Storage of Water Samples for Bacteriologic Examinations

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This laboratory study conducted for the Committee on Standard Methods for the Examination of Water and Sewage will be of immediate interest to all concerned with water treatment and other areas of environmental sanitation.

The validity of the results from stored bacterial samples has always been a matter of concern to the bacteriologist, the public health engineer, and now the statistician. This is particularly true in our control laboratories, since the bulk of the samples they receive are necessarily in transit for an indefinite period of time, and since the evidence in regard to coliforms obtained from such samples is to a very great degree used in the sanitary evaluation of water supplies. At the present moment this subject may be particularly pertinent in light of the great interest in the membrane filter field monitors and the proposed holding media.

All the editions of Standard Methods from 1928 to 1945 made the following statement in regard to the collection of bacterial samples: "The time allowed for storage or transportation between the filling of the sample bottle and the beginning of analysis should not be more than six hours for impure water and not more than 12 hours for relatively pure water. During storage the temperature shall be kept between 6° and 10° C."

During the preparation of the tenth edition the committee, in view of the information available to them, felt that these instructions might not be fully realistic. In the time available several short but intensive studies on the effect of storage were carried on under the supervision of the committee, but the evidence was still far from conclusive. When the tenth edition was finally prepared the committee broadened the temperature range to read from 0° to 10° C and any mention of time was omitted in favor of a statement that "all samples should be examined as soon as possible after collection."

The committee set up a program for further experiments on stored water and requested several representative laboratories to carry on these experiments. The present paper presents the results on this study from two central laboratories, those of the New York and the Massachusetts Health Department laboratories. Over a period of a year the New York department laboratory collected nearly 200 samples from a surface water source, of which 69 had determinate coliform values in both initial and in stored samples. The Lawrence Experiment Station collected over 200 samples from Haggetts Pond, which is the raw water supply of the town of Andover, Mass., and of these, 190 gave determinate values. The committee also received a report on nearly 100 samples of ground water from Connecticut, but very few of these had determinate values after storage.

In the case of both New York and

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Massachusetts the samples were collected three or four times a week over a 12-month period so that the effect of seasons, of temperature, and of runoff was included in the samples studied in both laboratories. Both laboratories followed the same procedure. Duplicate five-tube MPN determinations on the initial sample were initiated one-half hour after the time of sampling and the master sample was then divided into two portions. One of these was stored at room temperature and the other in a refrigerator. After 24 hours of storage, duplicate five-tube MPNs were made on each stored sample. All tubes positive after either 24 or 48 hours incubation at 35° C were confirmed in brilliant green bile according to present Standard Methods procedure. From the confirmed results the MPN was calculated for each sample analyzed. Temperatures were recorded at the time of sampling and at the end of the 24-hour storage period. On these same samples determinations were also made after six-hour storage on both room and refrigerator samples but, for the present, the results of this phase of the study are not presented.

Discussion of Results

The data from both laboratories were analyzed by the ratio method. The arithmetic average of the duplicate MPN's on the initial sample, which we have called the master sample, was compared with the average MPN of both stored pairs and the ratio in each case was calculated. From both laboratories these ratios were divided: (1) into summer and winter collection periods, and (2) into groups of increasing initial coliform density. In Massachusetts there were enough samples to divide into four initial coliform groups with about the same numbers in each, but it was possible to divide the results from the New York samples into only three groups according to initial density. From the number of analyses for all the samples and for each seasonal, or MPN grouping, the median ratios and the 95 per cent confidence limits were calculated, and these are presented in Tables 1 and 2. From this method of analysis, the following conclusions have been drawn:

The grouping of samples according to their initial MPN produced several interesting trends. The samples from Haggetts Pond with an initial coliform count of 23 or less gave a median ratio of 0.89 for the room and 1.07 for the refrigerated samples. For the samples with an initial MPN between 24 and 79 the figures were 0.92 and 1.02; with somewhat more pollution—that is, MPN between 80 and 230—the figures were 0.71 and 0.755; and for still more polluted samples—that is, of those with an initial MPN above 230—the median ratios were 0.61 and 0.77. Because of their smaller numbers, the New York samples were divided into only three groups. Those samples with an initial MPN of 23 or less had a median ratio of 1.00 for the room samples and 1.02 for the refrigerated samples. Waters with an initial MPN between 23 and 230 had median ratios of 0.51 and 0.67, and those with an initial MPN greater than 230 gave results of 0.51 and 0.52.

This evidence seems to indicate that 24-hour storage results in both laboratories are somewhat more reliable for samples initially containing a low coliform pollution than with samples of higher numbers. Our analysis does not indicate whether this reduced recovery is associated with high initial density or the initial temperature or both. There appears, however, to be no definite trend associated with the initial temperature alone, although we did not make a detailed investigation. If we assume that the effect of the initial temperature may be disregarded, the explanation may well be that samples of low coliform density

Comparison of the Median Ratios of Stored MPN to Initial MPN

Table 1—Massachusetts Water

	No. of Samples	24 hr. Room Storage MPN to Initial MPN: Median Ratio	24 hr. Room Storage MPN to Initial MPN: 95 Per cent Confidence Limits	24 hr Refrigerator Storage MPN to Initial MPN: Median Ratio	24 hr Refrigerator Storage MPN to Initial MPN: 95 Per cent Confidence Limits
Year	190	0.915	0.77 – 1.00	0.86	0.76 – 0.97
Summer (May–Oct.)	100	0.74	0.63 – 0.92	0.88	0.77 – 1.10
Winter (Nov.–Apr.)	90	1.12	0.85 – 1.20	0.825	0.73 – 0.93
MPN — 23 or less	59	1.07	0.90 – 1.8	0.89	0.63 – 1.3
24–79	53	1.02	0.75 – 1.39	0.92	0.78 – 1.21
80–230	52	0.71	0.53 – 0.92	0.755	0.46 – 0.97
over 230	26	0.61	0.31 – 1.1	0.77	0.50 – 1.0

Table 2—New York Water

Year	69	0.66	0.51 – 0.96	0.74	0.60 – 1.00
Summer (May–Oct.)	32	0.67	0.39 – 1.28	0.78	0.52 – 1.23
Winter (Nov.–Apr.)	37	0.66	0.50 – 0.96	0.67	0.48 – 1.00
MPN — 23 or less	27	1.02	0.65 – 1.49	1.00	0.67 – 1.77
23–230	23	0.51	0.29 – 0.97	0.67	0.42 – 1.22
over 230	19	0.51	0.39 – 0.74	0.52	0.45 – 1.09

tend to include only organisms which are hardy and acclimated and thus better able to survive the storage interval, while in the more polluted samples there were included a number of weak and perhaps less acclimated organisms which more readily succumbed during storage.

As reported in the paper on comparative coliform densities by the membrane filter test,[1] we have found that, with the Haggetts Pond water, samples with initial low coliforms generally also had fewer noncoliform organisms, whereas those exhibiting greater pollution tended to contain noncoliform organisms in a much higher ratio. The greater antagonistic competition for survival present in polluted water and perhaps the greater number of predators we might expect to find in such water might also account for the decrease of coliform organisms in the stored samples which initially had a higher coliform density. A report by Berry [2] on ground waters, which are normally of low coliform content and very apt to contain very small numbers of noncoliforms, appears to substantiate our findings that 24-hour storage of low coliform samples does not materially affect the survival of the coliform bacteria.

For all the Massachusetts samples the ratio in both room and refrigerator samples is only slightly less than 1.00, indicating some loss in recovery of coliforms after the 24 hours. While this statement is true in a numerical sense, the relatively narrow confidence limits for the annual ratios indicate that the loss of coliforms is not significant in any practical sense. The ratios for the New York results for all 69 samples show somewhat greater loss. For the least contaminated waters in New York the ratios are quite similar to those in Massachusetts, but 28 per cent of all the New York samples had coliform densities over 230 as compared to only 12 per cent for the Massachusetts samples and this factor tends to make the general median ratio somewhat low.

A comparison of the ratios for the entire year indicates no demonstrated advantage for refrigeration of samples for the Haggetts Pond water. The same appears to be true for the New York samples. In the Massachusetts samples it appears that a higher ratio of coliform recovery is obtained in the winter from samples stored in the room, while in the summer months a greater ratio of coliform recovery is obtained from refrigerated samples. In the New York results the recovery in the winter samples is the same for both conditions, but in the summer samples a better recovery is obtained in the refrigerated portions.

The degree of reproducibility of the results of the study has been examined by two methods of treatment. First the ratios of all 380 analyses were arrayed in order of magnitude and plotted on log probability paper against the log normal line predicted by the Halvorson-Ziegler [3] model for replicate MPN's. An excellent agreement was found with no overdispersion. Second, the "per cent agreement" between duplicate values was determined and it was found to coincide with that predicted by the Halvorson-Ziegler theory.

The authors are preparing for future presentation a more detailed analysis of the results of the study which it is believed will offer very significant evidence in regard to the reproducibility of the standard MPN test.

Conclusions

The many physical, chemical, and other environmental factors affecting the survival of the coliform group in stored water samples seem to make it improbable that an all-inclusive finding of the effect of storage on samples will ever be established. From this present investigation a general pattern is indicated in regard to waters like these from which these studies have been made.

The indication seems to be that bacterial samples with relatively low coliform densities which have been in shipment up to 24 hours at any reasonable temperature will give results which statistically correlate very well with the degree of pollution existing at the sample point at the time of collection. Samples with higher initial coliform densities, perhaps those with MPN's of 230 or more, may be more likely to exhibit a decrease in coliform content in 24-hour storage.

It might be fairly inferred that samples which normally have no positives in 10 ml portions would not be at all affected by 24 hours of storage. Such waters make up the very great majority of samples coming to Albany and to Lawrence.

It is our belief that the difference in results depending on the initial coliform density deserves further study in the general question of the effect of storage.

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