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Interval Plating: A Simplified Method to Determine Suitability of Distilled Water as a Dilution Fluid

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ABSTRACT

A simplified procedure has been developed for routine evaluation of distilled water used in milk, food, and water service laboratories. It is simple to do, requires no additional equipment or reagents, and can be done along with the routine daily workload. It requires no "control" water, and has a sensitivity which is appropriate for the intended use of the distilled water.

In both *Standard Methods for the Examination of Dairy Products* (*1*) and *Standard Methods for the Examination of Water and Wastewater* (*2*), recognition is given to the fact that in bacteriological determinations the quality of distilled water used to prepare media and dilution blanks is of considerable importance. To assist laboratories in determining the acceptability of their distilled water supply for use in Standard Methods bacteriological determinations, a procedure known as the Distilled Water Suitablility Test (DWST) (*3*) is detailed in both of the publications just mentioned. Neither publication requires that the DWST be done as a component of standard bacteriological procedures, but by virtue of stating that the distilled water source be acceptable, and by including only one procedure to determine this, the DWST by implication almost becomes required.

Certainly any requirement, whether stated or implied, which provides a reliable indication of the quality of a laboratory's distilled water contributes to continued assurance that laboratory analyses are being done competently. However, the DWST has some features which may make its inclusion in Standard Methods publications more of a disservice to users than a service.

The DWST basically involves incorporating the distilled water in question into a minimal growth medium, doing the same with a "control" double distilled water, inoculating both with a strain of *Enterobacter aerogenes*, and comparing the two for total amount of growth. This procedure lacks applicability in most small laboratories doing routine analyses because (*a*) the length and tedium of the procedure is prohibitive, (*b*) the quality of double-distilled "control" water varies with each laboratory doing the DWST, and (*c*) the DWST is probably too sensitive for the applications required in most routine milk and water analysis laboratories (*6*).

We are reporting the results of efforts to develop a more simple, valid, and applicable system for determining the quality of distilled water. The method is an Interval Plating Procedure, and is intended to measure the water quality in a more meaningful manner in relation to the procedure for which the distilled water will be used, i.e., routine Standard Methods bacteriological counts.

MATERIALS AND METHODS

Interval plating procedure

The distilled water to be examined was made into stock phosphate buffer by the procedure described in *Standard Methods for the Examination of Dairy Products* (*1*). This was then dispensed in 99-ml amounts into standard dilution bottles and autoclaved at 121 C for 15 min and stored at room temperature.

Escherichia coli (ATCC 25922) was used as the test organism. On the day before the interval plating procedure was to be done, 2-3 ml of Nutrient Broth (Difco) was inoculated from a nutrient agar slant culture and incubated at 32 C for 18 h. One-tenth milliliter of the broth culture was then added to 100 ml of Nutrient Broth in a flask and incubated for 4 h to obtain a culture in the mid-log phase of growth. The 4-h culture was then diluted serially to 10^{-6} in three of the dilution blanks prepared as indicated above. The 10^{-6} dilution was immediately plated as the "zero" time dilution by measuring 2 ml of the dilution into each of five plates and mixing with Violet Red Bile Agar (Difco) maintained in a water bath at 45 C. The culture was allowed to remain in the dilution water at room temperature for 60 min, and then plated again using the same protocol as for the "zero" time plating. Plates were then incubated at 32 C for 18-24 h. Colonies were enumerated and the following calculation done:

$$\frac{\text{Mean colony count (60 min)-Mean colony count (0 min)}}{\text{Mean colony count (0 min)}} \times 100 = \%\ \text{change between 0-60 min}$$

Arbitrarily we have established that the percent change in colony count between zero and 60 min should not exceed ± 15% for consideration of the distilled water as acceptable for application in routine milk, food, and water bacteriological analysis. Figure 1 illustrates schematically the Interval Plating Procedure.

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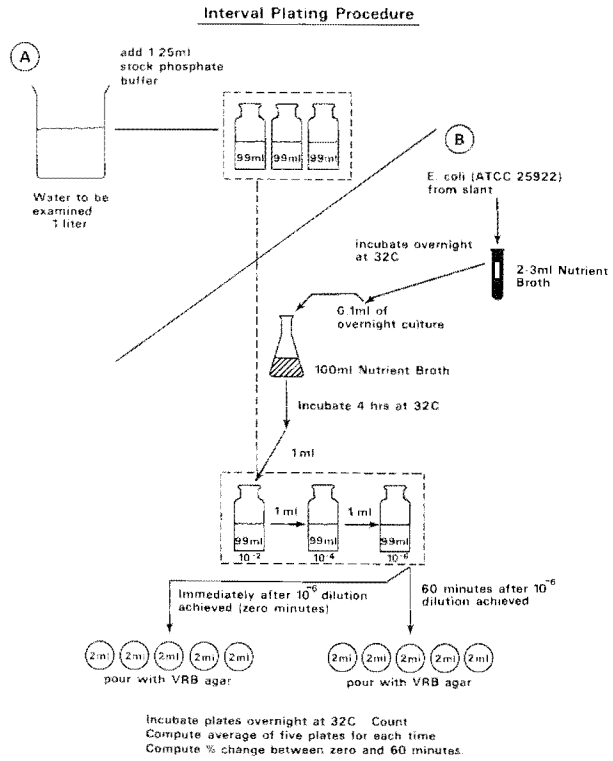

Figure 1. *Interval Plating Procedure.*

Comparison of the Distilled Water Suitability Test and the Interval Plating Procedure

To compare the DWST with the Interval Plating Procedure two identical comparative studies were done. Distilled water samples were requested from 19 milk testing laboratories approved by the Iowa State Department of Agriculture. These were representative of the water supply used in routine standard plate counts and other bacteriological testing. The water samples were submitted by mail, split, and evaluated on the same day by both methods.

RESULTS

Interval Plating Procedure

As a preliminary indication of the ability of the Interval Plating Procedure to detect toxic qualities in distilled water, several waters of known source and quality were analyzed. Table 1 shows the sources of distilled water and the results of interval plating. One of the distilled waters (Water #5) used to prepare dilution blanks in an unrelated experiment, caused bacterial population reduction after remaining at room temperature for 45 min. This water was from a building distribution line and was passed through a mixed-bed resin long overdue for recharge. Water #2 was the same water as #5 after recharging the resin column. Water #1 was the same building water passed through a Millipore Super Q (Millipore Corporation, Bedford, MA 01730) water treatment unit. Water #3 was again the same water but specially processed for use in tissue culture, and Water #4 was the untreated building line distilled water.

TABLE 1. *Detection of toxic properties of distilled water by the Interval Plating Procedure*

Water	Ohms specific resistance	%Change 0-60 min
1[a]	1,200,000	— 1.6
2[b]	630,000	— 4.5
3[c]	500,000	—10.3
4[d]	320,000	—99.9
5[e]	200,000	—99.9

[a]Building line water, through Millipore Super Q.
[b]Building line water, through recharged resin.
[c]Building line water, specially processed for tissue culture.
[d]Building line water, untreated.
[e]Building line water, through exhausted resin, suspected as toxic from previous experience.

Results of the interval plating showed that the procedure agreed with subjective judgements, essentially ohms specific resistance data previously made on the quality of distilled water used to prepare dilution water blanks and correlated directly with a population reduction.

As assurance that VRB Agar should be used in the procedure rather than a general purpose medium such as Plate Count (PC) Agar, known "good" and "bad" distilled waters were subjected to interval plating using both SPC and VRB Agars. Results of this evaluation are presented in Table 2.

TABLE 2. *Use of Violet Red Bile Agar vs Plate Count Agar in Interval Plating Procedure*

Water	Medium	%Change, 0-60 min
"Good" water (1,200,00 ohms-cm²)	PC[a]	— 6.1
	VRB[b]	+ 4.8
"Bad" water (330,000 ohms-cm²)	PC	— 5.8
	VRB	—98.4

[a]PC = Plate Count Agar.
[b]VRB = Violet Red Bile Agar.

As data in Table 2 show, PC Agar produced comparable results for both qualities of water, while the use of VRB Agar provided greatly divergent values. This appears to indicate that remaining in the diluent for 60 min did not cause cell death, but apparently injury resulted, preventing growth in the more selective VRB Agar. Since VRB Agar is apparently more capable of detecting toxic effects, while PC Agar permits growth regardless of injury, VRB Agar was chosen for use in the procedure.

Comparative study between DWST and the Interval Plating Procedure

Results of two separate comparative studies between the DWST and the Interval Plating Procedure are presented in Table 3. The results of trial #1 point out one of the inherent deficiencies of the DWST in that there are no standard specifications for the control water other than being double-distilled. In trial #1, all 17 waters by the DWST appeared to be extremely toxic (acceptable ratio is 0.8-3.0). That this is indeed the case from a practical standpoint is highly unlikely. Also, water #10 was submitted from the State Hygienic Laboratory as one of the approved milk laboratories in the state and was taken after passage through the Millipore Super Q system where it had a resistance measurement in excess of 1,000,000 ohms before shipment to the State

TABLE 3. *Comparative study between the Distilled Water Suitability Test and Interval Plating Procedure*

Water	Trial 1		Trial 2	
	DWST ratio	Int plating % , 0-60 min	DWST ratio	Int plating % , 0-60 min
1	0.04T[a]	−50.6T	0.82	+ 8.8
2	0.03T	−42.7T	1.15	−63.4T
3	0.01T	−99.9T	1.09	+ 4.7
4	0.08T	+80.0T	1.96	− 1.5
5	0.01T	−99.9T	0 T	−99.9T
6	0.02T	+2.6	2.42	+ 4.0
7	0.02T	+18.7T	1.28	+ 6.5
8	0.01T	−99.9T	0.65T	−99.9T
9	0.01T	+53.6T	2.5	− 7.8
10	0.02T	− 5.6	2.13	+ 4.6
11	0.01T	−41.4T	0.18T	−19.1T
12	0.05T	+10.5	0.93	+ 5.7
13	0.01T	−99.9T	0.19T	−91.6T
14	0.14T	+12.8	0.26T	− 6.1
15	0.01T	− 4.8	0.16T	−42.3T
16	0.01T	−73.6T	0.17T	−10.4
17	0.03T	+ 8.5	0.21T	+ 1.2
18			0.26T	− 2.7
19			0.10T	−24.0T

[a]T indicates unacceptable by established DWST criteria (either toxic or nutritive) or by suggested Interval Plating criteria (not exceeding ± 15% change at 60 min).

Department of Agriculture's laboratory. Thus it appears that the control water used in the DWST contained growth promoting substances making all of the samples appear to be extremely toxic.

In trial #2 the control distilled water used in the DWST appeared to have returned to normal, since a likely range of ratio values resulted. Only one sample was judged "false positive" by the Interval Plating Procedure because the DWST result indicated an acceptable ratio. Four water specimens were toxic by the DWST and acceptable by interval plating. The interval plating and DWST methods agreed upon the quality of 14 of the samples (74% agreement).

DISCUSSION

Any service laboratory, regardless of size, whose users rely upon the results generated is bound professionally and ethically to routinely do quality assurance procedures. Ideally, these should be done not monthly or yearly, but daily as an incorporated part of the customary workload. There are many quality control procedures, some more expensive and time consuming than others, but the method sought should give the most significant results for a given application for the least amount of expense and effort.

The quality of culture media used and the performance of laboratory personnel has been monitored to various degrees for some time. However, the quality of distilled water used to prepare media and, specifically, dilution water blanks is for the most part taken for granted, although it has been suggested in some jurisdictions that periodically small laboratories submit samples of their distilled water to a central laboratory for evaluation by the DWST (*4*). Poor water quality can tend to mask, complicate, or misinterpret results just as much as a poorly performing technician or unacceptable culture medium, and only occasional surveillance certainly cannot give an adequate view of the over-all quality of a distilled water source.

The routine users of Standard Methods publications use distilled water primarily to prepare dilution blanks and media. Culture media provide considerable protection from toxic effects of any water (*6*); therefore, the primary interest in distilled water quality is in its use as a dilution fluid. If distilled water is to be used for production of a minimal medium for bacterial cultivation, then the DWST would be justified for that particular application. However, we are concerned whether the dilution system will leave cells uninjured for a reasonable period, not whether or not the water in question will permit actual growth.

The DWST is complicated, time-consuming, relies on essentially another "unknown" water as a control, and is too sensitive biologically for the use to which the water will be put in routine water and milk analysis.

Interval plating, on the other hand, is simple and can be easily included in the daily routine and requires no additional materials. With the Interval Plating Procedure, the unknown distilled water is applied to a standard system and its effect on that system determines its acceptability, not how an unknown and a so-called "control" compare to each other in their effect on a standard system. Finally, the Interval Plating Procedure is a modification of the very system it was designed to monitor—survival in dilution blanks and, therefore, does not assess unnecessary limits of sensitivity.

Specific chemical parameters (specific resistance, copper, chlorides, etc.) provide an indication of quality, but overlook the potential presence of any number of both recognized and as yet unidentified factors which may either adversely affect a biological system or provide undesired nutritive supplements. The best way to determine biological suitablility is by biological assay.

The use of a pure culture for this procedure rather than a naturally contaminated source, such as raw milk, is justified since it is an attempt to develop a system as standardized as possible. The use of organisms other than *E. coli* was not evaluated and this particular organism was selected because of ease of cultivation and wide availability. However, a study has been done (*5*) in which *Staphylococcus aureus, Streptococcus pyogenes, E. coli,* and *Salmonella typhimurium* were allowed to remain in various diluents for 60 min. The *E. coli* strain used in that study was considerably less affected by any toxic effects in the dilution systems. Therefore it can be assumed that one of the less sensitive organisms has been used in this study, and that the distilled water in question is at least being given the benefit of the doubt.

A potential argument to undermine the importance of distilled water quality in dilution blank preparation is the suggestion that in an actual analytical situation the presence of the food or milk itself will tend to protect the organism while in the diluent. Straka and Stokes (*7*) have

shown that the flora of frozen meat and turkey pies, when diluted to between 10^{-3} and 10^{-5} underwent considerable decline when allowed to remain in phosphate buffered dilution blanks for 60 min. Thus, protection of organisms by the product itself is still in doubt.

To conclude, an interval plating method is suggested as an alternative to the DWST for use in milk, food, and water analysis laboratories. It is simple to do, can be easily included in the daily routine, does not rely on comparison with a "control" water, and is more meaningful for the evaluation of the distilled water for use as a dilution fluid.

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