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# A-1 Medium: Alternative Technique for Fecal Coliform Organism Enumeration in Chlorinated Wastewaters

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A 24-h most-probable-number technique using A-1 medium for detecting fecal coliforms in chlorinated wastewaters was evaluated. The A-1 medium technique, using 3 h of preincubation at 35°C, gave results statistically equivalent to those obtained with the American Public Health Association *Standard Methods* two-step most-probable-number technique.

The accepted method for determining disinfection efficiency of wastewater treatment systems is routinely to perform fecal coliform bacteria analyses on the final effluent (5). *Standard Methods for the Examination of Water and Wastewater* outlines two procedures for fecal coliform analysis: the membrane filtration technique and the most-probable-number (MPN) method (1). For chlorinated effluents, *Standard Methods* specifies that only the MPN method be used. However, the U.S. Environmental Protection Agency now accepts data on chlorinated effluents obtained by the membrane filtration technique but still requires any legal questions concerning fecal coliform data to be answered by using the MPN technique (5).

Several objections to the MPN technique can be raised. Among them are: test length (48 to 72 h), the extensive record keeping for inexperienced laboratory personnel, reagent quantities, glassware needs, and the number of extra steps involved.

In 1972, a simplified 24-h MPN technique was introduced for determining fecal coliforms in estuarine waters (2). The method used a new medium formulation called A-1. The medium differs from lauryl tryptose in that tryptose is replaced by tryptone, the phosphate buffer system is eliminated, and salicin and Triton X-100 are added. The A-1 technique gave results similar to the *Standard Methods* two-step fecal coliform MPN procedure. In 1978, two reports indicated that the A-1 medium gave satisfactory fecal coliform results for marine waters when used with a 3-h, 35°C preincubation (6, 7). The A-1 medium was also used without preincubation in a study by Dutka et al. (4). Many of the disadvantages of traditional MPN testing were eliminated with this new procedure, i.e., record keeping was simplified, the test could be completed within 24 h, and the confirmation step, including the use of extra media and glassware, was eliminated.

Theoretically, all of the methods for bacteria enumeration in *Standard Methods* should work on all types of waters (1). The purpose of our study was to determine the suitability of the 24-h A-1 medium technique for testing chlorinated secondary sewage treatment plant effluents and to assess its equivalency to the *Standard Methods* technique currently in use.

Samples were collected from 12 Wisconsin sewage treatment facilities in phase 1 and from 13 facilities in phase 2. Facilities were chosen that provide various levels of secondary (biological) treatment. Since all of the plants practice chlorination to disinfect the final effluent, samples were collected in sterile, wide-mouthed polyethylene bottles containing a sufficient amount of sodium thiosulfate to neutralize any excess chlorine (1). Samples were immediately chilled to less than 4°C and tested within 24 h of collection time. Although *Standard Methods* mandates a maximum 6- to 8-h holding time for this type of sample, we chose the 24-h limit since the original A-1 medium work by Andrews and Presnell (2) used a 24-h limit, and Standridge and Lesar (8) showed that carefully iced samples from chlorinated domestic sewage effluents, similar to those used in this study, showed little difference in fecal coliform counts between 8 and 24 h of storage.

In the first phase of the study, five replicates of each of 12 effluent samples were tested for fecal coliform organisms using the standard lauryl tryptose broth (LTB)/EC medium MPN technique (1). Five replicates of each sample were also tested by the A-1 medium technique described by Miescier et al. (7) with the exception that a 2-h preincubation at 22°C was used rather than 3 h at 35°C.

In the second phase of the study, five repli-

cates were made on a new series of samples taken from 13 sampling sites, using both the standard LTB/EC MPN technique and the A-1 medium technique employing the 3-h preincubation at 35°C as specified by Miescier et al. (7).

In this study, we wanted to test the hypothesis that the A-1 medium one-step technique was suitable for chlorinated wastewater effluent monitoring. The first phase of the study evaluated a simplified version of the A-1 technique in comparison with the standard MPN method. The simplification involved 2 h of preincubation at room temperature (22°C) instead of 35°C. If the technique proved to be successful with preincubation at 22°C, small wastewater treatment plant laboratories might be able to monitor their effluents without needing both 35 and 44.5°C incubator equipment. The second phase of the study compared the standard LTB/EC technique with the A-1 technique using 35°C preincubation, which is as it originally appeared in the literature (7).

The number and percentage of positive tube reactions observed in this study were compared using the criteria and techniques described by Andrews and Presnell (2) (Table 1). In the first phase of the study, the LTB/EC method produced a substantially higher number of positive tubes than did the A-1 medium with a 22°C preincubation. However, in the second phase, the A-1 technique with 35°C preincubation gave results very similar to those of the standard technique.

A more rigorous statistical analysis confirms these intuitive observations. After log transformations were made on the data to normalize the coliform population distribution, the means and standard deviations for each set of replicates were calculated (3). These data are presented in Table 2 for phase 1 and in Table 3 for phase 2. The differences between the means were then used in paired $t$ tests which tested the hypothesis that the average difference between the methods was zero (3).

The calculated difference of the method means in phase 1 was 0.28 with a $t$ value of 3.88. The hypothesis of equality had to be rejected at the $\alpha$ levels of 0.05 and 0.005 (3). The paired $t$ test on data from phase 2 yielded a difference of the method means of 0.067 and a $t$ value of 1.373. These values validated the hypothesis of method equality at an $\alpha$ level of 0.05, demonstrating that these two methods provide statistically equivalent data.

The data presented in Tables 2 and 3 show that the A-1 medium technique with 35°C preincubation, as originally designed for enumerating fecal coliforms in marine waters, is a viable technique for the chlorinated wastewater effluents of the type tested in this study. The 24 h of total time required for the test, combined with the simplified record keeping and labora-

TABLE 1. *Positive tube reactions*

| Test method | No. (%) of positive tubes |
|---|---|
| Phase 1 | |
| LTB/EC | 646 (62) |
| A-1, 22°C preincubation | 555 (53) |
| Phase 2 | |
| LTB/EC | 607 (62) |
| A-1, 35°C preincubation | 582 (60) |

TABLE 2. *Comparison of LTB/EC and A-1 (22°C preincubation) fecal coliform methods after log transformations and averaging*

| Site[a] | Log mean ± standard deviation | |
|---|---|---|
| | LTB/EC method | A-1 method at 22°C |
| Mazomanie | 1.94 ± 0.41 | 1.79 ± 0.23 |
| Lake Mills | 5.31 ± 0.10 | 5.19 ± 0.15 |
| Marshall | 6.21 ± 0.21 | 6.20 ± 0.15 |
| Waterloo | 1.87 ± 0.11 | 1.59 ± 0.36 |
| Brooklyn | 3.70 ± 0.17 | 3.63 ± 0.22 |
| Mt. Horeb | 3.13 ± 0.26 | 2.89 ± 0.13 |
| Madison | 4.05 ± 0.24 | 3.21 ± 0.22 |
| Cross Plains | 4.94 ± 0.31 | 4.69 ± 0.28 |
| Verona | 1.73 ± 0.16 | 1.56 ± 0.14 |
| Sun Prairie | 6.27 ± 0.17 | 5.58 ± 0.18 |
| Oregon | 6.19 ± 0.21 | 5.78 ± 0.22 |
| Stoughton | 4.68 ± 0.36 | 4.54 ± 0.32 |

[a] Sewage treatment plant sampling sites in Wisconsin.

TABLE 3. *Comparison of LTB/EC and A-1 (35°C preincubation) fecal coliform methods after log transformations and averaging*

| Site[a] | Log mean ± standard deviation | |
|---|---|---|
| | LTB/EC method | A-1 method at 35°C |
| Mazomanie | 2.51 ± 0.33 | 2.44 ± 0.089 |
| Lake Mills | 4.76 ± 0.32 | 4.59 ± 0.15 |
| Marshall | 6.68 ± 0.31 | 6.52 ± 0.19 |
| Waterloo | 3.64 ± 0.14 | 3.86 ± 0.32 |
| Brooklyn | 6.27 ± 0.42 | 6.01 ± 0.15 |
| Mt. Horeb | 4.58 ± 0.23 | 4.49 ± 0.20 |
| Madison | 4.72 ± 0.15 | 4.76 ± 0.10 |
| Cross Plains | 5.80 ± 0.32 | 5.95 ± 0.32 |
| Verona | 6.47 ± 0.31 | 6.51 ± 0.20 |
| Sun Prarie | 6.15 ± 0.17 | 5.99 ± 0.28 |
| Oregon | 7.01 ± 0.32 | 6.67 ± 0.22 |
| Stoughton | 3.83 ± 0.27 | 3.99 ± 0.15 |
| Lodi | 2.75 ± 0.24 | 2.52 ± 0.14 |

[a] Sewage treatment plant sampling sites in Wisconsin.

tory manipulations, makes this an efficient and suitable technique for both small and large laboratories.

By reporting these results, we hope to generate further interest in the A-1 medium technique with preincubation at 35°C so it can be evaluated more extensively on a greater variety of water and wastewater sample types in different geographical locales.

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