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Water quality control trials: statistical tables for direct comparison between membrane filtration bacterial counts and the multiple tube method with a description of the bacteriological method

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SUMMARY

Experiments in the quality control of water samples are being conducted in the Public Health Laboratory Service and the water industry in the United Kingdom. The number of distributions which have been made is 7 and 92 laboratories are now participating. The methods used for preparing and distributing samples are described. Some participating laboratories use the multiple tube method and some use membrane filtration to assess the presence of coliforms and *Escherichia coli*. The results are, therefore, a mixture of estimated numbers and direct colony counts.

In order to compare results from these two different laboratory methods statistical tables have been compiled to show the most likely multiple tube result corresponding to each colony count. Tables relating to two commonly used tenfold dilution series are presented.

To illustrate how these tables may be used we present results from a typical quality control distribution. The analyses of these results are generally satisfactory but show a tendency for lower counts using the membrane filtration method and more false negative results with *E. coli* counts.

INTRODUCTION

The purpose of this paper is to show that statistical tables may be used to compare counts by membrane filtration with those by the multiple tube method in quality control trials and to describe how samples are prepared and distributed.

In the 1970s the Public Health Laboratory Service used methods developed by Gray & Lowe (1976) to prepare and distribute simulated water for quality control to constituent laboratories. Three media proved equally successful in preserving *Escherichia coli* and coliform organisms for periods of 7–10 days at ambient temperature. The media used were deionized water with sodium thiosulphate and boric acid, Gray's own improved formate lactose glutamate medium without lactose but with added boric acid and nutrient broth plus boric acid. As the last seemed the cheapest and simplest to prepare we chose it for a series of

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experiments. These confirmed the efficacy of the medium but also that not all strains of *E. coli* and coliforms are equally suitable for preservation. Suitable strains were therefore sought amongst those routinely isolated in this laboratory from water samples and from infected urine.

Samples were prepared and distributed as described below and participating laboratories invited to use one or both of the multiple tube and filtration methods. The results were collected centrally for comparison although this led to difficulties because of the two counting methods used. If a sample is moderately or heavily contaminated then there are only a limited number of results which the multiple tube method can give – the differentiation between levels of estimated bacterial density will depend on the numbers of tubes showing reaction at the lowest level of dilution. Membrane filtration uses actual counts of colonies which develop from viable organisms in the sample and therefore allows greater differentiation between samples. In a quality control trial it is necessary to decide whether laboratories are achieving similar results, regardless of method.

MATERIALS AND METHODS

Preservation media

Nutrient broth at a pH of 7·5 plus 1·8% boric acid sterilized at 121 °C for 20 min.

Suspension of organisms

The selected organism was inoculated into nutrient broth and incubated at 37 °C for 18 h. One drop (33 μl) of the overnight broth was added to 200 ml of the preservation medium in a 500 ml bottle, mixed well and counted by the Miles and Misra method. The suspension was stored at room temperature in the dark and the following day an aliquot of the suspension diluted in a further volume of preservation medium. The amounts used depended upon the count and the quantity required for distribution to the participating laboratories. The number of organisms varied from time to time to test the ability of the laboratories to detect differing numbers of organisms. Ideally an occasional distribution would be of the order of only 1–2 *E. coli* or coliform organisms per 100 ml but this was rarely, if ever, attainable.

To confirm that the number of organisms present was at a suitable level for distribution, 3 ml of the suspension was added to 400 ml of distilled water in a 500 ml bottle, mixed well and examined by the multiple tube and membrane filtration methods. If the counts were satisfactory the suspension was distributed aseptically into screw-capped bijou bottles ensuring that the suspension was well mixed by using a magnetic stirrer. These bottles were filled to the brim and three of these simulated samples packed into a small box for distribution by post. One box was posted back to the distributing laboratory. The procedure to be adopted by the participating laboratory was the same as that recommended by Gray & Lowe (1976) as shown in the appendix.

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Table 1. *Range of counts per 100 ml for which i, j, k is the most likely result*

(11-tube series 1 × 50 ml : 5 × 10 ml : 5 × 1 ml where i, j and k tubes show growth)

	i, j, k
1	010
	100
2–3	110
4–6	120
7–10	130
10–17	140
18–19	150
20–40	151
41–68	152
69–110	153
111–175	154
176–infinity	155

Table 2. *Range of counts per 100 ml for which i, j, k is the most likely result*

(15-tube series 5 × 10 ml : 5 × 1 ml : 5 × 0·1 ml where i, j and k tubes show growth)

	i, j, k
1–3	100
4–5	200
6–9	300
10–15	400
16–18	410
19–38	510
39–67	520
68–109	530
110–179	540
180–184	550
185–404	551
405–694	552
695–1099	553
1100–1800	554
1800–infinity	555

Statistical method for comparison of results of multiple tube and membrane

For a dilution series modern computers allow the calculation of exact conditional probabilities, defined as the probability that a certain combination of the multiple tubes will show growth given that there were n relevant organisms in the volume examined (Tillett & Coleman, 1985; Tillett, 1987). These probabilities have been studied to find the most probable dilution series result that could be expected from a sample containing n viable organisms.

RESULTS

Tables have been prepared to compare direct colony counts with multiple tube results. Table 1 and 2 relate to the dilution series 1 × 50 ml : 5 × 10 ml : 5 × 1 ml and 5 × 10 ml : 5 × 1 ml : 5 × 0·1 ml respectively. The first column shows the number of organisms (expressed, conventionally, as number per 100 ml) for which this dilution series would be the most likely result. The second column in each table

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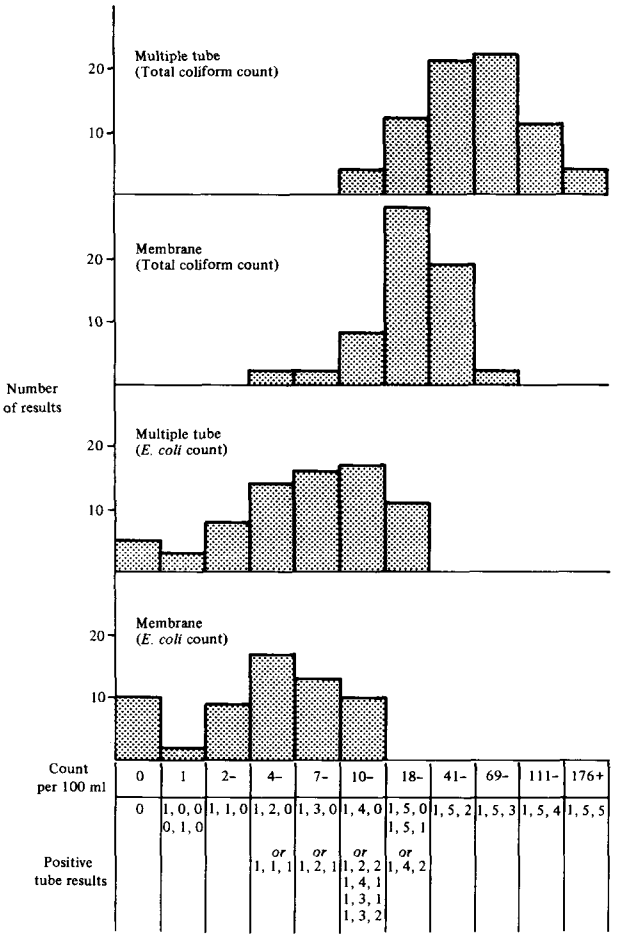

Fig. 1. Chart showing results from a quality control trial.

shows the numbers of tubes showing growth in the multiple tube series (i, j and k tubes at the three consecutive dilution levels). For example, in Table 1 a true density in the range 41–68 organisms per 100 ml would be most likely to give a multiple tube result of 1, 5, 2.

In one quality control distribution, one of the three bottles was intended to be sterile and none of the 50 laboratories returning results reported the presence of any coliforms. The two other bottles contained the same number of organisms. Results for them have been pooled. These are shown in Fig. 1 which was prepared to allow comparison between the methods. Membrane counts are grouped according to the most probable combination of positive tube reactions that they would give, as shown in Table 1. (Laboratories using the multiple tube method were asked to use the 11-tube series.) Membrane filtration results can thus be plotted directly below the equivalent multiple tube results (Fig. 1). The less common multiple tube results, which do not appear in Table 1 but which were recorded for 15% of the samples examined, have been plotted with the range of

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counts which correspond with the most probable numbers of organisms for that combination of positive tube results (Fig. 1).

Participating laboratories were supplied with a summary of results and a copy of the chart shown in Fig. 1, with their own counts marked on – thus giving a quick visual assessment of their performance.

Eighteen laboratories used both methods and so can be compared directly. Nineteen used multiple tube only and 13 membrane filtration only. The membrane results (in one distribution) gave median counts of 28 for total coliforms and 5 for *E. coli*. Such counts would be expected to yield tube results of 1, 5, 1 and 1, 2, 0. However, the multiple tube results gave a median coliform result of 1, 5, 3 and a median *E. coli* of 1, 3, 0.

There were more false zero *E. coli* counts with the membrane method than with the multiple tube method (10 of 61 compared with 5 of 74, $P = 0{\cdot}1$). Although this difference is not statistically significant it confirms the trend seen in other quality control trials (Tillett, 1986).

DISCUSSION

Distributions have now been made to 53 Public Health Laboratories in the UK and recently extended to 39 Water Authority Laboratories making a total of 92 participants in the quality control programme. The method employed appears to have been satisfactory and it is hoped to continue distributions at the rate of 4–5 per year. In any comparison between methods used to quantify the bacterial density in a sample it must be remembered that the methods will have been applied to different portions of that sample. Therefore, however thoroughly the sample is mixed, the methods can quite correctly give different answers due to random variation. However, with a large series of trials the two methods should, on average, give comparable results if they are of equal competence. There is some evidence to suggest that membranes give more false negatives than the multiple tube method when counts are low.

In quality control trials of simulated public health water samples the tables in this paper can be used to compare counts by membrane filtration with those by the multiple tube method.

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APPENDIX 1

Three simulated specimens of water are enclosed. On receipt please store at room temperature in a dark cupboard and examine on the day stated.

Preparation of Samples – it is important that the instructions given are meticulously carried out.

(1) Half the contents of each bijou bottle should be decanted into a sterile universal bottle.

(2) The bijou bottle should then be shaken vigorously and mixed well by aspiration, using a pasteur pipette. The contents are then pipetted into the universal bottle.

(3) The universal bottle should then be vigorously shaken for at least 2 min and 3 ml of the contents added to 400 ml of sterile distilled or deionized water.

This constitutes the simulated water sample which should be mixed and examined, if possible by both multiple tube and membrane filtration methods.

Please examine on: date specified.

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