FISHERIES AND ENVIRONMENT CANADA
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# EVALUATION OF THE A-1 MEDIUM FOR RAPID RECOVERY OF FECAL COLIFORMS FROM MARINE WATERS



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## ABSTRACT

The efficiency of the A-1 medium in the recovery of fecal coliforms from marine waters was compared with the APHA Standard Method Test. The modified A-1 method, which included a 3 hour resuscitation period at 35°C, was found to be more productive in the recovery of E.coli from the marine environment than both the standard method or the A-1 method, and equally as productive for the recovery of fecal coliforms as the standard method. The A-1 method was slightly more selective for E.coli than was the modified A-1 method, with the standard method being the least selective.

Statistical analysis using the Analysis of Variance (F) test on 273 sample results demonstrated there was no significant difference in the results obtained for each method.

## Résumé

On a comparé l'efficacité du milieu A-1 à la méthode d'essai usuelle APHA pour récupérer les bactéries coliformes d'origine fécale contenues dans les eaux marines. La méthode A-1 modifiée, suivie d'une période de réanimation de trois heures à 35°C, s'est révélée, à cet égard, aussi productive que la méthode normale et plus productive que les méthodes normale et A-1 pour récupérer les Escherichia coli.

Les méthodes normale, A-1 modifiée et A-1 se sont révélées, dans l'ordre, plus sélectives pour l'E. coli.

L'analyse statistique de variance (F), appliquée à 273 résultats échantillonnés, démontre qu'il n'existe pas de différence significative entre les résultats obtenus pour chaque méthode.

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# 1 INTRODUCTION

During the 1971 National Shellfish Sanitation Workshop, the Microbiology Task Force recommended that an interagency laboratory program be established to review and evaluate rapid test procedures for the bacteriological examination of shellfish growing waters. The U.S. Food and Drug Administration was requested to develop and coordinate the program.

Four procedures of potential value were reviewed and are listed as follows:

1) A Single Medium for the Rapid Detection of Escherichia coli at 44°C, Mara, D.D., J. Hyg. Camb. (1973), 71, 783.

2) Rapid Recovery of Escherichia coli from Estuarine Water, Andrews, W.H. and Presnell, M.W., Applied Microbiology, March 1972. (A-1 Procedure)

3) a Membrane Filtration Technique for the Enumeration of Escherichia coli in Seawater, Halls, S. and Ayres, P.A., J. of Applied Bacteriology, 37, 1974.

4) L.E.S. (Lawrence Experiment Station) Two-Step, Two-Day Procedure for Fecal Coliforms in Estuarine Water [See: Measurement of Fecal Coliform in Estuarine Water - Presented at the Eighth National Shellfish Sanitation Workshop, New Orleans, Louisiana, January 1974].

Two of the procedures were membrane filtration procedures and were not further considered for several reasons. Firstly, the L.E.S. method, although showing considerable promise with regard to comparable recoveries to the Standard Method, was a 48 hour, rather than 24 hour test. Secondly, some types of samples cannot be filtered because of

the presence of high concentrations of suspended colloidal matter. Finally, since the fecal coliform standard has such a low median value (14 MPN) a comparatively large sample volume may be required for the MF test.

Of the remaining two procedures, the method of Andrews and Presnell was chosen for further investigation as the A-1 medium used in this method had been shown to give E.coli recovery in 24 hours comparable to that of the standard methods procedure of the APHA (96 hour test).

The purpose of this study was to evaluate the A-1 rapid method for the enumeration of fecal coliforms in shellfish growing area waters and to compare these with the conventional standard APHA method. The methods investigated were:

(a) a 24 hour elevated temperature (44.5°C) test with A-1 medium,

(b) A-1 modified method with preincubation of A-1 medium for three hours at 35°C, and

(c) the 72 to 96 hr APHA standard method procedure.

The study consisted of three steps. Firstly, a small scale sampling program was initiated in a routine growing area to obtain preliminary test data. Secondly, a series of six split samples was sent to all participating laboratories for analysis via the three methods listed above. Thirdly, a more intensive study of one year's length was conducted by participating laboratories to obtain a large enough data block for statistical analysis.

The three part study began on May 20, 1975 and was completed on June 17, 1976.

## 2 MATERIALS AND METHODS

### 2.1 Sampling Procedure

All water samples were collected in sterile 200 ml wide mouth glass bottles, approximately 15 to 30 cm below the water surface by means of a rod sampling device. Samples were stored in coolers at 10°C and were analyzed by the EPS Regional Microbiology laboratory within two hours of collection.

### 2.2 Methods of Examination

2.2.1 Standard Method. The five-tube decimal dilution (MPN) method, as described in Part 908 of the 14th edition of Standard Methods for the Examination of Water and Wastewater (1) was used. Bacto-Lauryl Tryptose Broth was used as the presumptive test medium with incubation at 35 ± 0.5°C for 24 and 48 hours, and positive tubes were transfered to Bacto-EC medium and incubated in a water bath at 44.5 ± 0.2°C for 24 hours.

2.2.2 A-1 Method. Three decimal dilutions of water sample were pipetted into each of five tubes of A-1 medium. The A-1 medium was prepared according to the formula of Andrews and Presnell (2). The inoculated tubes were transferred immediately into a water bath maintained at temperature of 44.5 ± 0.2°C. Tubes showing any amount of gas after 24 hours incubation were recorded as positive.

2.2.3 Modified A-1 Method. Water samples were pipetted directly into A-1 medium in three decimal dilutions using five tubes per dilution. The inoculated tubes were first incubated in an air incubator at 35°C for three hours before being transferred directly into a water bath at 44.5 ± 0.2°C for 21 hours.

2.2.4 Differentiation of the Fecal Coliform Types. All positive tubes from representative test media were streaked on Levine eosin methylene blue (EMB) agar plates and incubated at 35 ± 0.5°C for 24 hours. Each colony type was picked and transferred to lactose broth and incubated at 35 ± 0.5°C for 24 to 48 hours. All cultures from gas-positive lactose tubes were subjected to the Indole, Methyl Red, Voges-Proskauer and Simmon's Citrate Agar tests.

## 3 RESULTS AND DISCUSSION

Results from each step of the study will be presented separately, followed by a general discussion.

### 3.1 Step 1

Forty marine samples taken from the Cates Park-Deep Cove area were analyzed using the three different methods. IMViC analyses were performed on positive tubes from the first eight samples. One colony from each EMB plate was chosen. The MPN results are presented in Table 1. The data obtained indicated that the modified A-1 method gave results which were more compatible with those obtained using the standard method EC medium. The specificity of the A-1 medium for E.coli was superior to the EC medium with recoveries of IMViC type ++-- of 83%, 100% and 100% from the EC, A-1 and A-1 modified tests. The geometric means of the three tests were 15.8, 10.4 and 12.9 respectively, with the geometric mean of the A-1 method differing significantly from the geometric mean of the standard procedure ($p<0.01$).

When the data from all participating laboratories were examined and subjected to statistical analysis, it was found that, while results of both the A-1 and modified A-1 test showed good correlation with the standard method, a statistically significant difference existed between all three methods ($p<0.01$). Both the A-1 and modified A-1 tests showed a higher recovery of E.coli than the standard test. The data are plotted on log-probability paper in Figure 1. From this graph it can be demonstrated that the A-1 modified method shows comparable results with the standard method around an MPN of 14/100 ml (shellfish growing water standard), but tends to drop below the standard method result in the higher MPN ranges (approx. >100/100 ml). This however, would not appear to be a concern in the classification of shellfish growing waters, as the upper limit for the standard is an MPN of 43/100 ml. A complete summary report of the results from Step 1 of the evaluation is found in Appendix I.

TABLE 1 SUMMARY OF SEAWATER SAMPLES ANALYSES - STEP 1
(MPN/100 ml)

| Date | Location | | Standard Method EC | | A-1 | | A-1 Plus Pre-incubation | |
|---|---|---|---|---|---|---|---|---|
| May 20 | Cates Park | Stn # 1 | 5:1:0 | 33 | 4:0:0 | 13 | 4:2:0 | 22 |
| | | 2 | 5:1:0 | 33 | 3:3:0 | 17 | 3:1:0 | 11 |
| | | 3 | 5:1:0 | 33 | 4:3:0 | 27 | 3:0:0 | 8 |
| | | 4 | 4:2:0 | 22 | 1:0:0 | 2 | 0:0:0* | <2 |
| May 30 | Deep Cove | Stn # 1 | 2:1:0 | 7 | 0:0:0* | <2 | 2:0:0 | 5 |
| | | 2 | 4:1:0 | 17 | 4:1:0 | 17 | 4:1:0 | 17 |
| | | 3 | 5:4:3 | 280 | 5:5:1 | 350 | 5:5:1 | 350 |
| | | 4 | 1:0:0 | 2 | 2:0:0 | 5 | 2:0:0 | 5 |
| May 21 | Cates Park | Stn # 1 | 4:1:0 | 17 | 4:1:0 | 17 | 4:1:0 | 17 |
| | | 2 | 5:1:0 | 33 | 4:0:0 | 13 | 3:1:0 | 11 |
| | | 3 | 5:2:0 | 49 | 5:0:0 | 23 | 5:4:0 | 130 |
| | | 4 | 5:0:0 | 23 | 3:0:0 | 8 | 5:1:0 | 33 |
| May 21 | Deep Cove | Stn # 1 | 4:1:0 | 17 | 1:1:0 | 4 | 4:0:0 | 13 |
| | | 2 | 5:1:0 | 33 | 5:1:0 | 33 | 5:1:0 | 33 |
| | | 3 | 5:5:0 | 240 | 5:2:1 | 70 | 5:2:2 | 94 |
| | | 4 | 4:0:0 | 13 | 1:0:0 | 2 | 1:0:0 | 2 |
| May 22 | Cates Park | Stn # 1 | 2:0:0 | 5 | 0:0:0* | <2 | 3:1:0 | 11 |
| | | 2 | 3:0:0 | 8 | 0:0:0* | <2 | 1:0:0 | 2 |
| | | 3 | 5:1:0 | 33 | 5:4:0 | 130 | 5:1:0 | 33 |
| | | 4 | 3:1:0 | 11 | 4:1:0 | 17 | 5:1:0 | 33 |

TABLE 1 SUMMARY OF SEAWATER SAMPLES ANALYSES - STEP 1 (cont.)
(MPN/100 ml)

| Date | Location | | Standard Method EC | | A-1 | | A-1 Plus Pre-incubation | |
|---|---|---|---|---|---|---|---|---|
| May 22 | Deep | Stn # 1 | 5:4:1 | 170 | 5:4:0 | 130 | 5:5:1 | 350 |
| | Cove | 2 | 2:0:0 | 5 | 2:0:0 | 5 | 1:0:0 | 2 |
| | | 3 | 1:0:0 | 2 | 0:0:0* | <2 | 1:0:0 | 2 |
| | | 4 | 4:1:0 | 17 | 4:0:0 | 13 | 4:0:0 | 13 |
| May 23 | Cates | Stn # 1 | 5:1:1 | 46 | 4:2:0 | 22 | 5:2:0 | 49 |
| | Park | 2 | 4:2:0 | 22 | 3:2:0 | 14 | 5:4:0 | 130 |
| | | 3 | 4:1:0 | 17 | 5:0:0 | 23 | 5:1:0 | 33 |
| | | 4 | 4:1:0 | 17 | 4:2:0 | 22 | 5:0:0 | 23 |
| May 23 | Deep | Stn # 1 | 5:1:0 | 33 | 5:2:0 | 49 | 5:2:0 | 49 |
| | Cove | 2 | 2:1:0 | 7 | 3:0:0 | 8 | 1:1:0 | 4 |
| | | 3 | 2:1:0 | 7 | 0:0:0* | < 2 | 0:0:0* | < 2 |
| | | 4 | 4:0:0 | 13 | 2:0:0 | 5 | 3:0:0 | 8 |
| May 26 | Cates | Stn # 1 | 3:2:0 | 14 | 1:1:0 | 4 | 2:0:0 | 5 |
| | Park | 2 | 4:1:0 | 17 | 4:1:0 | 17 | 4:1:0 | 17 |
| | | 3 | 4:3:0 | 27 | 4:1:0 | 17 | 5:0:0 | 23 |
| | | 4 | 4:0:0 | 13 | 3:1:0 | 11 | 1:0:0 | 2 |
| May 26 | Deep | Stn # 1 | 2:0:0 | 5 | 3:0:0 | 8 | 2:0:0 | 5 |
| | Cove | 2 | 1:1:0 | 4 | 0:0:0* | < 2 | 1:0:0 | 2 |
| | | 3 | 1:0:0 | 2 | 0:0:0* | < 2 | 4:0:0 | 13 |
| | | 4 | 1:0:0 | 2 | 0:0:0* | < 2 | 0:1:0 | 2 |

* These analyses, because of partial indeterminate results were not used in the evaluation of the method.

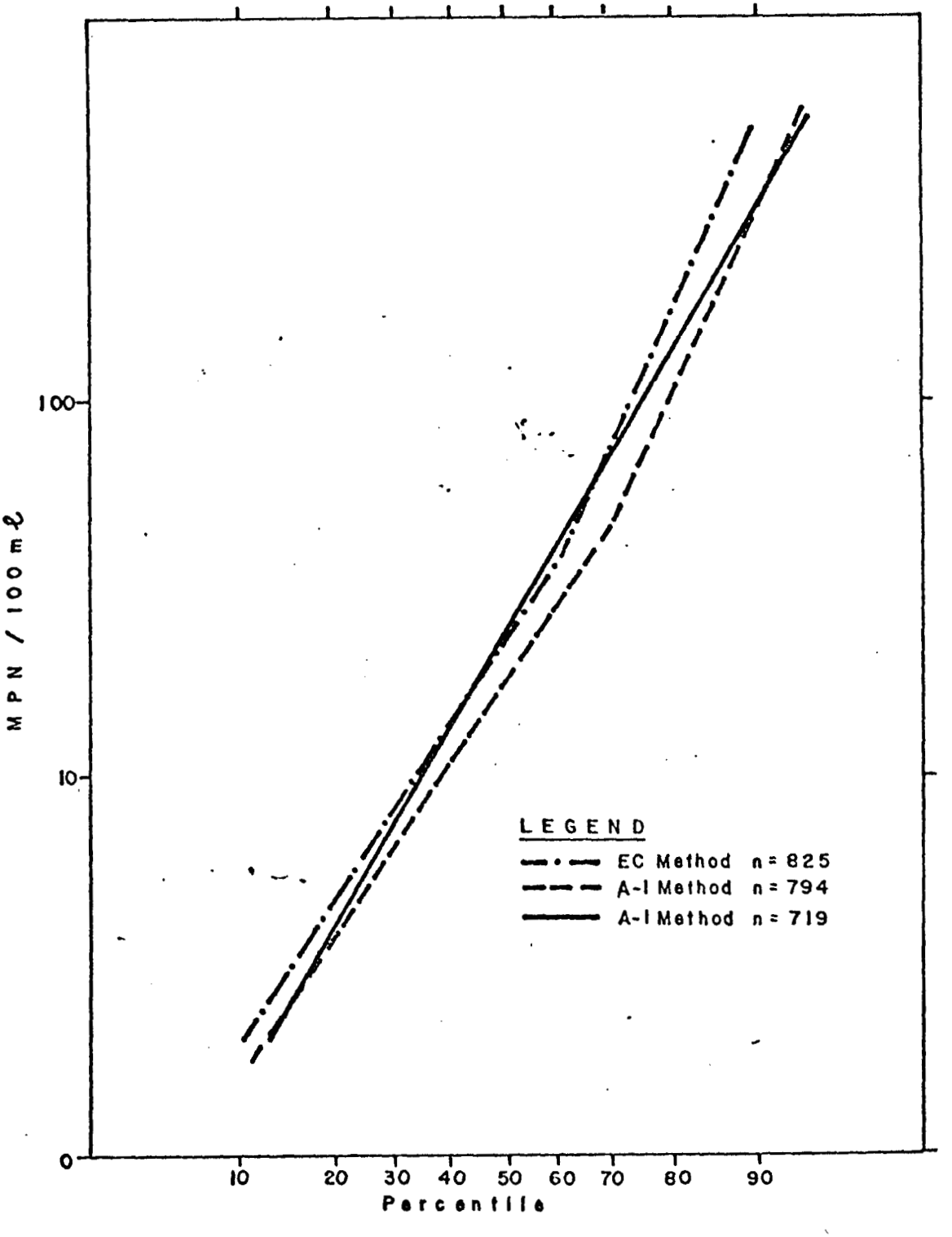


FIGURE 1 LOG PLOTS OF THE DATA FROM THE THREE METHODS - STEP 1

3.2 Step 2

The second phase of the collaborative study was conducted to determine the comparative recovery of E. coli from a series of split artificial seawater samples using the standard fecal coliform test, the A-1 test and the modified A-1 test.

Salt was added to water to produce a salinity of 15 parts per thousand and peptone was added to a level of 20 mg/liter. The solution was divided into three equal volumes. They were subsequently spiked with a pure culture of E. coli to obtain expected recoveries of 0-10, 10-100, and 100-1000 organisms per 100 ml, respectively. Replicate subsamples of each of these solutions were sent to each of twenty-four laboratories from the Northeast Technical Services Unit (FDA) in Rhode Island. Three laboratories received their samples too late to be analyzed.

The results for the EPS-Pacific laboratory are presented in Table 2 and compared favourably with those obtained from all other

TABLE 2 BACTERIOLOGICAL DATA - SPLIT SAMPLE ANALYSIS

| Sample Number | MPN per 100 ml | | |
|---|---|---|---|
| | Standard Method | A-1 | A-1 Modified |
| 19 | 8 | 5 | 5 |
| 36 | 5 | 11 | 8 |
| 58 | 70 | 46 | 130 |
| 81 | 79 | 110 | 79 |
| 115 | 350 | 220 | 540 |
| 134 | 350 | 540 | 240 |
| Geometric mean | 55 | 57 | 61 |

participating laboratories. Table 3 summarizes geometric means for each method within each laboratory and across all laboratories.

The results from the EPS Atlantic laboratory were noticeably different from those of the other labs. This laboratory had received the samples three or four days later than did the other laboratories, and the temperature of the samples was 25°C, five degrees higher than the highest temperature recorded by the other laboratories. Table 3 indicates that there was no consistent difference between methods; that is, no single method showed a consistently higher or lower recovery than any of the other methods in all laboratories.

An analysis of variance of the data (Table 4), showed no difference between method means ($p>0.50$). When EPS Atlantic results were included, the analysis of variance showed a significant difference between laboratories ($p<0.01$). However, when these results were excluded from the analysis, no significant difference ($p>0.50$) between laboratory mean recoveries was found. This analysis of variance had a replicate subsampling error variance of 0.063. The expected variability of the 5-tube 3-dilution MPN test itself, when 10-fold dilutions are used, is 0.060, indicating that the subsampling variability was totally accounted for by the variability of the MPN test.

It was concluded from the split sample data that all three methods were equally effective in recovering pure culture E. coli from a standardized seawater sample, and that all labs were comparable in their ability to recover these bacteria with the three methods employed.

### 3.3 Step 3

At the completion of Steps 1 and 2 of the A-1 media evaluation study, all data were reviewed by FDA and the following protocol was recommended for Step 3:

TABLE 3 GEOMETRIC MEANS - SPLIT SAMPLE ANALYSIS

| Laboratory | Lab Code | Date Sent | Date Arrive | Time Arrived | Temp. °C | Geometric Mean Methods Within Each Lab. N=6 for Each Method | | | Geometric Mean Levels Within Each Lab. N=6 for Each Level | | | Geometric Means All Levels, All Methods-N=18/Lab |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Std | A-1 | A-1 Mod | Level 1 | Level 2 | Level 3 | |
| GCTSU-FDA | 1 | 6-17 | 6-20 | 1355 | 5.3 | 48 | 52 | 88 | 8 | 50 | 578 | 60 |
| New York | 2 | 6-17 | 6-18 | 1400 | 5.1 | 38 | 58 | 68 | 5 | 57 | 568 | 53 |
| Conn. | 3 | 6-17 | 6-18 | 1145 | 15.0 | 84 | 57 | 65 | 8 | 55 | 680 | 68 |
| Washington | 4 | 6-17 | 6-20 | 0950 | 10.0 | 96 | 55 | 60 | 8 | 59 | 638 | 63 |
| Louisiana | 5 | 6-17 | 6-20 | 1230 | 18.0 | 69 | 59 | 56 | 6 | 70 | 554 | 61 |
| Dartmouth-EPS | 6 | 6-16 | 6-25 | 0845 | 25.0 | 2016 | 1998 | 2236 | 312 | 1731 | 16702 | 2081 |
| Mass. | 7 | 6-17 | 6-19 | 0805 | 7.0 | 71 | 70 | 34 | 6 | 59 | 502 | 55 |
| Texas | 8 | 6-17 | 6-19 | 1100 | 8.0 | 76 | 63 | 79 | 6 | 93 | 675 | 72 |
| Alabama | 9 | 6-17 | 6-20 | 1100 | 16.5 | 87 | 45 | 39 | 6 | 40 | 590 | 53 |
| NETSU-FDA | 10 | 6-17 | 6-18 | 1900 | 6.4 | 64 | 59 | 65 | 7 | 62 | 586 | 63 |
| South Carolina | 11 | 6-17 | 6-19 | 1555 | 9.5 | 50 | 53 | 76 | 8 | 67 | 402 | 59 |
| Virginia | 12 | | | | | | | | | | | |
| N. British Columbia-EPS | 13 | 6-16 | 6-17 | 1700 | 3.5 | 55 | 57 | 61 | 7 | 81 | 352 | 58 |
| British Columbia-Fish | 14 | 6-16 | 6-17 | 1620 | 3.0 | 58 | 46 | 48 | 4 | 60 | 527 | 50 |
| Florida | 15 | | | | | | | | | | | |
| Quebec-Fish | 17 | 6-16 | 6-19 | 0930 | 6.0 | 64 | 65 | 61 | 9 | 55 | 534 | 63 |
| Longeuil-EPS | 18 | 6-16 | 6-19 | | 9.0 | 59 | 67 | 88 | 6 | 90 | 658 | 70 |
| Longeuil-Fish | 19 | 6-16 | 6-19 | | 4.0 | 67 | 125 | 75 | 10 | 55 | 1110 | 85 |
| NETSU-FDA-PR | 20 | | | | | | | | | | | |
| Maryland | 21 | 6-17 | 6-19 | 1445 | 7.8 | 59 | 35 | 41 | 4 | 56 | 382 | 44 |
| St. John's | 22 | | | | | | | | | | | |
| FDA-Wash. | 23 | 6-17 | 6-18 | 1157 | 4.6 | 74 | 106 | 62 | 8 | 83 | 740 | 79 |

TABLE 4 ANALYSIS OF VARIANCE - SPLIT SAMPLES

| | EFFECT | SS | DF | MS | SIGNIFICANCE |
|---|---|---|---|---|---|
| EXCLUDING DARTMOUTH EPS | LABS | 1.278 | 19 | 0.067 | N.S. |
| | METHODS | .1025 | 2 | 0.051 | N.S. |
| | LEVELS | 211.21 | 2 | 105.6 | ** |
| | LAB X METHODS | 2.416 | 36 | 0.067 | N.S. |
| | LEVELS X METHODS | 0.211 | 4 | 0.053 | N.S. |
| | LABS X LEVELS | 1.892 | 36 | 0.053 | N.S. |
| | LABS X LEVELS X METHODS | 4.405 | 72 | 0.061 | N.S. |
| | REPLICATE SUBSAMPLES | 10.251 | 171 | 0.060 | N.S. |
| INCLUDING DARTMOUTH EPS | LABS | 41.45 | 20 | 2.073 | ** |
| | METHODS | 0.0436 | 2 | 0.022 | N.S. |
| | LEVELS | 232.5 | 2 | 116.24 | ** |
| | LABS X METHODS | 2.737 | 40 | 0.068 | N.S. |
| | LEVELS X METHODS | 0.226 | 4 | 0.057 | N.S. |
| | LABS X LEVELS | 2.431 | 40 | 0.061 | N.S. |
| | LABS X LEVELS X METHODS | 4.721 | 80 | 0.059 | N.S. |
| | REPLICATE SAMPLES | 11.925 | 189 | 0.063 | N.S. |

N.S. - not significant

** - significant difference ($p<0.01$)

1. Each participating laboratory was requested to analyze at least 10 samples per month from routine sampling stations for a period of 12 months.

2. The standard E.C. test, the A-1 and A-1 modified should be done on all samples.

3. IMViC tests should be performed on all EMB colony types arising from gas-positive tubes in each of the three tests.

Sampling and analysis in this laboratory began on September 22, 1976 and continued until June 17, 1977. During this period, 100 marine samples were analyzed, representing 295 MPN and 3,619 IMViC analyses. Sampling was carried out in two areas of the lower mainland, Cates Park and Sunset Beach (False Creek). The location of stations sampled is shown in Figure 2.

All of the gas positive tubes from each method were subjected to IMViC analysis, and on the basis of these data, the presence or absence of E. coli of either IMViC type (++--) was determined. The tubes from which E. coli were not isolated were considered "false positives", in that the fermentation of lactose was due to an organism or organisms other than E. coli. The number of tubes from which E. coli was isolated, was calculated as a percentage of the total number of positive tubes in each sample, and this percentage represented the effective recovery of E. coli. Omitting the false positive tubes, the MPN/100 ml was determined (IMViC MPN), and this was compared with the MPN/100 ml resulting from the inclusion of all gas positive tubes, the figure which would normally be reported using that method (METHOD MPN).

In Appendix II, the effective recovery of E. coli, and the "Method MPN" vs. the "IMViC MPN" are summarized for each month. The percent recovery of E. coli, has also been averaged on a monthly basis,

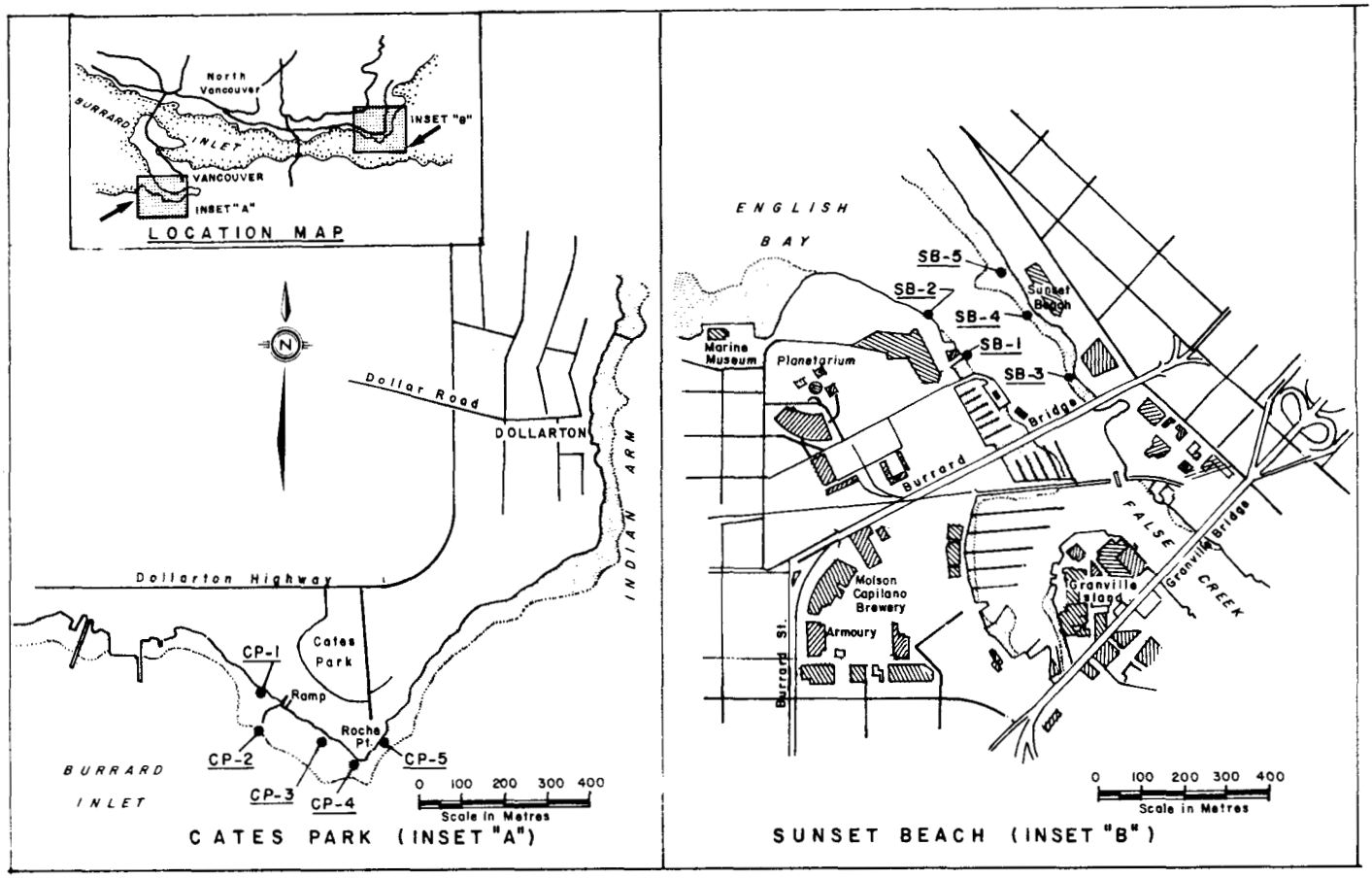


FIGURE 2 SAMPLE STATION LOCATIONS - A-I METHOD EVALUATION (STEP 3)

and this is presented on each page. The cumulative averages of recovery for each method, over 100 samples (95 in the standard method) are:

| | |
|---|---|
| Standard Method: | 90.4% |
| A-1 Method: | 96.8% |
| Modified A-1 Method: | 96.7% |

The highest recovery of E. coli from gas positive tubes was achieved using the A-1 method. The modified A-1 Method gave slightly lower recovery while the standard method was the least effective in recovering E. coli.

These data, although indicating the increased selectivity of A-1 medium for E. coli, did not indicate which method gave the best recovery of E. coli from the marine environment, as opposed to recovery from gas-positive tubes (i.e., the productivity of the method).

The productivity of the methods was examined in two ways. Firstly, the productivity with respect to the recovery of E. coli was examined by totalling the number of E. coli positive tubes in each method. Secondly, the fecal coliform productivity for each method was determined by totalling the number of 44.5°C gas-positive tubes for each method. The results in Table 5 clearly demonstrate that the modified A-1 method was the most productive in recovering E. coli from the marine

TABLE 5* PRODUCTIVITY OF THE THREE METHODS

| | Number of positive tubes | | |
|---|---|---|---|
| Productivity | Standard Method | A-1 Method | Modified A-1 Method |
| E. coli | 744 | 757 | 799 |
| Fecal coliforms | 823 | 719 | 822 |

*Results in this table do not include data for September 29/75 sampling.

environment and was comparable to the standard method in recovering fecal coliforms. The data suggest that the modified A-1 method has fewer false positive reactions than does the standard method.

The data were also examined statistically using the Analysis of Variance (ANOVA) test to determine whether there was any significant difference between the results obtained from each method. Both the method MPN's and the IMViC MPN's were compared between each method and across all three methods. IMViC MPN's were included in the ANOVA to compensate for any false positive reactions which occurred. The results are presented in Table 6. At the 95% level of confidence, there was no significant difference between methods or across all three methods. The

TABLE 6 ANALYSIS OF VARIANCE TEST RESULTS (STEP 3)

| Methods compared | Degrees of Freedom | F value | Significance |
|---|---|---|---|
| X vs Y | 181 | 0.505 | not significant |
| X vs Z | 181 | 0.5877 | not significant |
| Y vs Z | 181 | 0.0078 | not significant |
| $X_1$ vs $Y_1$ | 181 | 2.6182 | not significant |
| $X_1$ vs $Z_1$ | 181 | 2.2579 | not significant |
| $Y_1$ vs $Z_1$ | 181 | 0.06 | not significant |
| X vs Y vs Z | 272 | 0.3433 | not significant |
| $X_1$ vs $Y_1$ vs $Z_1$ | 272 | 1.7942 | not significant |

X = Standard Method IMViC MPN
Y = A-1 Method IMViC MPN
Z = Modified A-1 Method IMViC MPN

$X_1$ = Standard Method MPN
$Y_1$ = A-1 Method MPN
$Z_1$ = Modified A-1 Method MPN

F values for the first three comparisons indicate that, when the false positive reactions are corrected for by using the IMViC MPN's, the

methods are higly comparable. ANOVA testing was also performed on all data by FDA for each of the American regions, Canada, the seasons within regions, and the overall pooled data. The FDA interpretation of Canadian data indicated that there were significant differences between the three method means in winter but the summer data were comparable between all three methods. The winter mean MPN's for the Canadian data were 501.6/100 ml (Standard method), 356.5/100 ml (A-1 method) and 424.3/100 ml (Modified A-1 method). The higher counts provided by the standard method were perhaps due to false positive reactions which resulted in the significant difference in means. The higher incidence of false positive EC reactions during high precipitation (i.e., winter) conditions has been observed in this and other Canadian laboratories. The sanitary significance of coliform counts obtained under these conditions is therefore questionable. Bearing in mind the observed superior productivity of the A-1 medium in recovering E. coli from the marine environment, this further suggests that false positive reactions were the cause of the discrepancy between the means, as the modified A-1 test results would have approximated more closely the actual E. coli density in the sample, and the relative sanitary significance of the sample. Additional interpretation of the Canadian data was done by EPS, Atlantic Region, using the standard student's t test. At the 98% level of confidence, there was no significant difference between the standard method and the modified A-1 method for total, winter or summer data, although there was a significant difference between the standard method and the A-1 method.

## 4 SUMMARY

The evaluation of a new culture medium and method requires the consideration of several factors: accuracy, productivity, comparability to existing method and selectivity for the organism(s) being examined. The accuracy of A-1 medium was established by the collaborative split sample study (Step 2) which concluded that all three methods were equally proficient for the recovery of E. coli in pure culture. Productivity of the A-1 medium was examined in this laboratory and was found to be more productive in recovering E. coli from the marine environment than was the standard method, particularly when the 3 hour 35°C pre-incubation period was used. The A-1 methods were compared to the standard method using both the ANOVA and student's t tests. The results indicated no significant difference between the modified A-1 test and the standard method. The A-1 medium was found to be more selective for E. coli than was the standard methods media, the greatest selectivity being observed with the modified A-1 test method.

# 5 CONCLUSIONS

The modified A-1 test method was found to be superior in recovery and productivity for E. coli in sea water while producing results which were statistically comparable with the standard method.

In addition to its comparability with the standard method, the modified A-1 test method has several other practical advantages. The method requires less time, is more convenient and is less costly than the standard method. Also, by using this method, a more comprehensive shellfish water quality survey can be conducted, as it will permit the analysis of greater numbers of samples.

Based on the data presented in this and other reports (3), the modified A-1 test method is a viable alternative to the present method for the routine bacteriological examination of shellfish growing waters.

REFERENCES

1. "Standard Methods for the Examination of Water and Wastewater." 14th edition. American Public Health Association, Washington, D.C. (1975).

2. Andrews, W.H. and Presnell, M.W., "Rapid Recovery of Escherichia coli from Estuarine Water." Applied Microbiology, (March 1972).

3. Menon, A.S., Evaluation of A-1 Medium for the Rapid Recovery of Fecal Coliforms from Marine Waters. Environmental Protection Service, Atlantic Region. Technology Development Report [Draft], (1977).

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# APPENDICES

# APPENDIX I

## SUMMARY OF RESULTS OF DATA OF COMPARATIVE TESTS ON ROUTINE GROWING AREA SAMPLES

- Step 1

(U.S. Food and Drug Administration
Memorandum to participating laboratories)

## Summary Of Results Of Data Comparative Test On Routine Growing Area Samples

Table 3 shows a comparison of method recoveries by the Standard, A-1, and A-1 Modified methods. The geometric means shown for each method are the means for the total number of water samples analyzed for each method. In 13 of the 20 laboratories, the method mean recoveries of fecal coliforms were significantly different from each other. The overall mean of 21.9 for the A-1 method and 26.7 for the A-1 Modified method were both significantly different from the Standard method. The ranking of the method recoveries shows that except for NETSU and three of the Canadian laboratories (Texas has too few analyses and did not use the A-1 Modified method), all laboratories recovered the highest number of fecal coliforms by the standard method, the next highest by the A-1 Modified method, and the lowest by the A-1 method.

## Conclusion

The standard method recovered significantly higher numbers of "fecal coliforms" as defined by the standard procedure than either the A-1 or A-1 Modified procedure recovered as defined by gas fermentation in 24 hours.

Results for the A-1 and A-1 Modified methods, based upon the geometric mean of the MPN values were 75% and 91% respectively of the geometric mean of the recoveries by the standard method (Table 3). Results of both tests showed good correlation with the standard method although a statistically significant difference exists between all three methods. Both the A-1 and A-1 Modified tests showed a higher recovery of *E. coli*, the principal coliform organism, than the standard test (Table 6). The interaction of the analytical methods with the geographic and physical variables of the waters such as temperature, salinity, and turbidity, was not delineated by the data presented. Both the A-1 and A-1 Modified tests indicated differences in the sanitary quality of the waters tested which corresponded to the differences indicated by the standard methods (Graphs 1 & 2).

TABLE 3 COMPARISON OF METHODS USING DATA FROM ROUTINE GROWING AREA SAMPLES

| Laboratory | Lab Code | Geometric Mean Of Method Recoveries Of Fecal Coliform: Stand. | n[1] | Al | n | Al-Mod | n | Sign | Ranking Of Method Recoveries[2]: Stand. | Al | Al-Mod |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gulf Coast | 1 | 48.5 | 42 | 29.3 | 42 | 34.9 | 42 | ** | 1 | 3 | 2 |
| New York | 2 | 3.7 | 40 | 2.4 | 41 | 3.6 | 39 | ** | 1 | 3 | 2 |
| Conn. | 3 | 181.4 | 32 | 88.5 | 35 | 125.6 | 34 | ** | 1 | 3 | 2 |
| Washington | 4 | 67.3 | 50 | 36.8 | 50 | 45.7 | 50 | ** | 1 | 3 | 2 |
| Louisiana | 5 | 27.7 | 43 | 23.6 | 43 | 26.2 | 43 | N.S. | 1 | 3 | 2 |
| Dartmouth | 6 | 37.0 | 42 | 28.3 | 42 | 36.6 | 42 | * | 2 | 3 | 2 |
| Massachusetts | 7 | 18.1 | 49 | 11.8 | 49 | 16.8 | 49 | ** | 1 | 3 | 2 |
| Texas | 8 | 194.1 | 12 | 213.3 | 12 | | | N.S. | 3 | 1 | 2 |
| Alabama | 9 | 35.2 | 41 | 27.6 | 41 | 35.2 | 41 | N.S. | 1.5 | 3 | 1.5 |
| NETSU | 10 | 21.9 | 39 | 14.3 | 39 | 23.3 | 39 | ** | 2 | 3 | 1 |
| S. Carolina | 11 | 63.7 | 40 | 36.6 | 40 | 36.1 | 40 | ** | 1 | 2 | 3 |
| Virginia | 12 | 38.8 | 44 | 31.0 | 41 | 35.5 | 44 | N.S. | 1 | 3 | 2 |
| N. Vancouver, B.C. | 13 | 15.8 | 40 | 10.4 | 40 | 12.9 | 40 | ** | 1 | 3 | 2 |
| British Columbia | 14 | 13.6 | 101 | 10.5 | 101 | 13.1 | 36 | ** | 1 | 3 | 2 |
| Florida | 15 | 96.1 | 30 | 58.9 | 30 | 82.4 | 30 | ** | 1 | 3 | 2 |
| Provincial-Quebec | 17 | 33.1 | 35 | 50.4 | 28 | 45.7 | 30 | N.S. | 3 | 1 | 2 |
| Longueiel-EPS | 18 | 52.6 | 50 | 52.8 | 25 | 49.8 | 26 | N.S. | 2 | 1 | 3 |
| Fisheries-Longueiel | 19 | 51.4 | 50 | 51.9 | 51 | 32.5 | 50 | ** | 2 | 1 | 3 |
| Parker River, Mass. | 20 | 16.5 | 29 | 24.4 | 29 | 29.6 | 29 | ** | 3 | 2 | 1 |
| Maryland | 21 | 10.2 | 58 | 8.7 | 57 | 10.1 | 57 | N.S. | 1 | 3 | 2 |
| Over All States | | 29.3 | 867 | 21.9* | 836 | 26.7* | 761 | | | | |

[1]no. of samples analyzed for each method
[2]1 = method recovering highest no. of fecal coliforms
2 = method recovering second highest no. of fecal coliforms
3 = method recovering lowest no. of fecal coliform
* - indicates methods are significantly different at p 0.01
N.S. - indicates no significant difference between methods

TABLE 4 PERCENTILES - FECAL COLIFORM - 21 STATES

| | Standard | A1 | A1-Mod |
|---|---|---|---|
| 10 | 2.0 | 2.0 | 2.0 |
| 20 | 4.5 | 2.0 | 4.0 |
| 30 | 7.8 | 6.8 | 7.8 |
| 40 | 17.0 | 11.0 | 14.0 |
| 50 | 23.0 | 17.0 | 23.0 |
| 60 | 33.0 | 33.0 | 46.0 |
| 70 | 79.0 | 49.0 | 79.0 |
| 80 | 170.0 | 110.0 | 130.0 |
| 90 | 540.0 | 350.0 | 350.0 |
| 100 | 4900.0 | 13,000.0 | 4900.0 |
| n | 825 | 794 | 719 |

TABLE 5 COMPARISON OF GEOMETRIC MEAN OF EACH TEST AGAINST THE STANDARD PROCEDURE

| Lab. No. | Standard | A-1 | A-1 Mod. |
|---|---|---|---|
| 1 | 48.5 | 29.3** | 34.9** |
| 2 | 3.7 | 2.4** | 3.6 N.S. |
| 3 | 181.4 | 88.5** | 125.6 * |
| 4 | 67.3 | 36.8** | 45.7 * |
| 5 | 27.7 | 23.6 N.S. | 26.2 N.S. |
| 6 | 37.0 | 28.3 * | 36.6 N.S. |
| 7 | 18.1 | 11.8** | 16.8 N.S. |
| 8 | 194.1 | 213.3 N.S. | |
| 9 | 35.2 | 27.6 N.S. | 35.2 N.S. |
| 10 | 21.9 | 14.3** | 23.3 N.S. |
| 11 | 63.7 | 36.6** | 36.1** |
| 12 | 38.8 | 31.0 N.S. | 35.5 N.S. |
| 13 | 15.8 | 10.4** | 12.9 N.S. |
| 14 | 13.6 | 10.5 * | 13.1 N.S. |
| 15 | 96.1 | 58.9** | 82.4 N.S. |
| 17 | 33.1 | 50.4 N.S. | 45.7 N.S. |
| 18 | 52.6 | 52.8 N.S. | 49.8 N.S. |
| 19 | 51.4 | 51.9 N.S. | 32.5 * |
| 20 | 16.5 | 24.4 * | 29.6** |
| 21 | 10.2 | 8.7 * | 10.1 N.S. |

N.S. - no significant difference $p<0.05$

* - significantly different from standard ($p<0.05$)

** - significantly different from standard ($p<0.01$)

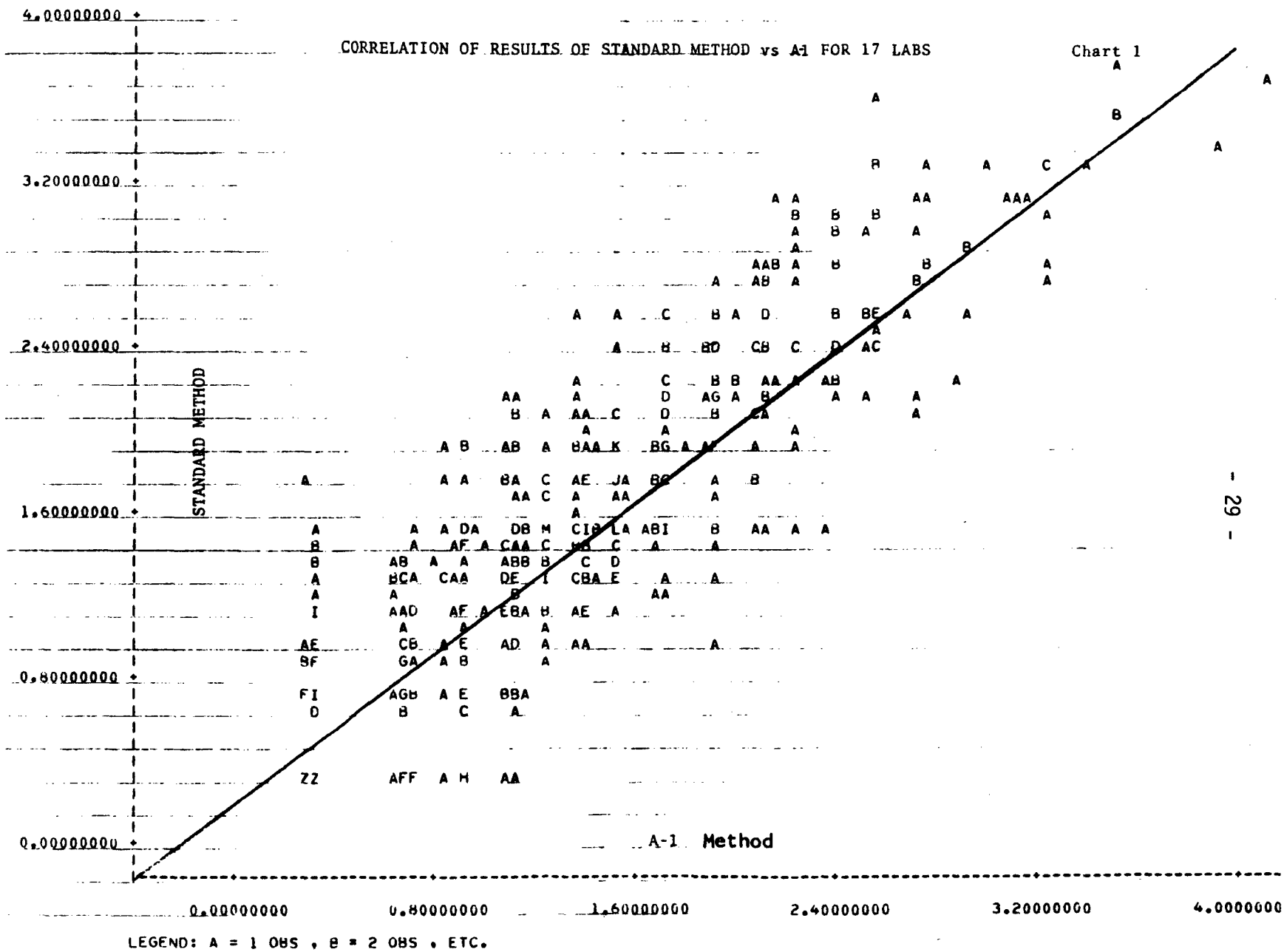
TABLE 6 SUMMARY OF IMViC DATA

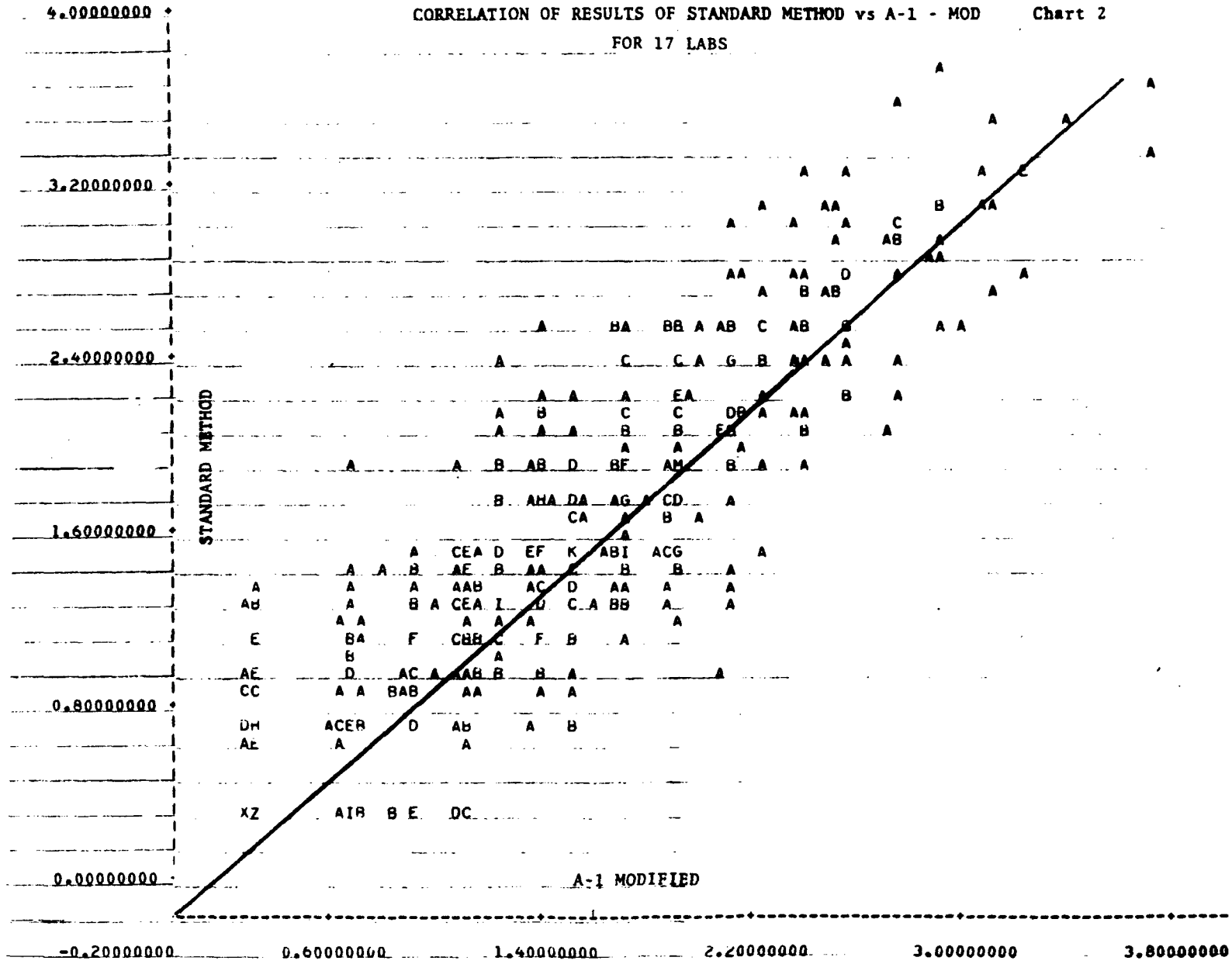
| Lab Code # | TUBES Standard # positive | total | % | TUBES A-1 # pos. | total | % | TUBES A1-Modified # pos. | total | % |
|---|---|---|---|---|---|---|---|---|---|
| 21 | 141 | 165 | 85.5 | 146 | 158 | 92.4 | 146 | 163 | 89.6 |
| 9 | 25 | 39 | 64.1 | 28 | 32 | 87.5 | 28 | 32 | 87.5 |
| 19 | 11 | 11 | 100 | 12 | 12 | 100 | 12 | 12 | 100 |
| 17 | n.r. | n.r. | 82 | n.r. | n.r. | 92 | n.r. | n.r. | 91 |
| 14 | n.r. | n.r. | 83 | n.r. | n.r. | 75 | n.r. | n.r. | 100 |
| 18 | 55 | 70 | 78.6 | 42 | 56 | 82.1 | 26 | 26 | 100 |
| 20 | 39 | 50 | 78.0 | 32 | 39 | 80 | 42 | 43 | 97.7 |
| 6 | 34 | 40 | 85 | 68 | 85 | 97.5 | 69 | 71 | 97.2 |
| 3 | | | | 115 | 118 | 92 | 118 | 118 | 100 |
| 1 | 207 | 260 | 79 | 198 | 215 | 98.7 | 210 | 233 | 90 |
| 5 | 247 | 259 | 95 | 236 | 239 | | 246 | 251 | 98 |

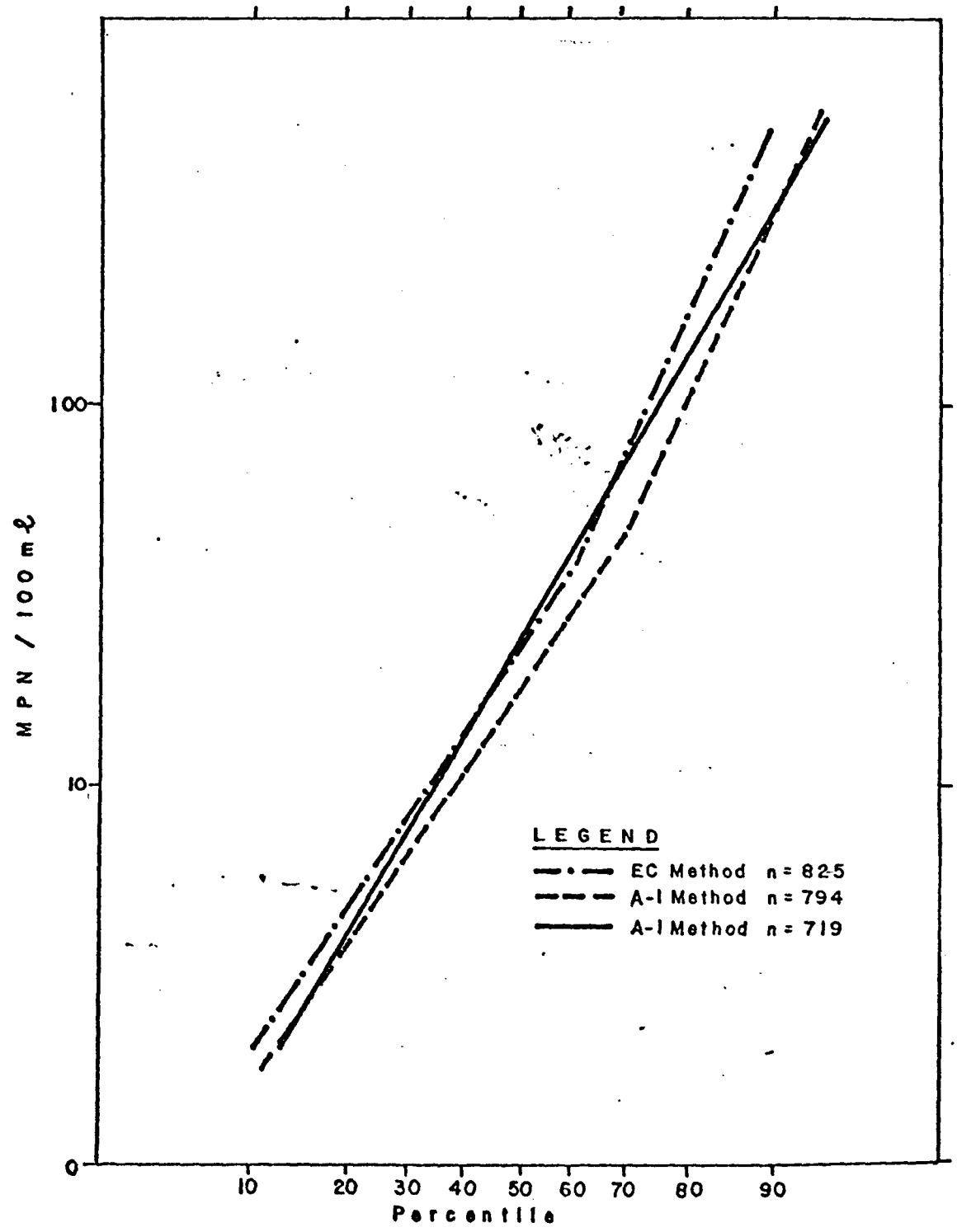
#positive - No. tubes positive for E. coli IMViC ++-- or -+--

total - total number of gas positive tubes IMViC tested

n.r. - not reported


CORRELATION OF RESULTS OF STANDARD METHOD vs A-1 FOR 17 LABS
Chart 1
STANDARD METHOD
A-1 Method
4.00000000
3.20000000
2.40000000
1.60000000
0.80000000
0.00000000
0.00000000
0.80000000
1.60000000
2.40000000
3.20000000
4.0000000
LEGEND: A = 1 OBS , B = 2 OBS , ETC.


CORRELATION OF RESULTS OF STANDARD METHOD vs A-1 - MOD Chart 2
FOR 17 LABS
4.00000000
3.20000000
2.40000000
1.60000000
0.80000000
0.00000000
STANDARD METHOD
A-1 MODIFIED
-0.20000000
0.60000000
1.40000000
2.20000000
3.00000000
3.80000000
LEGEND: A = 1 OBS , B = 2 OBS , ETC.




LOG PLOTS OF THE DATA FROM THE THREE METHODS - STEP 1

# APPENDIX II

## A-1 MEDIA EVALUATION - DATA SURVEY

- Step 3

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| Sept. | CP1 | 5/5 | 100 | 23 | 23 | 1/1 | 100 | 2 | 2 | 3/3 | 100 | 8 | 8 |
| 22/75 | CP2 | 2/2 | 100 | 5 | 5 | 3/3 | 100 | 8 | 8 | 2/2 | 100 | 5 | 5 |
| | CP3 | 1/1 | 100 | 2 | 2 | 2/2 | 100 | 5 | 5 | 1/1 | 100 | 2 | 2 |
| | CP4 | 3/3 | 100 | 8 | 8 | 1/1 | 100 | 2 | 2 | 2/2 | 100 | 7 | 7 |
| | CP5 | 2/2 | 100 | 5 | 5 | 4/4 | 100 | 13 | 13 | 2/3 | 66.6 | 7 | 4 |
| Sept. | SB1 | - | - | 79 | - | 7/8 | 87 | 70 | 46 | 9/9 | 100 | 140 | 94* |
| 29/75 | SB2 | - | - | 170 | - | 5/6 | 83 | 33 | 17 | 8/8 | 100 | 79 | 79 |
| | SB3 | - | - | 350 | - | 9/10 | 90 | 240 | 41 | 8/10 | 80 | 240 | 25* |
| | SB4 | - | - | 920 | - | 9/9 | 100 | 130 | 130 | 9/12 | 75 | 540 | 28 |
| | SB5 | - | - | 23 | - | 8/9 | 89 | 110 | 33 | 6/6 | 100 | 49 | 22* |
| Average % Recovery | | | 100 | | | | 94.9 | | | | 92.2 | | |

* One or more tubes lack IMViC data.

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| Oct. | SB1 | 8/9 | 39 | 110 | 33 | 11/11 | 100 | 350 | 350 | 10/10 | 100 | 240 | 240 |
| 6/75 | SB2 | 10/10 | 100 | 170 | 170 | 9/9 | 100 | 130 | 130 | 10/10 | 100 | 240 | 240 |
| | SB3 | 10/10 | 100 | 240 | 240 | 9/9 | 100 | 110 | 110 | 11/11 | 100 | 350 | 350 |
| | SB4 | 6/7 | 86 | 49 | 33 | 7/7 | 100 | 49 | 49 | 8/8 | 100 | 79 | 79 |
| | SB5 | 8/9 | 89 | 130 | 79 | 8/8 | 100 | 79 | 79 | 10/10 | 100 | 240 | 240 |
| Oct. | SB1 | 14/14 | 100 | 1600 | 1600 | 13/13 | 100 | 920 | 920 | 12/13 | 92 | 920 | 64 |
| 13/75 | SB2 | 10/12 | 83 | 540 | 48 | 14/14 | 100 | 1600 | 1600 | 11/12 | 92 | 920 | 180* |
| | SB3 | 14/14 | 100 | 1600 | 1600 | 11/12 | 92 | 540 | 350 | 14/15 | 93 | 1600 | 1600 |
| | SB4 | 13/14 | 93 | 1600 | 350 | 13/13 | 100 | 920 | 920 | 12/13 | 92 | 1600 | 280* |
| | SB5 | 11/11 | 100 | 350 | 350 | 12/12 | 100 | 540 | 540 | 10/12 | 83 | 540 | 47 |
| Average % Recovery | | | 94.0 | | | | 99.2 | | | | 95.2 | | |

* One or more tubes lack IMViC data

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| Oct. | SB1 | 10/11 | 91 | 3500 | 1700 | 14/14 | 100 | 1600 | 1600 | 11/12 | 92 | 540 | 56 |
| 27/75 | SB2 | 10/11 | 91 | 3500 | 1700 | 9/9 | 100 | 920 | 920 | 11/11 | 100 | 350 | 350 |
| | SB3 | 7/8 | 87 | 790 | 270 | 10/11 | 91 | 350 | 240 | 13/13 | 100 | 920 | 920 |
| | SB4 | 8/8 | 100 | 790 | 790 | 11/11 | 100 | 240 | 240 | 12/12 | 100 | 540 | 540 |
| | SB5 | 6/7 | 86 | 460 | 210 | 11/11 | 100 | 350 | 350 | 10/10 | 100 | 240 | 240 |
| Nov. | SB1 | 9/13 | 69 | 920 | 28 | 10/11 | 91 | 220 | 47 | 10/10 | 100 | 240 | 240 |
| 3/75 | SB2 | 7/11 | 67 | 350 | 21 | 11/12 | 92 | 540 | 56 | 9/10 | 90 | 240 | 41 |
| | SB3 | 12/13 | 92 | 920 | 64 | 14/14 | 100 | 1600 | 1600 | 13/13 | 100 | 920 | 920 |
| | SB4 | 11/12 | 92 | 540 | 350 | 9/11 | 82 | 540 | 40* | 10/11 | 91 | 540 | 48 |
| | SB5 | 9/11 | 82 | 220 | 39 | 10/11 | 91 | 220 | 56 | 12/13 | 92 | 920 | 540 |
| Average % Recovery | | | 85.7 | | | | 94.7 | | | | 96.5 | | |

*One or more tubes lack IMViC data

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| Nov. | SB1 | 8/10 | 80 | 2400 | 340 | 8/8 | 100 | 790 | 790 | 9/9 | 100 | 1300 | 1300 |
| 25/75 | SB2 | 7/7 | 100 | 490 | 490 | 10/10 | 100 | 1700 | 1700 | 8/8 | 100 | 790 | 790 |
| | SB3 | 7/7 | 100 | 490 | 490 | 7/8 | 87 | 790 | 490 | 9/9 | 100 | 1300 | 1300 |
| | SB4 | 9/9 | 100 | 1700 | 1700 | 6/6 | 100 | 330 | 330 | 8/8 | 100 | 700 | 700 |
| | SB5 | 6/6 | 100 | 330 | 330 | 8/8 | 100 | 790 | 790 | 10/10 | 100 | 1700 | 1700 |
| Dec. | SB1 | 8/10 | 80 | 2400 | 340 | 9/9 | 100 | 1300 | 1300 | 9/10 | 90 | 1700 | 1100 |
| 2/75 | SB2 | 10/10 | 100 | 2400 | 2400 | 10/10 | 100 | 1400 | 1400 | 10/10 | 100 | 2400 | 2400 |
| | SB3 | 10/10 | 100 | 2400 | 2400 | 9/9 | 100 | 1300 | 1300 | 10/11 | 91 | 3500 | 480 |
| | SB4 | 8/10 | 80 | 2400 | 250 | 8/8 | 100 | 790 | 790 | 9/9 | 100 | 1300 | 1300 |
| | SB5 | 8/9 | 89 | 1300 | 790 | 12/12 | 100 | 5400 | 5400 | 10/10 | 100 | 1400 | 1400 |
| Average % Recovery | | | 92.9 | | | | 98.7 | | | | 98.1 | | |

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| Jan. | SB1 | 13/14 | 93 | 1600 | 920 | 13/14 | 93 | 1600 | 350 | 11/11 | 100 | 350 | 350 |
| 12/76 | SB2 | 13/15 | 87 | 2400 | 340 | 14/14 | 100 | 1600 | 1600 | 13/13 | 100 | 920 | 920 |
| | SB3 | 13/14 | 93 | 1600 | 350 | 16/16 | 100 | 2200 | 2200 | 14/14 | 100 | 1600 | 1600 |
| | SB4 | 14/15 | 93 | 1700 | 400 | 12/12 | 100 | 540 | 540 | 14/14 | 100 | 1100 | 1100 |
| | SB5 | 15/16 | 94 | 2200 | 470 | 14/14 | 100 | 1600 | 1600 | 15/15 | 100 | 1400 | 1400 |
| Jan. | SB1 | 11/11 | 100 | 350 | 350 | 10/10 | 100 | 170 | 170 | 10/11 | 91 | 350 | 48 |
| 19/76 | SB2 | 10/10 | 100 | 240 | 240 | 10/11 | 91 | 350 | 170 | 12/12 | 100 | 280 | 280 |
| | SB3 | 12/12 | 100 | 540 | 540 | 12/12 | 100 | 350 | 350 | 13/14 | 93 | 1600 | 920 |
| | SB4 | 14/14 | 100 | 1600 | 1600 | 12/12 | 100 | 540 | 540 | 13/13 | 100 | 920 | 920 |
| | SB5 | 11/11 | 100 | 350 | 350 | 10/10 | 100 | 240 | 240 | 12/12 | 100 | 540 | 540 |
| Average % Recovery | | | 96.0 | | | | 98.4 | | | | 98.4 | | |

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| Feb. 2/76 | CP1 | 10/10 | 100 | 170 | 170 | 7/7 | 100 | 49 | 49 | 9/9 | 100 | 110 | 110 |
| | CP2 | 7/7 | 100 | 49 | 49 | 7/7 | 100 | 49 | 49 | 9/9 | 100 | 130 | 130 |
| | CP3 | 8/8 | 100 | 79 | 79 | 6/6 | 100 | 33 | 33 | 7/7 | 100 | 46 | 46 |
| | CP4 | 7/10 | 70 | 240 | 27 | 11/11 | 100 | 350 | 350 | 10/10 | 100 | 140 | 140 |
| | CP5 | 6/8 | 75 | 70 | 17 | 6/7 | 86 | 49 | 33 | 7/9 | 78 | 130 | 49 |
| Feb. 16/76 | CP1 | 9/9 | 100 | 110 | 110 | 8/8 | 100 | 33 | 33 | 8/8 | 100 | 70 | 70 |
| | CP2 | 10/10 | 100 | 240 | 240 | 8/8 | 100 | 95 | 95 | 8/8 | 100 | 79 | 79 |
| | CP3 | 9/10 | 90 | 170 | 110 | 8/8 | 100 | 79 | 79 | 8/8 | 100 | 79 | 79 |
| | CP4 | 10/10 | 100 | 140 | 140 | 7/7 | 100 | 27 | 27 | 7/7 | 100 | 49 | 49 |
| | CP5 | 8/9 | 89 | 130 | 34 | 7/7 | 100 | 49 | 49 | 7/7 | 100 | 49 | 49 |
| Average % Recovery | | | 92.4 | | | | 98.6 | | | | 97.8 | | |

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| March | CP1 | 7/7 | 100 | 49 | 49 | 4/4 | 100 | 11 | 11 | 8/9 | 88.8 | 130 | 79 |
| 2/76 | CP2 | 5/6 | 83.3 | 33 | 17 | 5/6 | 83.3 | 22 | 14 | 5/5 | 100 | 23 | 23 |
| | CP3 | 6/6 | 100 | 33 | 33 | 5/5 | 100 | 14 | 14 | 5/5 | 100 | 23 | 23 |
| | CP4 | 7/7 | 100 | 46 | 46 | 7/7 | 100 | 49 | 49 | 6/6 | 100 | 33 | 33 |
| | CP5 | 5/5 | 100 | 23 | 23 | 7/7 | 100 | 49 | 49 | 5/5 | 100 | 23 | 23 |
| March | CP1 | 8/8 | 100 | 79 | 79 | 6/6 | 100 | 33 | 33 | 5/6 | 83.3 | 22 | 14 |
| 8/76 | CP2 | 6/6 | 100 | 33 | 33 | 6/6 | 100 | 33 | 33 | 7/8 | 87.5 | 79 | 27 |
| | CP3 | 6/6 | 100 | 33 | 33 | 4/4 | 100 | 11 | 11 | 5/5 | 100 | 17 | 17 |
| | CP4 | 5/5 | 100 | 17 | 17 | 4/4 | 100 | 13 | 13 | 4/4 | 100 | 13 | 13 |
| | CP5 | 4/4 | 100 | 11 | 11 | 5/5 | 100 | 17 | 17 | 3/3 | 100 | 8 | 8 |
| Average % Recovery | | | 98.3 | | | | 98.3 | | | | 95.9 | | |

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | #E. coli/ | | Method | IMViC | #E. coli/ | | Method | IMViC | #E. coli/ | | Method | IMViC |
| | | Total # | % | MPN/ | MPN/ | Total # | % | MPN/ | MPN/ | Total # | % | MPN/ | MPN/ |
| Date | Sample | Tubes | Recovery | 100 ml | 100 ml | Tubes | Recovery | 100 ml | 100 ml | Tubes | Recovery | 100 ml | 100 ml |
| April | CP1 | 6/6 | 100 | 31 | 31 | 5/5 | 100 | 23 | 23 | 6/6 | 100 | 33 | 23 |
| 20/76 | CP2 | 7/8 | 87.5 | 79 | 27 | 6/6 | 100 | 22 | 22 | 5/5 | 100 | 17 | 17 |
| | CP3 | 5/5 | 100 | 23 | 23 | 3/3 | 100 | 7 | 7 | 7/7 | 100 | 49 | 49 |
| | CP4 | 7/7 | 100 | 49 | 49 | 5/5 | 100 | 23 | 23 | 6/6 | 100 | 33 | 33 |
| | CP5 | 7/7 | 100 | 46 | 46 | 7/7 | 100 | 46 | 46 | 9/9 | 100 | 130 | 130 |
| April | CP1 | 6/9 | 66.6 | 130 | 17 | 6/6 | 100 | 17 | 17 | 4/4 | 100 | 9 | 9 |
| 27/76 | CP2 | 6/6 | 100 | 17 | 17 | 2/2 | 100 | 5 | 5 | 3/4 | 75 | 11 | 8 |
| | CP3 | 6/6 | 100 | 33 | 33 | 2/2 | 100 | 5 | 5 | 3/4 | 75 | 13 | 8 |
| | CP4 | 8/8 | 100 | 33 | 33 | 10/10 | 100 | 170 | 170 | 10/10 | 100 | 170 | 170 |
| | CP5 | 1/2 | 50 | 4 | 2 | - | - | 2 | - | - | - | 2 | - |
| Average % Recovery | | | 90.4 | | | | 100 | | | | 94.4 | | |

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| May | SB1 | 5/8 | 62.5 | 70 | 12 | 10/10 | 100 | 240 | 240 | 10/10 | 100 | 240 | 240 |
| 3/76 | SB2 | 4/6 | 66.6 | 330 | 110 | 8/9 | 88.8 | 130 | 34 | 10/10 | 100 | 240 | 240 |
| | SB3 | 3/7 | 42.9 | 490 | 80 | 10/10 | 100 | 240 | 240 | 11/11 | 100 | 350 | 350 |
| | SB4 | 2/8 | 25 | 700 | 40 | 10/10 | 100 | 170 | 170 | 10/10 | 100 | 170 | 170 |
| | SB5 | 3/6 | 50 | 220 | 70 | 11/11 | 100 | 350 | 350 | 12/12 | 100 | 540 | 540 |
| May | SB1 | 7/9 | 77.7 | 130 | 27 | 8/8 | 100 | 79 | 79 | 5/6 | 83.3 | 31 | 17 |
| 10/76 | SB2 | 7/7 | 100 | 46 | 46 | 6/6 | 100 | 33 | 33 | 7/7 | 100 | 49 | 33 |
| | SB3 | 5/6 | 83.3 | 330 | 170 | 5/5 | 100 | 230 | 230 | 6/6 | 100 | 330 | 330 |
| | SB4 | 4/10 | 40 | 140 | 8 | 8/8 | 100 | 79 | 79 | 8/8 | 100 | 79 | 79 |
| | SB5 | 6/7 | 85.7 | 49 | 22 | 4/4 | 100 | 13 | 13 | 3/3 | 100 | 7 | 7 |
| Average % Recovery | | | 63.4 | | | | 98.8 | | | | 98.3 | | |

APPENDIX II A-1 MEDIA EVALUATION - DATA SURVEY (continued)

| | | Standard Method | | | | A-1 Method | | | | Modified A-1 Method | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Sample | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml | #E. coli/ Total # Tubes | % Recovery | Method MPN/ 100 ml | IMViC MPN/ 100 ml |
| May | SB1 | 5/6 | 83.3 | 33 | 17 | 5/6 | 83.3 | 33 | 17 | 5/5 | 100 | 23 | 23 |
| 31/76 | SB2 | 5/6 | 83.3 | 33 | 23 | 6/6 | 100 | 33 | 33 | 7/7 | 100 | 49 | 49 |
| | SB3 | 7/7 | 100 | 49 | 49 | 6/6 | 100 | 33 | 33 | 8/8 | 100 | 79 | 79 |
| | SB4 | 9/10 | 90 | 240 | 48 | 4/4 | 100 | 13 | 13 | 5/5 | 100 | 23 | 23 |
| | SB5 | 8/8 | 100 | 70 | 70 | 2/2 | 100 | 5 | 5 | 4/4 | 100 | 13 | 13 |
| June | SB1 | 8/10 | 80 | 2400 | 790 | 12/12 | 100 | 540 | 540 | 10/10 | 100 | 240 | 240 |
| 7/76 | SB2 | 11/11 | 100 | 350 | 350 | 9/9 | 100 | 110 | 110 | 11/11 | 100 | 350 | 350 |
| | SB3 | 9/11 | 81.8 | 220 | 39 | 11/12 | 91.6 | 540 | 350 | 13/13 | 100 | 920 | 920 |
| | SB4 | 6/8 | 75 | 79 | 33 | 7/8 | 87.5 | 70 | 26 | 10/10 | 100 | 240 | 240 |
| | SB5 | 4/9 | 44.4 | 130 | 9 | 9/10 | 90 | 240 | 41 | 10/10 | 100 | 240 | 240 |
| Average % Recovery | | | 83.5 | | | | 95.2 | | | | 100 | | |