in direct microscopic count, indicating lack of conformity with Standard Methods for the Examination of Dairy Products (eighth edition)*

	Total			New England			Middle Atlantic			East North Central			West North Central			South Atlantic			East South Central			Mountain		
Number of laboratories surveyed	38			6			6			7			7			8			1			3		
	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No
14. Sample agitation—agitate vigorously	0			0			0				1		0			0			0			0		
Mix thoroughly	0			0			0				1		0			0			0			0		
Immediately before removing portion	0			0			0				1		0			0			0			0		
Before opening container remove all material from closure which may contaminate sample		4		0			0				1			2		0			0				1	
Wholesale and process samples: shake 25 times	1	7	6	0			0				4				4	1	2	2	0				1	
Up and down excursion	9	9	6	1	2		2	1		3	4		1		4	1	2	2	0			1		
About a foot	11	12	6	1	4		1	2		2	4		2		4	2	2	2	0			3		
Within 7 seconds	2	7	6		1		0			2	3			1	4		2	2	0			0		
15. Sample measurement—use aseptic technique	0			0			0			0			0			0			0			0		
Rinse bore of pipette thoroughly in fresh warm (40°-45° C.), clean water between samples		8	2		1			2			5		0					2	0			0		
Rinse bore in subsequent milk sample	3	10	2	1	1			2			5		2	2				2	0			0		
Draw sample into pipette above graduation mark		8	1		1			2			5		0					1	0			0		
Use clean towel to wipe exterior of pipette	2	10	1		1			2			5		1	1		1		1	0				1	
And absorb milk at tip to reduce column to graduation	2	10	1		1			2			5		1	1		1	1	1	0			0		
16. Making films—completely deposit 0.01 ml. in proper place on clean slide	6	4		1			1	1		3	1		1				1		0				1	
Spread liquid over exact sq. cm. area with point of bent needle	14	1		0				1		4			6			3			0			1		
Needle cleaned by wiping with clean cloth between samples (or flamed)		6	3	0			0				5				3		1		0			0		
Work rapidly to prevent bacterial growth while making films		1		0			0			0				1		0			0			0		
Film dried in warm place upon level surface protected from dust and insects		5			1		0				1			2		0			0				1	
Dried completely within 5 minutes	2	6		0			0				2		1	3		1			0				1	
Not heated so rapidly—preparation cracks or peels			1	0			0			0					1	0			0			0		
Cream- free from air bubbles		22	12		6			5			5			1	6		4	3		1				3
Dissolve all fat out of film (double treatment xylene, drying after each)	1	5	32			6			6			7	1		6		5	3			1			3
Treat film with alcohol and dry so as to flatten until thin and transparent before staining (double treatment alcohol, drying after each)	1	5	32			6			6			7	1		6		5	3			1			3
Renew xylene frequently		5	32			6			6			7		1	6		4	3			1			3

17. Staining films—(*a*) dip in suitable fat solvent for sufficient time, drain, dry, immerse in suitable alcohol for 1 to 2 minutes, dry, dip in staining solution for sufficient time, rinse (decolorize in alcohol if overstained), dry thoroughly; or (*b*) dip in combined solvent, fixing and staining solution for sufficient time, remove, dry thoroughly, dip in water to remove excess stain	1	1	----	----	1	----	0	----	----	0	----	----	1	----	----	0	----	----	0	----	----	0	----	----
18. Counting films—place proper ocular diaphragms or micrometer discs in proper oculars to obtain suitable field diameters	1	1	6	----	1	----	0	----	----	0	----	----	----	----	4	1	----	----	0	----	----	----	----	2
Use immersion oil between slide and objective	1	----	----	0	----	----	0	----	----	0	----	----	0	----	----	1	----	----	0	----	----	0	----	----
Count the bacteria in not less than the number of fields specified by Standard Methods	11	17	----	5	1	----	----	6	----	3	1	----	1	4	----	1	2	----	1	----	----	----	3	----
Determine the average number of bacteria per field	1	1	----	0	----	----	0	----	----	----	1	----	0	----	----	1	----	----	0	----	----	0	----	----
Multiply by the proper factor for the estimated number of bacteria or clumps per ml	2	2	----	0	----	----	0	----	----	1	1	----	1	----	----	----	1	----	0	----	----	0	----	----
Preserve microscopic preparations for a reasonable interval after official reports are rendered	8	5	----	2	----	----	----	1	----	4	----	----	----	2	----	1	----	----	0	----	----	1	2	----
At intervals have a second technician check results	4	17	3	----	5	----	----	1	----	----	4	----	1	2	3	3	1	----	----	1	----	----	3	----
19. Reporting counts—report as estimated number of individual bacteria or clumps per ml	1	1	1	0	----	----	0	----	----	----	1	----	----	----	1	1	----	----	0	----	----	0	----	----
Use only 2 significant figures	7	5	----	1	2	----	0	----	----	----	1	----	3	2	----	2	----	----	0	----	----	1	----	----
Raise if figure dropped is 5 or more; lower if 4 or less	9	12	----	1	3	----	----	1	----	----	1	----	3	3	----	4	3	----	0	----	----	1	1	----

De=Deviations. Un=Undetermined. No=Not used.

TABLE 3.—*Summary of items pertaining to apparatus, preparation, and procedure used in methylene blue reduction test, indicating lack of conformity with Standard Methods for the Examination of Dairy Products (eighth edition)*

	Total			East North Central			West North Central			South Atlantic			East South Central			West South Central			Mountain			Pacific		
Number of laboratories surveyed	61			9			26			14			1			7			3			1		
	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No	De	Un	No
4. Pipette—straight-sided graduated to deliver 10 ml. or dipper calibrated to deliver 10 ml	1	----	1	0	----	----	1	----	----	0	----	----	0	----	----	----	----	1	0	----	----	0	----	----
5. Burette—graduated, or pipette graduated to deliver 1 ml	1	----	----	0	----	----	1	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
6. Tubes—without lip, 150 mm. × 12–15 mm., rubber stoppers to fit (or leakproof closures)	24	1	----	5	----	----	9	1	----	6	----	----	0	----	----	2	----	----	2	----	----	0	----	----
7. Tube racks—rust-resistant metal, single or double rows, hold tubes in vertical position	7	10	----	----	2	----	5	2	----	1	3	----	0	----	----	1	2	----	0	----	----	----	1	----
8. Water bath or incubator—thermostatically controlled at 37° C.± 0.5 C°	13	6	----	1	----	----	8	2	----	2	1	----	0	----	----	2	1	----	----	1	----	----	1	----
9. Stains—use only methylene blue thiocyanate tablets, certified suitable for reduction	15	4	----	2	1	----	6	2	----	3	----	----	1	----	----	1	----	----	2	----	----	----	1	----
Dissolve one tablet (over night if necessary) in 200 ml. of sterile or freshly boiled distilled water	1	5	----	----	3	----	----	1	----	0	----	----	----	1	----	0	----	----	1	----	----	0	--	----
Aqueous solution protected from light by storage in amber bottles in dark	25	8	5	4	1	----	11	5	3	6	----	1	0	----	----	1	2	1	3	----	----	0	--	----
Use fresh solutions whenever old has deteriorated	----	2	----	0	----	----	----	1	----	0	----	----	0	----	----	----	1	----	0	----	----	0	--	----
10. Equipment—glassware scrupulously clean	1	----	----	0	----	----	1	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	--	----
Free from etching	1	----	----	0	----	----	1	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	--	----
Containers for dye solution, pipettes, test tubes, and rubber stoppers sterilized, or boiled immediately before use (allowing retained heat to dry equipment)	10	1	1	2	----	----	4	----	1	3	----	----	0	----	----	1	1	----	0	----	----	0	--	----
11. Identification—identify each tube legibly and indelibly immediately before or after addition of sample with number or symbol corresponding to sample, or by chart	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	--	----
12. Dye solution—with a burette or 1 ml. pipette, add 1 ml. to each tube either shortly before or after sample	1	----	----	0	----	----	1	----	----	0	----	----	0	----	----	0	----	----	0	----	----	0	----	----
13. Sample—measure 10 ml. with sterile (or subjected to practical sterilization) pipette or dipper (or use tubes graduated to contain 10 ml.)	9	2	----	1	1	----	4	1	----	3	----	----	0	----	----	0	----	----	1	----	----	0	----	----
(If practical sterilization, chlorine sterilizer not used)	15	23	14	----	9	----	10	5	7	2	8	----	----	1	----	3	----	4	----	----	3	0	----	----
Place sample in sterilized or boiled test tubes	5	1	----	1	----	----	1	1	----	2	----	----	0	----	----	1	----	----	0	----	----	0	----	----
Close or stopper immediately with sterilized or boiled rubber stoppers	27	2	----	5	----	----	11	1	----	6	----	----	----	1	----	3	----	----	2	----	----	0	----	----
And avoid recontamination of part of stopper inserted into tube	1	13	7	----	5	----	1	4	4	----	1	1	0	----	----	----	2	1	----	1	1	0	----	----

14. Incubation—place tubes in water bath at 37° C.±0.5° C	15	4	----	1	----	----	10	4	----	0	----	----	0	----	----	2	----	----	2	----	----	0	----	----
After 5 minutes invert tubes a few times	38	17	----	----	8	----	22	----	----	5	8	----	1	----	----	7	----	----	3	----	----	----	1	----
Avoid further agitation which might disturb cream layer	13	1	----	1	----	----	3	----	----	6	----	----	0	----	----	1	----	----	2	----	----	----	1	----
If water bath is not of sufficient capacity to warm samples to 37° C. within 5 minutes or if air incubator is used, warm samples to 37° C. in a water bath not over 40° C.	17	1	3	0	----	----	11	----	3	4	----	----	0	----	----	----	1	----	2	----	----	0	----	----
Determine temperature by immersing bulb of thermometer in blank milk tube	35	3	17	----	----	9	21	1	1	4	2	5	----	----	1	7	----	----	2	----	1	1	----	----
Completely protect samples from light rays (including heat source and pilot light)	16	8	----	----	3	----	8	2	----	2	----	----	1	----	----	3	2	----	1	1	----	1	----	----
15. Observation—observe without lowering temperature of milk	1	----	----	0	----	----	0	----	----	0	----	----	0	----	----	1	----	----	0	----	----	0	----	----
Examine samples for reduction at end of each hour but not beyond 8 hours*	5	8	8	----	4	----	2	1	6	2	2	2	----	1	----	1	----	----	0	----	----	0	----	----
16. Reduction time—express results as number of elapsed full hours when decolorization is first observed*	3	4	5	----	2	----	----	1	2	2	1	2	0	----	----	1	----	----	----	----	1	0	----	----
Samples not reduced at the end of 8 hours are to be reported as reduced in 9 hours*	10	12	16	----	6	----	6	4	10	1	1	3	----	1	----	3	----	----	----	----	3	0	----	----

*Indicates modification required by P.H.S. Milk Code. De=Deviations. Un=Undetermined. No=Not used.

in item 9. Some failure to sterilize equipment was observed, as reported in item 10.

In technique, deviations in manipulation of the sample included not using sterile pipettes or dippers in some instances, and in about half of the laboratories, failure to stopper the test tubes. The requirement of not using chlorine sterilizer appeared in the eighth edition, resulting in the larger numbers recorded in the *undetermined* and *not used* columns shown in item 13. Deviations in incubation were frequent (item 14), over one-fourth of the laboratories not incubating within the required temperature range. There was general deviation as to time and extent of agitation of samples, and general failure to warm samples as prescribed when air incubators were used. The item pertaining to checking the temperature of the water bath appeared in the eighth edition, hence the larger numbers in the *not used* column.

The requirements under items 15 and 16 pertain only to communities operating under the Milk Ordinance and Code recommended by the United States Public Health Service; consequently, this information for laboratories in other communities was noted as *not used.*

DISCUSSION

Observations made during the survey indicate that improvement is necessary if laboratory results are to have the significance attributed to them, not only in milk but also in water analysis and in the diagnostic tests themselves. In general, those tests used primarily for sanitation are a minor interest of many public health laboratories and do not receive the consideration due them. This is particularly unfortunate since laboratory tests are an essential part of a sanitation program, and sanitation is the very basis of public health. However, observations indicate that even the diagnostic tests, probably a major interest in most public health laboratories, are too often done routinely, even poorly.

With special reference to milk analysis, the blame for the present situation seems to be rather equally divided into four aspects. First, the person doing the analysis usually has not been taught standard methods, primarily the responsibility of the teacher who, for various reasons, apparently largely because he neither knows nor understands the reasons for the requirements, pays little attention to this. Secondly, the individual worker himself is commonly to be blamed for not looking into Standard Methods. All too often he has turned to his laboratory manual or textbook used when he studied such laboratory work, not to Standard Methods, possibly again because he was not familiarized with or referred to this material originally. Thirdly, Standard Methods itself is partly to blame, for if it is turned to by

the worker seeking guidance, he is frequently confused by the discussion of pros and cons, alternatives, discussion of an item in one section and then the appearance some pages later of something else pertinent to the same item, so that the worker seeking information may well become confused even if he attempted to follow Standard Methods. Lastly, a considerable share of the responsibility falls upon public health administrators who commonly take laboratory results for granted, notwithstanding the fact that they must realize that such personnel are ordinarily poorly paid and sometimes poorly trained. If some interested administrative official had visited local laboratories and had given them some aid and encouragement in facilities, equipment, procedures, and in-service or additional training, the commonly low caliber of bacteriological milk analysis would have been materially higher. This is borne out by the fact that the only two States really having some system of inspecting the laboratories and the personnel doing milk analysis ranked highest among the 48 States in compliance with Standard Methods pertaining to agar plate counts.

Some such responsibility would logically seem to be a function of an effective public health laboratory. An excellent presentation of "The Need for Official Supervision of Laboratories," with many worth while suggestions, has been published by Mickle (*2*); those interested are referred to this paper.

COMPARATIVE VALUE OF METHODS

Direct microscopic (*Breed's method*).—To return to a brief discussion of the relative merits of the agar plate count compared to the results obtained by direct microscopic examination or by methylene blue reduction, "count" is defined by Webster's Dictionary as "an accurate enumeration of that which is contained by a representative sample; as, a bacterial count." As suggested in the discussion of the second application of the direct microscopic test in Standard Methods, namely, "the making of actual numerical estimates," it might be pointed out that Webster defines "estimate" as "a judgment made by calculation, especially from incomplete data; rough or approximate." Estimates by the direct microscopic method are thus, by definition, only approximations. To equal in accuracy that of a plate count from lower count milk, with from 3,000 to 30,000 colonies per ml., where a 1:100 dilution should show 30 to 300 colonies on the plate, the resulting growth from the entire 0.01 ml. of milk being observed on the plate in counting, theoretically the entire 0.01 ml. of milk on the entire film on the slide used for the direct microscopic method should similarly be observed, amounting to 5,000 to 6,000 fields. Whereas in Standard Methods the number of fields to be examined for such milk is stipulated to be 60 or 120, depending upon

the field diameter used, in practice our observation was that rarely 30 and usually 10 fields were used. Again, compared to the agar plate count above, 10 such microscopic fields would roughly be comparable to estimating a bacterial plate count from only one of the nine smallest divisions of only one of the average 65 sq. cm. areas on the agar plate, whereas actually it is required that the entire 65 sq. cm. be counted for such a plate.

This is mentioned to emphasize the fact that the small amount of milk observed in microscopic methods necessitates high multiplication factors, with consequent larger potential errors. In a comprehensive study of such methods some years ago, Robertson, Moody, and Frayer (*3*) compared agar plate, reductase test, and both individual and clump microscopic counts, and concluded, "The microscopic methods as herein described and used are not as accurate as the agar plate count in milk containing relatively few bacteria because of the failure to examine more microscopic fields on each preparation." Also "However, the counting of a sufficient number of fields to make the microscopic method as accurate as the plate method on a large number of samples of milk is impractical, particularly when one is dealing with milk containing few bacteria."

There are numerous other poorly controlled factors that might be mentioned relative to microscopic methods. As already pointed out, in the surveys considerable errors in technique were noted, even when suitable equipment was available. In addition to errors from not using proper pipettes for measurement and not having the microscope accurately standardized, errors were frequently made in measurement of the 0.01 portion, and in preparation of the film lack of care was not infrequently observed in spreading the portion approximately over the required area. According to a recent report (*4*) both the nature of the stain and the nature of the illumination are unsuitable, permitting numerous bacteria present to be overlooked. Furthermore, workers commonly estimate the number per field and do not enumerate; if they did, fatigue would play an even larger role than at present. Human errors in multiplication and calculation are also a potential hazard. One laboratory in a small city had used the test for a number of years and, instead of observing the bacterial cells, had been enumerating holes in the preparation itself, no immersion oil being used and the appearance of bacteria themselves not being known. In another city where an especially thorough effort was made, each milk sample was analyzed in triplicate, with the usual 10 fields examined on each of the three smears; the results were averaged and the factor was found to be just 25 percent of the correct value. As additional evidence of the great irregularity of the direct microscopic method when applied, had any one of the three preparations alone been used, as is the ordinary practice, the count reported on a sample demonstrated to us

would have been, respectively, 120,000, 30,000, or 60,000 per ml., depending upon which one of the triplicate smears was utilized, inasmuch as actually 4, 1, or 2 bacterial clumps were reported in the 10 representative fields examined.

When it is realized that bacteria present may not be seen or recognized, because backgrounds are frequently confusing, and if attempts are made to count individual cells, there may be many others that are obscured, it might be concluded that the direct microscopic examination is not as simple as some of its proponents imply. Actually, all too often this procedure is turned over to someone in the laboratory without experience in milk sanitation, with the expectation that he or she will observe and report to the milk sanitarian or inspector the type, nature, or probable source of bacteria present. This is sometimes carried out by individuals with so little experience that the organisms are reported by name!

Reports of other investigators indicate that hazards exist in interpretation, too. In discussing the direct microscopic examination of raw milk as indicative of source of contamination, Bryan et al. (*5*) stated, "The suspected trouble was indicated only in those cases where the bacterial count exceeded 100,000 per cubic centimeter of milk," the counts reported being clump counts in which groups or individuals were counted as one. In discussing comparisons with other tests it was stated, "The microscopic count of low count milk is inaccurate, and indicates only that the milk has a very low bacteria count. The accuracy increases as the count of the milk increases." The bacteria counts were divided into four classes, and in class 1, with a (clump) count of less than 100,000 bacteria per cc., it was stated, "In this count range, no suspected trouble is indicated except cells and mastitis streptococci as noted; the scarcity of bacteria does not permit indicating any suspected source of excess contamination."

Another report (*6*) is of interest in that it suggests that the laboratory worker could foresee "how clumps will break up during the plating process." In a report of a comparative study of plate and microscopic counts, counting 20 fields on each sample, it was stated, "Clumps of the same type of bacteria were counted as one. If the clumps showed signs of breaking up, the groups were counted individually." Also, "The close correlation between the average microscopic and plate counts on Grade A milk (microscopic range 0–100,000 per ml.) may be due to the fact that low count milk contains very few clumps of bacteria. It was observed in this study that the bacteria occurred for the most part singly and in pairs." Where differences occurred they suggested no explanation except "possibly, the inability of the technician to judge more accurately how clumps of bacteria will break up during the plating process."

Whereas in the preceding report the bacteria in clumps occurred singly and in pairs, another recent article on the microscopic examination of pasteurized milk (*7*) reported ratios between plate and microscopic counts ranging from 1:260 to 1:25 for street samples, 1:163 to 1:38 for plant samples, and 1:265 to 1:15 for laboratory samples. Mallmann, Bryan, and Fox (*8*) made agar plate counts and microscopic counts before and after pasteurization. They reported that in some instances there was agreement and in others not, both living and dead bacteria being stained, contrary to the reports of others that microscopic counts from pasteurized milk represented mostly viable bacteria, and concluded, "These data would indicate that the microscopic count of pasteurized milk is not always a measure of the viable bacteria present in the sample." Other data presented showed that many dead bacteria did stain and they concluded, "The microscopic count of pasteurized milk, taken directly after pasteurization, represents both living and dead bacteria."

Robertson and Frayer (*9*) in a fifth bulletin summarized their previous studies on variability and discussed at some length applications to raw milk, milk for which premiums are paid for low bacterial counts, and pasteurized milk. With regard to pasteurized milk samples, they stated, "These should always be plated to show the number of viable organisms at 37° C. In addition to this the microscopic method is exceedingly useful in revealing: (1) something of the past history of the milk before pasteurization, (2) the presence of bacterial growth subsequent to pasteurization, and (3) the presence of thermophilic or thermoduric bacteria which enter or grow during the pasteurizing process but which may or may not grow on the plates."

The direct microscopic test has been advocated recently as the sole bacteriological method for pasteurized milk, with any pasteurized milk designated as Grade A if it has not over 200,000 clumps per ml. In addition to the inherent inaccuracies of the procedure itself, it should be pointed out that an excessively high count milk mixed with a larger mass of lower count milk might still result in an average for the whole of under 200,000 clumps per ml., assuming all cells still stained after pasteurization. Since only a varying percentage do so stain, the original count of the milk might have still further exceeded any standard in any individual instance, yet be thinned out in the larger volume so that the entire supply would appear to be of acceptable quality. This would seem to obviate the use of this test as indicative of the original condition of the milk prior to pasteurization, just as the variable proportion staining would render it an inexact estimate of viable organisms after pasteurization.

Similarly, the practice of adding formalin to pasteurized milk samples prior to shipment to a laboratory, with the idea that after microscopic examination a constant factor can be applied to estimate

the number viable before the formalin was added, cannot be substantiated.

Methylene blue reduction.—It would seem unnecessary to discuss at any length the relative accuracy of the agar plate count and the methylene blue test. The methylene blue reduction test is commonly used in England, and a statistical study there (*10*) concluded that the plate count was slightly more sensitive than the methylene blue reduction test observed every half hour. Bearing in mind the deviations reported upon agar plate counts in a preceding paper, it would appear that surely deviations in the agar plate count would have been present even in such a comparative study, and if such plate counts were better done an even more favorable result would be expected. Where no other laboratory facilities are available, and possibly in metropolitan areas with excessively large numbers of producers, the reduction test has some value. Unfortunately it furnishes less information than is frequently desirable, especially where with increased sanitation most of the samples may not be reduced within the time observed. Under these conditions if some of the samples were analyzed by a more accurate method they might be found to reflect approaching breakdowns in sanitation, which the rougher method might not reveal, thus permitting correction before the situation was out of bounds. A rougher test such as methylene blue reduction might continue to pass such milk as satisfactory for some time beyond that indicated by more accurate tests.

Robertson, Moody, and Frayer (*3*) compared agar plate, reductase test, and both individual and clump microscopic counts, and concluded, " * * * the reductase test is more variable than either the agar plate or microscopic methods. This is particularly true when milk containing few bacteria is under observation."

Other deviations are indicated in the discussion of potential material to be studied by the subcommittee on methylene blue test procedure (*11*), including variations in the quantity of the sample, icing of some samples for considerable periods before analysis, failure to stopper tubes, sterilize stoppers, or avoid their contamination in handling, variation in the number, time, and nature of agitations of samples, and variations in temperature of incubation.

Robertson and Frayer (*9*) concluded: "(1) That the agar plate method (logarithmic relations between the counts) is the most feasible method for use (*a*) where premiums are paid for low-count milk and (*b*) where control agencies are examining milk samples; and (2) That the microscopic method and reduction test should be reserved for rapid field work where the object is chiefly to determine whether the milk is good, medium or poor." They also stated, "When workers fully realize

and accept the limitations of any one technique and reserve the use of that technique for the purpose or type of milk sample to which it is best adapted, there will be fewer arguments about accuracy and variability."

The necessity of laboratory control in milk production and handling is generally agreed to, and while the results may not permit immediate segregation of milk, laboratory results are particularly helpful in showing deficiencies in practice frequently overlooked in inspection, or may confirm observations of inspection with tangible results. No doubt improved tests could be developed: however it is evident that many places are not using the present tests to the best advantage, particularly when they have departed from the Standard Methods procedures. The present surveys have shown that most laboratories have failed to conform to such procedures; however, it should be pointed out that the unsatisfactory conditions referred to at the time of the original surveys have been corrected in many instances, and continued improvement should be expected if proper emphasis is placed upon correct performance of such analyses.

(NOTE.—Part III, on sampling and health department practice, will appear in an early issue.)

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PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

REPORTS FROM STATES FOR WEEK ENDED OCTOBER 30, 1943

Summary

The incidence of poliomyelitis continues to decline, but it has not yet reached the median expectancy based on the 5 years 1938–42. A total of 363 cases was reported for the current week, as compared with 438 for the preceding week and a 5-year median of 247. Decreases were recorded in all geographic areas except the East South Central, which area reported 9 cases as compared with 3 for the preceding week. Only 6 States reported more than 20 cases and only 10 States reported more than 10 cases for the week. The following-named States reported more than 30 cases (last week's figures in parentheses): Illinois 38 (57), California 58 (84), and Washington 37 (24). A total of 11,120 cases has been reported to date.

The incidence of meningococcus meningitis also declined, but remained at a level more than five times the 5-year median expectancy. A total of 198 cases was reported currently, as compared with 224 last week, 68 for the corresponding week in 1942, and a 5-year median of 35. A total of 15,380 cases has been reported to date this year, as compared with 2,911 for the same period last year and a 5-year median of 1,705 cases.

Only 3 cases of smallpox and 88 cases of typhoid fever were reported during the current week, as compared with 5-year medians of 25 and 239, respectively. The incidence of both diseases to date is below that for last year, in which year the lowest incidence of record was established for these diseases.

A total of 3,588 cases of endemic typhus fever has been reported to date this year, as compared with 3,013 for the same period last year. For the current week, 109 cases were reported, of which 35 occurred in Georgia, 25 in Alabama, and 21 in Texas.

Deaths recorded for the week in 87 large cities in the United States totaled 8,636, as compared with 8,393 in the same cities last week, and a 3-year average of 8,041 for the week. The cumulative figure to date is 380,683, as compared with 353,104 for the same period last year.

Telegraphic morbidity reports from State health officers for the week ended October 30, 1943, and comparison with corresponding week of 1942 and 5-year median

In these tables a zero indicates a definite report, while leaders imply that, although none was reported, cases may have occurred.

Division and State	Diphtheria			Influenza			Measles			Meningitis, meningococcus		
	Week ended		Median 1938–42	Week ended		Median 1938–42	Week ended		Median 1938–42	Week ended		Median 1938–42
	Oct. 30, 1943	Oct. 31, 1942		Oct. 30, 1943	Oct. 31, 1942		Oct. 30, 1943	Oct. 31, 1942		Oct. 30, 1943	Oct. 31, 1942	
NEW ENGLAND												
Maine	0	0	1				46	1	5	3	2	0
New Hampshire	0	0	0		4		1	8	1	0	1	0
Vermont	0	0	0				66	63	6	0	0	0
Massachusetts	5	1	5				176	171	101	12	4	1
Rhode Island	0	2	1				26	15	9	3	1	0
Connecticut	0	0	0	5	7	1	6	34	21	8	1	0
MIDDLE ATLANTIC												
New York	6	20	18	[1] 5	[1] 9	[1] 8	166	83	89	26	17	1
New Jersey	2	6	8	4	6	3	132	22	22	6	4	0
Pennsylvania	13	21	21	1			68	112	112	15	7	4
EAST NORTH CENTRAL												
Ohio	15	25	25	2	6	6	262	23	23	4	2	1
Indiana	12	4	17	12	14	10	56	16	14	2	1	1
Illinois	12	17	17	9	5	8	23	16	16	10	2	2
Michigan [2]	10	6	6		1		255	39	44	7	0	2
Wisconsin	6	0	1	6	22	22	390	43	53	5	0	0
WEST NORTH CENTRAL												
Minnesota	8	3	2			2	292	12	12	2	1	1
Iowa	2	1	9		3	2	7	14	14	3	0	0
Missouri	4	6	13	6	2	2	5	1	7	6	0	0
North Dakota	2	0	0			1	99	1	7	0	0	0
South Dakota	4	1	2				5	1	2	1	0	0
Nebraska	6	2	2	3	5		6	29	4	1	0	0
Kansas	4	5	4			1	3	16	16	3	0	0
SOUTH ATLANTIC												
Delaware	0	0	0				14	1	1	2	0	0
Maryland [2]	4	4	5	2	1	4	5	8	8	7	1	1
District of Columbia	1	0	1		1	1	5	0	1	7	1	0
Virginia	15	46	49	128	182	60	85	12	12	[3] 12	4	1
West Virginia	2	14	15		7	7	60	2	2	0	0	0
North Carolina	43	59	125	5	2	2	45	1	51	2	2	2
South Carolina	8	30	31	249	201	201	21	4	2	1	0	1
Georgia	19	33	53	17	2	30	13	1	2	3	1	0
Florida	21	33	8	1	8	2	14	2	2	2	1	1
EAST SOUTH CENTRAL												
Kentucky	9	22	22	2	1	1	6	2	11	4	0	2
Tennessee	14	15	24	1	37	19	38	5	5	6	3	3
Alabama	37	41	44	30	68	33	16	1	3	2	2	1
Mississippi [2]	8	17	17							2	1	1
WEST SOUTH CENTRAL												
Arkansas	3	20	20	15	23	27	2	9	8	1	1	0
Louisiana	2	5	20	1	2	4	1	0	1	3	0	0
Oklahoma	2	23	23	20	62	51	4	2	6	0	0	0
Texas	45	54	54	737	503	217	17	12	17	2	0	0
MOUNTAIN												
Montana	1	4	2			4	70	9	9	0	0	0
Idaho	0	0	0		2		0	26	9	0	0	0
Wyoming	2	0	1	2	26	2	7	3	3	0	1	0
Colorado	12	10	9	15	34	14	11	6	16	4	0	0
New Mexico	6	1	1	1	2	1	1	7	7	1	0	0
Arizona	3	6	5	79	44	65	5	14	14	1	0	0
Utah [2]	0	0	0		1	2	3	112	7	2	0	0
Nevada	0	0	0				1	1	0	1	0	0
PACIFIC												
Washington	9	0	1	31			25	264	11	7	1	1
Oregon	2	6	4	9	13	13	23	83	17	1	1	1
California	30	33	23	19	33	28	57	24	73	8	5	2
Total	409	596	668	1,417	1,339	1,093	2,639	1,331	1,359	[3] 198	68	35
43 weeks	10,712	11,789	12,218	91,225	89,696	56,891	551,026	474,381	474,381	15,380	2,911	1,705

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended October 30, 1943, and comparison with corresponding week of 1942 and 5-year median—Con.

Division and State	Poliomyelitis			Scarlet fever			Smallpox			Typhoid and paratyphoid fever [4]		
	Week ended—		Median 1938–42	Week ended—		Median 1938–42	Week ended—		Median 1938–42	Week ended—		Median 1938–42
	Oct. 30, 1943	Oct. 31, 1942		Oct. 30, 1943	Oct. 31, 1942		Oct. 30, 1943	Oct. 31, 1942		Oct. 30, 1943	Oct. 31, 1942	
NEW ENGLAND												
Maine	0	2	0	17	11	9	0	0	0	0	2	1
New Hampshire	0	3	0	8	9	3	0	0	0	0	0	0
Vermont	1	3	1	10	1	5	0	0	0	2	0	0
Massachusetts	7	1	2	121	189	73	0	0	0	4	2	2
Rhode Island	5	0	0	1	6	3	0	0	0	0	1	1
Connecticut	7	0	0	31	19	21	0	0	0	1	0	1
MIDDLE ATLANTIC												
New York	26	6	12	168	200	163	0	0	0	7	8	14
New Jersey	4	11	5	48	60	59	0	0	0	1	1	2
Pennsylvania	6	3	5	139	115	115	0	0	0	3	6	10
EAST NORTH CENTRAL												
Ohio	3	4	8	257	184	171	0	0	0	4	14	6
Indiana	4	4	5	67	51	51	1	0	1	1	0	1
Illinois	38	8	8	108	160	178	0	1	1	2	13	15
Michigan [3]	17	4	8	117	53	122	0	0	0	1	4	4
Wisconsin	13	1	3	126	122	104	0	0	0	1	1	1
WEST NORTH CENTRAL												
Minnesota	7	3	13	61	53	56	0	0	0	0	0	1
Iowa	4	3	3	57	54	58	0	0	1	5	0	2
Missouri	0	1	1	33	59	59	0	0	0	2	1	5
North Dakota	1	0	1	9	12	10	0	0	0	0	0	1
South Dakota	0	2	2	12	20	20	0	2	0	1	0	1
Nebraska	3	6	1	43	13	15	0	1	1	0	0	0
Kansas	21	11	1	55	60	60	0	0	0	0	3	1
SOUTH ATLANTIC												
Delaware	0	0	0	1	11	7	0	0	0	1	1	2
Maryland [2]	1	0	2	35	32	21	0	0	0	0	3	6
District of Columbia	2	0	0	18	22	13	0	0	0	0	1	1
Virginia	1	1	4	34	77	52	0	0	0	6	9	10
West Virginia	0	0	1	63	48	51	0	0	0	1	0	4
North Carolina	1	2	1	113	128	123	1	0	0	2	3	3
South Carolina	0	2	1	13	10	17	0	0	0	0	4	8
Georgia	0	1	1	49	50	38	0	0	0	4	5	9
Florida	0	2	1	11	7	4	0	0	0	0	3	1
EAST SOUTH CENTRAL												
Kentucky	6	1	5	50	62	73	0	0	0	4	3	12
Tennessee	0	1	1	38	81	80	0	0	0	4	9	6
Alabama	3	4	4	38	36	39	0	0	0	5	3	11
Mississippi [3]	0	2	3	11	14	15	1	0	0	5	3	4
WEST SOUTH CENTRAL												
Arkansas	0	2	2	7	5	7	0	0	0	0	6	6
Louisiana	0	1	1	8	5	10	0	0	0	0	3	7
Oklahoma	8	0	0	8	20	20	0	0	2	1	0	5
Texas	19	12	3	41	57	48	0	1	1	8	12	14
MOUNTAIN												
Montana	0	0	0	31	8	18	0	0	0	0	0	0
Idaho	2	0	1	13	4	12	0	0	1	0	0	1
Wyoming	1	0	0	1	1	5	0	0	0	0	0	0
Colorado	8	1	2	21	9	23	0	1	1	0	5	5
New Mexico	2	1	0	6	3	6	0	0	0	3	1	2
Arizona	3	1	1	15	0	1	0	0	0	1	4	1
Utah [3]	15	3	3	13	12	10	0	0	0	0	0	0
Nevada	2	0	0	1	0	0	0	0	0	0	0	0
PACIFIC												
Washington	37	8	3	61	19	27	0	2	1	2	1	3
Oregon	27	3	2	19	9	13	0	0	1	2	0	1
California	58	21	7	148	103	103	0	1	1	4	0	7
Total	363	140	247	2,355	2,284	2,284	3	9	25	88	135	239
43 weeks	11,120	3,519	6,245	113,474	102,851	131,066	648	683	2,089	4,827	6,001	8,399

See footnotes at end of table.

Telegraphic morbidity reports from State health officers for the week ended October 30, 1943, and comparison with corresponding week of 1942 and 5-year median—Con.

Division and State	Whooping cough			Week ended Oct. 30, 1943								
	Week ended—		Median 1938–42	Anthrax	Dysentery			Encephalitis, infectious	Leprosy	Rocky Mt. spotted fever	Tularemia	Typhus fever
	Oct. 30, 1943	Oct. 31, 1942			Amebic	Bacillary	Unspecified					
NEW ENGLAND												
Maine	8	46	29	0	0	0	0	0	0	0	0	0
New Hampshire	1	2	4	0	0	0	0	0	0	0	0	0
Vermont	27	49	24	0	0	0	0	0	0	0	0	0
Massachusetts	87	190	134	1	0	13	0	0	0	0	0	0
Rhode Island	13	4	30	0	0	0	0	0	0	0	0	0
Connecticut	32	58	58	0	0	0	0	0	0	0	0	0
MIDDLE ATLANTIC												
New York	250	444	405	1	1	35	0	1	0	1	0	0
New Jersey	69	169	145	0	1	0	0	0	0	0	0	0
Pennsylvania	154	238	238	1	1	2	0	1	0	0	0	0
EAST NORTH CENTRAL												
Ohio	86	189	169	0	0	0	0	0	0	0	0	0
Indiana	16	19	19	0	0	0	0	0	0	0	0	0
Illinois	137	171	192	0	3	1	0	0	0	0	0	0
Michigan [2]	128	154	228	0	0	2	0	0	0	0	0	0
Wisconsin	175	130	168	0	0	0	0	0	0	0	1	0
WEST NORTH CENTRAL												
Minnesota	55	18	52	0	2	0	0	0	0	0	0	0
Iowa	22	16	16	0	3	0	0	0	0	0	1	0
Missouri	16	8	22	0	0	0	2	0	0	0	0	0
North Dakota	8	6	18	0	0	0	0	0	0	0	0	0
South Dakota	5	0	0	0	0	0	0	0	0	0	0	0
Nebraska	21	10	5	0	0	0	0	0	0	0	0	0
Kansas	39	30	30	0	0	0	0	0	0	0	0	0
SOUTH ATLANTIC												
Delaware	0	4	4	0	0	0	0	0	0	0	0	0
Maryland [2]	31	87	56	0	0	0	1	0	0	0	0	0
District of Columbia	10	14	12	0	0	0	0	0	0	0	0	1
Virginia	58	11	35	0	0	0	53	0	0	2	1	1
West Virginia	2	22	22	0	0	0	0	0	0	0	0	0
North Carolina	130	42	61	0	0	0	0	0	0	0	0	6
South Carolina	32	6	21	0	0	2	0	0	0	6	0	4
Georgia	9	11	11	0	0	3	0	0	0	0	0	35
Florida	19	3	6	0	2	3	0	0	0	0	0	8
EAST SOUTH CENTRAL												
Kentucky	64	32	58	0	0	2	0	0	0	1	0	0
Tennessee	27	21	36	0	1	0	4	0	0	0	0	1
Alabama	6	31	28	0	0	0	0	0	0	0	0	25
Mississippi [3]	------	------	------	0	0	0	0	0	0	0	1	2
WEST SOUTH CENTRAL												
Arkansas	25	34	14	0	0	14	0	0	0	0	0	1
Louisiana	1	4	5	0	0	0	0	0	0	0	1	4
Oklahoma	1	6	6	0	0	0	0	0	0	0	0	0
Texas	68	69	69	0	15	183	0	0	1	0	3	21
MOUNTAIN												
Montana	23	18	17	0	0	0	0	0	0	0	0	0
Idaho	0	3	2	0	0	0	0	0	0	0	0	0
Wyoming	10	1	6	0	0	0	0	0	0	0	0	0
Colorado	52	7	20	0	0	0	0	0	0	0	0	0
New Mexico	3	6	13	0	0	5	5	0	0	0	0	0
Arizona	15	3	3	0	1	0	18	0	0	0	0	0
Utah [3]	16	9	15	0	0	0	0	0	0	0	0	0
Nevada	0	0	0	0	0	0	0	0	0	0	0	0
PACIFIC												
Washington	87	13	43	0	0	0	0	0	0	0	0	0
Oregon	54	2	10	0	0	0	0	0	0	0	0	0
California	85	187	155	0	3	12	0	4	0	1	0	0
Total	2,177	2,597	3,123	3	33	277	83	6	1	5	8	109
43 weeks	156,828	149,727	150,098	56	1,779	13,982	3,664	592	24	427	682	3,588
43 weeks, 1942	------	------	------	70	1,009	10,799	5,941	482	40	445	746	3,014

[1] New York City only.
[2] Period ended earlier than Saturday.
[3] Exclusive of delayed report (included only in cumulative total) of 4 cases in Virginia.
[4] Including paratyphoid fever cases reported separately as follows: Massachusetts, 4; New York, 1; New Jersey, 1; Michigan, 1; Georgia, 1; Tennessee, 1; Washington, 1.

WEEKLY REPORTS FROM CITIES

City reports for week ended Oct. 16, 1943

This table lists the reports from 86 cities of more than 10,000 population distributed throughout the United States, and represents a cross section of the current urban incidence of the diseases included in the table.

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
NEW ENGLAND												
Maine:												
Portland	1	0	-----	0	3	0	1	0	2	0	1	2
New Hampshire:												
Concord	0	0	-----	0	0	0	1	0	0	0	0	0
Vermont:												
Barre	0	0	-----	0	0	0	0	0	0	0	0	0
Massachusetts:												
Boston	1	0	-----	1	3	7	13	5	43	0	1	40
Fall River	0	0	-----	0	0	0	0	3	2	0	0	0
Springfield	0	0	-----	0	1	0	2	0	8	0	0	5
Worcester	0	0	-----	0	1	0	2	0	12	0	0	0
Rhode Island:												
Providence	0	0	-----	0	18	1	3	5	3	0	0	41
Connecticut:												
Bridgeport	0	0	-----	0	0	0	0	1	6	0	0	0
Hartford	1	0	-----	0	1	0	3	1	4	0	0	1
New Haven	0	0	-----	0	0	1	3	1	1	0	0	2
MIDDLE ATLANTIC												
New York:												
Buffalo	0	0	-----	0	4	1	5	7	2	0	0	10
New York	7	3	5	4	61	17	45	16	85	0	4	79
Rochester	0	0	-----	0	2	0	2	0	6	0	0	6
Syracuse	0	0	-----	0	0	0	2	0	1	0	0	19
New Jersey:												
Camden	0	0	-----	0	2	1	0	0	0	0	0	0
Newark	0	0	-----	1	1	1	4	1	7	0	0	18
Trenton	0	0	-----	0	0	0	2	0	1	0	0	3
Pennsylvania:												
Philadelphia	3	0	1	0	10	7	18	1	24	0	1	62
Pittsburgh	2	0	-----	1	15	6	24	1	30	0	1	11
Reading	0	0	-----	0	0	0	0	0	1	0	0	3
EAST NORTH CENTRAL												
Ohio:												
Cincinnati	1	0	-----	1	20	3	0	1	26	0	0	11
Cleveland	0	0	-----	0	0	9	14	4	33	0	1	23
Columbus	1	0	-----	0	8	0	1	2	13	0	0	4
Indiana:												
Fort Wayne	0	0	-----	0	0	1	1	0	4	0	0	0
Indianapolis	0	0	-----	0	1	0	4	0	12	0	0	5
South Bend	0	0	-----	0	2	0	0	0	1	0	0	0
Terre Haute	1	0	-----	0	1	0	1	0	1	0	0	0
Illinois:												
Chicago	2	0	-----	1	2	10	19	30	25	0	0	74
Springfield	0	0	-----	0	0	0	1	0	0	0	0	2
Michigan:												
Detroit	11	0	-----	0	9	5	9	5	36	0	2	38
Flint	0	0	-----	0	0	0	0	0	3	0	0	15
Grand Rapids	0	0	-----	0	1	0	0	2	3	0	0	1
Wisconsin:												
Kenosha	0	0	-----	0	0	0	0	0	5	0	0	0
Milwaukee	0	0	-----	0	2	1	0	1	28	0	0	44
Superior	0	0	-----	0	72	0	0	0	0	0	0	6
WEST NORTH CENTRAL												
Minnesota:												
Duluth	0	0	-----	0	5	0	3	0	2	0	0	15
Minneapolis	6	0	-----	1	18	0	2	2	25	0	0	4
St. Paul	0	0	-----	0	17	0	1	2	5	0	0	16
Missouri:												
Kansas City	3	0	-----	0	1	3	7	1	8	0	0	7
St. Joseph	0	0	-----	0	0	0	0	0	3	0	0	0
St. Louis	2	0	2	0	0	1	11	0	10	0	0	5
Nebraska:												
Omaha	2	0	-----	0	0	0	3	1	9	0	0	0
Kansas:												
Topeka	0	0	-----	0	0	0	0	1	0	0	0	2
Wichita	0	0	-----	0	1	0	1	1	5	0	0	2

City reports for week ended Oct. 16, 1943—Continued

	Diphtheria cases	Encephalitis, infectious, cases	Influenza		Measles cases	Meningitis, meningococcus, cases	Pneumonia deaths	Poliomyelitis cases	Scarlet fever cases	Smallpox cases	Typhoid and paratyphoid fever cases	Whooping cough cases
			Cases	Deaths								
SOUTH ATLANTIC												
Delaware:												
Wilmington	0	0	------	0	1	1	0	0	0	0	0	0
Maryland:												
Baltimore	2	0	1	1	4	4	6	1	8	0	0	30
Cumberland	0	0	------	0	0	0	0	0	0	0	0	0
Frederick	0	0	------	0	0	0	0	0	0	0	0	0
District of Columbia:												
Washington	0	0	------	0	1	1	3	0	17	0	0	9
Virginia:												
Lynchburg	0	0	------	0	21	0	2	0	1	0	0	12
Richmond	0	0	------	0	0	0	1	1	2	0	0	1
Roanoke	0	0	------	0	0	0	0	0	0	0	0	0
West Virginia:												
Charleston	0	0	------	0	2	0	0	0	3	0	0	0
Wheeling	0	0	------	0	0	2	3	0	1	0	1	1
North Carolina:												
Winston-Salem	1	0	------	0	0	1	2	0	4	0	0	6
South Carolina:												
Charleston	0	0	5	1	0	0	1	0	3	0	1	0
Georgia:												
Atlanta	2	0	8	0	1	1	1	0	5	0	0	2
Brunswick	1	0	------	0	2	0	1	0	1	0	0	0
Savannah	0	0	------	0	1	0	2	0	2	0	0	3
Florida:												
Tampa	0	0	------	0	0	0	0	0	0	0	0	1
EAST SOUTH CENTRAL												
Tennessee:												
Memphis	1	0	------	0	0	0	0	0	0	0	0	2
Nashville	0	0	------	0	0	0	0	0	3	0	0	4
Alabama:												
Birmingham	0	0	------	0	0	0	3	0	1	0	0	0
Mobile	0	0	1	0	0	0	2	0	0	0	0	0
WEST SOUTH CENTRAL												
Arkansas:												
Little Rock	0	0	------	0	0	0	0	0	1	0	0	0
Louisiana:												
New Orleans	0	0	1	1	1	0	6	0	2	0	4	1
Shreveport	1	0	------	0	0	0	0	0	0	0	0	0
Texas:												
Dallas	0	0	------	0	0	0	0	1	3	0	0	2
Galveston	0	0	------	0	0	0	0	0	0	0	0	3
Houston	1	0	------	0	1	1	8	0	2	0	2	3
San Antonio	1	0	2	2	1	0	5	1	1	0	0	0
MOUNTAIN												
Montana:												
Billings	3	0	------	0	0	0	0	0	0	0	0	0
Great Falls	0	0	------	0	15	0	0	0	1	0	0	3
Helena	0	0	------	0	0	0	0	0	0	0	0	0
Missoula	0	0	------	0	0	0	2	0	1	0	0	0
Idaho:												
Boise	0	0	------	0	0	0	1	0	0	0	0	6
Colorado:												
Denver	2	0	3	0	1	0	7	2	1	0	0	17
Pueblo	0	0	------	0	1	0	2	1	3	0	0	2
Utah:												
Salt Lake City	0	0	------	0	1	0	0	2	3	0	0	11
PACIFIC												
Washington:												
Seattle	0	0	------	0	2	0	1	5	0	0	0	11
Spokane	0	0	------	0	3	0	1	0	6	0	0	4
Tacoma	0	0	------	0	0	0	2	1	2	0	0	2
California:												
Los Angeles	4	0	3	0	2	3	3	7	12	0	0	12
Sacramento	0	0	------	0	1	0	2	4	4	0	1	1
San Francisco	0	0	4	0	0	3	10	10	18	0	0	11
Total	63	3	36	15	344	92	285	131	606	0	20	736
Corresponding week, 1942	101	0	83	19	199	31	313	45	515	0	22	939
Average, 1938–42	104	------	62	[1] 14	[2] 254	------	[1] 276	------	477	1	36	1,030

Anthrax.—Cases: Philadelphia, 1.

Dysentery, amebic.—Cases: Boston, 1; New York, 2.

Dysentery, bacillary.—Cases: New York, 6; Rochester, 1; Chicago, 4; Detroit, 4; St. Louis, 1; Baltimore, 1; Charleston, S. C., 3; Los Angeles, 9.

Dysentery, unspecified.—Cases: Baltimore, 1; Richmond, 4; Memphis, 1.

Rocky Mountain spotted fever.—Cases: Terre Haute, 1.

Typhus fever.—Cases: Boston, 1; New York, 2; Richmond, 1; Winston-Salem, 3; Charleston, S. C., 2; Atlanta, 2; Savannah, 9; Memphis, 1; Nashville, 1; Birmingham, 1; Mobile, 2; New Orleans, 2; Dallas, 1.

[1] 3-year average, 1940–42.

[2] 5-year median.

Rates (annual basis) per 100,000 population, by geographic groups, for the 86 cities in the preceding table (estimated population, 1942, 34,582,700)

	Diphtheria case rates	Encephalitis, infectious, case rates	Influenza		Measles case rates	Meningitis, meningococcus, case rates	Pneumonia death rates	Poliomyelitis case rates	Scarlet fever case rates	Smallpox case rates	Typhoid and paratyphoid fever case rates	Whooping cough case rates
			Case rates	Death rates								
New England	7.5	0.0	0.0	2.5	67.1	22.4	69.6	39.8	201.2	0.0	5.0	226
Middle Atlantic	5.4	1.3	2.7	2.7	42.4	14.7	45.5	11.6	70.0	0.0	2.7	94
East North Central	9.0	0.0	0.0	1.2	69.4	17.1	29.4	26.5	111.8	0.0	1.8	131
West North Central	25.7	0.0	4.0	2.0	83.1	7.9	55.4	15.8	132.5	0.0	0.0	101
South Atlantic	10.4	0.0	24.3	3.5	57.3	17.4	38.2	3.5	81.6	0.0	3.5	113
East South Central	5.9	0.0	5.9	0.0	0.0	0.0	29.7	0.0	23.8	0.0	0.0	36
West South Central	8.8	0.0	8.8	8.8	8.8	2.9	55.7	5.9	26.4	0.0	17.6	26
Mountain	40.2	0.0	24.1	0.0	144.7	0.0	96.5	40.2	72.4	0.0	0.0	314
Pacific	7.0	0.0	12.2	0.0	14.0	10.5	33.2	47.2	73.4	0.0	1.7	72
Total	9.5	0.5	5.4	2.3	51.9	13.9	43.0	19.8	91.4	0.0	3.0	111

TERRITORIES AND POSSESSIONS

Hawaii Territory

Honolulu—Dengue fever.—Up to October 21, 1943, a total of 662 cases of dengue fever was reported in Honolulu, Hawaii Territory.

FOREIGN REPORTS

CANADA

Provinces—Communicable diseases—Week ended October 2, 1943.—During the week ended October 2, 1943, cases of certain communicable diseases were reported by the Dominion Bureau of Statistics of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Chickenpox		1	9	51	70	7	21	10	30	199
Diphtheria	4	29	1	39	1	2			1	77
Dysentery (bacillary)				4						4
German measles		6		1	4	2	1	2	4	20
Influenza		15	1		27	1			6	50
Measles			1	107	34	33	1	14	35	225
Meningitis, meningococcus				6	3					9
Mumps		11		11	28	12	6	9	24	101
Poliomyelitis				12	7			1	1	21
Scarlet fever		12	1	72	61	21	43	21	22	253
Tuberculosis (all forms)		2	2	118	36	27	50	18	66	319
Typhoid and paratyphoid fever				30	3			3		36
Whooping cough		11		82	90	25		34	11	253

CUBA

Habana—Communicable diseases—4 weeks ended October 16, 1943.—During the 4 weeks ended October 16, 1943, certain communicable diseases were reported in Habana, Cuba, as follows:

Disease	Cases	Deaths	Disease	Cases	Deaths
Diphtheria	19	2	Measles	12	
Leprosy	1		Tuberculosis	7	3
Malaria	5		Typhoid fever	19	2

Provinces—Notifiable diseases—4 weeks ended October 9, 1943.—During the 4 weeks ended October 9, 1943, cases of certain notifiable diseases were reported in the Provinces of Cuba as follows:

Disease	Pinar del Rio	Habana [1]	Matanzas	Santa Clara	Camaguey	Oriente	Total
Cancer	2		3	4		8	17
Diphtheria	2	25	3	2			32
Hookworm disease		12					12
Leprosy		2					2
Malaria	53	21	16	34	14	192	330
Measles		9		1			10
Poliomyelitis			1	1			2
Tuberculosis	15	89	9	24	1	53	191
Typhoid fever	18	35	16	40	10	69	188
Whooping cough				1			1
Yaws						3	3

[1] Includes the city of Habana.

FINLAND

Notifiable diseases—August 1943.—During the month of August 1943, cases of certain notifiable diseases were reported in Finland as follows:

Disease	Cases	Disease	Cases
Cerebrospinal meningitis	18	Paratyphoid fever	313
Chickenpox	224	Pneumonia	460
Conjunctivitis	26	Poliomyelitis	36
Diphtheria	923	Puerperal fever	46
Dysentery	35	Rheumatic fever	232
Gastroenteritis	6,579	Scabies	1,857
Gonorrhea	764	Scarlet fever	410
Hepatitis, epidemic	636	Syphilis	442
Influenza	427	Tetanus	3
Laryngitis	32	Typhoid fever	50
Malaria	1	Vincent's angina	25
Measles	965	Whooping cough	719
Mumps	208		

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

NOTE.—Except in cases of unusual prevalence, only those places are included which had not previously reported any of the above-mentioned diseases, except yellow fever, during the current year. All reports of yellow fever are published currently.

A cumulative table showing the reported prevalence of these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

(Few reports are available from the invaded countries of Europe and other nations in war zones.)

Typhus Fever

Iran.—During the week ended August 14, 1943, 57 cases of typhus fever with 9 deaths were reported in Iran.

Yellow Fever

Gold Coast—Asuboi.—On September 12, 1943, 1 case of yellow fever with 1 death was reported at Asuboi, near Nsawam, Gold Coast.

DEATHS DURING WEEK ENDED OCTOBER 23, 1943

[From the Weekly Mortality Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended Oct. 23, 1943	Corresponding week, 1942
Data for 89 large cities of the United States:		
Total deaths	8,552	8,444
Average for 3 prior years	8,152	
Total deaths, first 42 weeks of year	379,677	351,391
Deaths under 1 year of age	562	605
Average for 3 prior years	559	
Deaths under 1 year of age, first 42 weeks of year	27,246	24,120
Data from industrial insurance companies:		
Policies in force	65,963,981	65,170,956
Number of death claims	12,199	12,473
Death claims per 1,000 policies in force, annual rate	9.6	10.0
Death claims per 1,000 policies, first 42 weeks of year, annual rate	9.7	9.1

COURT DECISION ON PUBLIC HEALTH

Public water supply—protection against pollution—reasonableness of State board of health regulations.—(New Hampshire Supreme Court; *Willis* v. *Wilkins et al.*, 32 A.2d 321; decided May 4, 1943.) The town of Pembroke, which obtained its public water supply from a certain great pond, filed with the New Hampshire State Board of Health a petition for water supply regulation under statutory provisions, alleging that the pond was in danger of contamination. The board of health held hearings on this petition and subsequently adopted regulations which prohibited swimming and bathing in the pond and forbade the erection and maintenance of any structure upon the ice of the pond. The owners of lands located on the shore of the pond sought to have these regulations declared void and concluded their petition with a prayer for equitable relief. Facts were found by a master who recommended that the plaintiffs' petition be dismissed. The plaintiffs excepted and the questions of law raised by their exceptions were transferred by the superior court without a ruling to the State supreme court.

The latter court first made several findings which may be summarized as follows:

(*a*) The rights affected by the regulations in question comprised rights which the plaintiffs were privileged to enjoy primarily as members of the public together with certain incidental rights which they possessed as owners of lands bordering the pond, but the plaintiffs were not entitled to compensation even though their lands might be less valuable because of the curtailment of such rights.

(*b*) It was unimportant that formal notice of the investigation by the State board of health was not served upon the plaintiffs as riparian proprietors. (The court pointed out that the statute contained no

provision for notice other than a requirement for the posting or publication of the regulations made, but that in the instant case, however, notice was given both by posting and publication and that some of the plaintiffs appeared at the hearings and participated therein.)

(*c*) The plaintiffs' contention that the board erred in not granting certain requests for findings of fact and rulings of law was without merit, inasmuch as the plaintiffs were not entitled to a hearing as of right and the board could, if it saw fit, adopt a summary procedure.

(*d*) It was immaterial that certain board members participating in the final deliberations did not hear all the testimony introduced at both hearings, as the board could act upon its own inspection and knowledge, was not obliged to hear any party, and could obtain its information from any source and in any way.

(*e*) The plaintiffs had failed, so far as the record was concerned, to show that the board had acted illegally in respect to jurisdiction, authority, or observance of the law.

After setting forth the above rulings the supreme court stated that the regulations, however, could not stand if they were indisputably unreasonable. The plaintiffs, being for the most part owners of cottages which they occupied in summer for recreational purposes, had rights incidental to the enjoyment of public rights in the pond which were more extensive than those of one who was only a member of the public, and, according to the court, although no part of their property was "taken" in the constitutional sense of the term by force of the regulations, the beneficial use of such property was unquestionably impaired thereby, with a resulting depreciation in value. "This fact is, in our opinion, a material circumstance to be considered in determining whether or not the prohibition can be found to be reasonable. But no such determination can be made on the present transfer, since the master in reaching his conclusion has failed to consider all the material evidence bearing on the issue." There was evidence to the effect that the bacteria in the pond were largely eliminated by natural self-purification; that the lowering of the intake of the water system to the depth of 20 feet would be an almost perfect safeguard against summer pollution, and that the cost of such lowering would be from $1,600 to $2,500; that chlorination rendered the water practically sterile, that the cost of installing an automatic chlorination plant would be about $2,500, and that the annual expense of maintaining and operating such a plant would be about $200. The plaintiffs, in support of their claim that the regulations were arbitrary and unreasonable, had offered to prove that the market value of their properties before the restrictions on the use of the pond were imposed was approximately $70,000 but that the board's action had reduced such value from 50 to 75 percent. This evidence was excluded by the

master as immaterial, but the supreme court held that such exclusion constituted reversible error and that the master's report had to be set aside and a new trial ordered. The court's view was that, in passing upon the reasonableness of regulations promulgated under legislative authority, the importance of the public benefit which the regulations sought to promote was to be balanced against the seriousness of the restriction of private right sought to be imposed.