EXECUTIVE SUMMARY

OBJECTIVES

The overall objectives of this project were to provide new information on the relative merits of the various procedures approved by the USEPA and the DWI for the microbiological examination of drinking water. The specific objectives of the project were:

1. Perform a literature review of microbiological methods that are being used for the detection of coliforms and *E.coli* in water,
2. Perform an initial assessment of the performance of coliform and *E.coli* methodologies which are approved for use in the US and the UK,
3. Perform in the field intensive investigations of the use of up to three methods with different sample volumes,
4. Apply selected methods for the examination of "follow up" samples after detection of coliforms by routine sampling, and
5. Round robin testing of selected methods at utilities within the US.

BACKGROUND

For many years, water microbiologists have sought improved methods for the detection of coliforms and *E. coli,* in a quest to utilize more sensitive, yet more specific tests. In the past twenty years there has been an explosion of interest in the development of methods and many laboratories have adopted procedures that rely upon the detection of expression of the enzymes β-D-galactosidase and β-D-glucuronidase for total coliforms and *E. coli* respectively. While these methods offer substantial benefits in terms of ease of use and simplicity, it remains unclear how the results generated using such methods relate to those generated using more traditional methodologies such as those based upon fermentation of lactose and growth at elevated temperatures.

The debate as to the significance of total coliform organisms with respect to public health has fuelled the fire in relation to choice of methods. Some workers believe that the newer technologies offer a more taxonomic approach to the detection of coliforms and *E. coli,* while others believe that the broader range of organisms detected by methods utilizing β-D-galactosidase means that the total coliform group has even less meaning for the indication of water safety. What is clear is that methods that utilize β-D-galactosidase for the detection of coliforms will detect a wider range of coliforms than methods that rely solely on the fermentation of lactose.

The ability to accurately, quickly and cost-effectively detect coliforms and *E. coli* is critical to water utilities in order to protect public health. This project focused on a comparison of the performance of the various methods that are approved by the U.S. Environmental Protection Agency (EPA) and the Drinking Water Inspectorate (DWI). While the ability to determine exactly which method would give the best overall performance would be an ideal achievement, the experiments described are an attempt to determine some of the performance characteristics of the various methods such that microbiologists can make more informed decisions about the methods that they use for microbiological monitoring.

The project was undertaken by a team of researchers from Analytical Services Inc, Williston, Vermont. The project was also supported by a number of microbiologists from a range of utilities in both the U.S. and the U.K.

APPROACH

The initial portion of this study involved an extensive literature review of microbiological methods used to detect the presence of coliforms and *E. coli* in drinking water. Following this initial phase, the study focused on comparing the performance of various culture media in recovering total coliforms and *E. coli* from drinking water and similar matrices. Pure cultures of accurately counted bacteria were used initially to determine differences in inherent sensitivity. Subsequent experiments utilized diluted raw water and disinfected sewage effluents to determine both sensitivity and specificity. These experiments were also used to determine the usefulness of the ISO procedure 17994 for the comparison of microbiological methods. In assessing the performance of microbiological methods, sensitivity and specificity were used in addition to other measurements such as false positive and false negative rates. After establishing some baseline information on a number of the methods, field trials were conducted using two procedures to analyze both 100 mL and 2 L samples. The differences in the number of samples found to contain coliforms using the different volumes and different methodologies were compared.

Different procedures were used to attempt to define the performance of the different media in recovering coliforms and *E. coli*. Accurately counted pure cultures of bacteria were used to investigate inherent sensitivity and some differences were seen between different media. However, this procedure actually measured the inhibitory nature of the media towards target organisms and while some useful information was produced, the performances of the methods were quite different when compared with samples containing competing flora. Competing flora have two major influences on the performance of culture media such as those used for the detection of coliforms in water. Firstly, bacteria such as *Aeromonas* and *Pseudomonas* can inhibit the growth of coliforms due to the production of bacteriocins. Secondly, the growth of competing flora can make the detection of target organisms difficult due to overgrowth.

Some effort was expended in looking at the performance of the various methodologies that use β-D-glucuronidase as a marker for *E. coli*. A distinct flaw in the design of one medium was noted.

Nine laboratories, including Analytical Services, Inc. (ASI), took part in a small round robin study to simply assess the ease of use of two procedures for analysis of 100 mL and 2 L volumes. The study was too small to look at the variation between laboratories, but did yield some interesting results with respect to the performance of the two selected methods.

During the course of the study an unusual phenomenon was encountered which had not been described previously. Essentially, the water from one participating utility was toxic in varying degrees to bacteria, but the toxicity was neither consistent nor regular. These data were eliminated from the study to avoid bias. ASI applied for a small amount of additional funding through the Project Continuation Research Fund to investigate the cause of the toxicity.

RESULTS AND CONCLUSIONS

During the course of the literature review it became clear that while there have been an extraordinary number of studies comparing the relative merits of various media, there has been little attempt to develop a standard protocol for the comparison of microbiological methods in the United States. There is an International Standards Organization (ISO) procedure for comparing methods and whilst the basis of this procedure gives an outline of how to compare methods, some of the detail (for example the number of target bacteria present in samples) needs to be amended.

This study did not determine that any one single method was superior to all others. However, it was clear that methods that utilize detection of β-D-galactosidase for identifying the presence of coliforms will detect more coliforms (a higher concentration and a broader range) than methods that rely upon lactose fermentation. This may be the result of methodological differences and also different definitions for the coliform group. There are differing opinions as to the potential benefits of the coliform group being expanded. Furthermore, media that are incubated at elevated temperature (44-44.5°C) tended to recover fewer *E. coli* than methods that rely upon detection of β-D-glucuronidase activity, and this is important for public health and safety. The methods that relied upon lactose fermentation as a phenotypic marker for the presence of total coliforms or *E. coli* tended to have more false positive "presumptive" results (i.e. organisms appeared to be the target organism on primary isolation but were subsequently shown to be a false positive) for both groups of organisms. This is of significance in dealing with positive microbiological tests since responses to finding total coliforms or *E. coli* in drinking water should be undertaken as soon as possible and should not be delayed pending the results of confirmatory tests. For total coliforms the determination of whether methods show high false negative rates depends upon the definition used for the total coliform group. Many authorities around the world now describe the total coliform group as "members of the *Enterobacteriaceae* that produce the enzyme β-D-galactosidase. That definition has been adopted by the International Standards Organization and has been used throughout this study. When using this definition, the lactose-based methods for total coliforms detect considerably fewer coliforms than the methods based upon β-D-galactosidase. For *E. coli*, the false negative rate is not so clear cut and differences between media seem to be based more upon the composition of the media rather than the phenotypic traits used to identify *E. coli*. It did appear however, that methods based upon incubation at elevated temperature detected fewer *E. coli* although this difference was not clear.

There were differences in the performance of liquid media (presence/absence tests) and also differences between different media that are based upon membrane filtration. There was no clear difference between membrane-based methods and presence-absence methods, although of the membrane-based methods, those that simultaneously detect coliforms and *E. coli* generally performed better than those that utilize two membranes to separately recover coliforms and *E. coli*.

The testing of 2 L volumes of water resulted in significantly more coliform detections when compared to 100 mL samples, which is not in itself surprising. However, the increase seen was very different in the two sites that were investigated during this study. The use of 2 L samples can be recommended for investigation of possible contamination events but examination of this larger volume cannot be considered appropriate for routine regulatory or operational sampling with the use of currently available methods.

During the course of the work performed it became clear that some dechlorinated tap water samples had a considerable bactericidal effect. This effect was only noted in samples processed in one laboratory. Investigation of the processes used at the water treatment plant supplying the laboratory and microbiological testing demonstrated that this was due to the presence of residual polyelectrolyte (Magnifloc 572C) in the municipal supply. While this may not have a significant impact on the overall microbiological data for a water system, the effect can significantly reduce the number of bacteria in specific samples, and may be especially important if samples are not analyzed immediately. Holding samples for up to 24 hours could impact the likelihood of detecting coliforms and hence utilities should do their utmost to eliminate or minimize residual polyelectrolyte in municipal water systems and minimize hold times if polyelectrolyte is present. Somewhat counter-intuitively, the bactericidal effect was inversely proportional to concentration of the polymer, with low concentrations having the largest effect on cell recovery.

APPLICATIONS / RECOMMENDATIONS

The researchers recommend that utilities use the following criteria to select their method for both regulatory and operational monitoring; the methodology used should be sensitive, specific and accurate, simple to use and with minimal time for a confirmed result. Sensitivity and specificity address the issues of matrix effects and the effects of interfering organisms. If the method used is simple to perform, requiring minimal microbiological expertise, smaller systems will derive additional benefit. This study has provided data that indicate that methods based upon detection of activity of the two enzymes β-D-galactosidase and β-D-glucuronidase tend to be more specific, sensitive and accurate than more traditional methods based upon lactose fermentation. However, there may be matrix effects which were not studied here that could be specific to any given water source. It is crucial therefore that any method is tested for its suitability with given water sources.

Whilst the more recently developed, enzyme-based procedures cost more in materials than the traditional, lactose-based membrane filtration methods, there are many potential savings to be made when using a method that gives a more rapid and accurate result. The cost of re-sampling can be reduced with fewer false positives and the ability to act quickly and decisively based on an accurate result can lead to considerable cost benefits. The enzyme-based methods generally require less time to prepare and quality assure and this in itself can be a substantial cost saving.

It is recommended that utilities with an interest in "background flora" decouple this interest from coliform / *E. coli* monitoring, and perform heterotrophic plate counts to gather these data. Information gathered regarding background flora as a by-product of coliform / *E. coli* monitoring is likely to be both incomplete and skewed. This is because media designed to detect coliforms contain inhibitors to prevent the growth of non-target organisms and the population of non-target organisms recovered is likely to be small and a variable proportion of the heterotrophic plate count.

The researchers do not recommend increasing the volume of samples used for regulatory monitoring. However, it is recommended that utilities strongly consider the use of larger sample volumes when investigating the source of coliform organisms in water supply systems. Confirmation of positive results in these situations is required. In this study, the use of two liter (2 L) samples facilitated the detection of considerably more coliform-positive samples than did

the use of 100 mL samples. It is unclear if this would lead to better public health decisions but the use of larger samples has been useful in determining the source of total coliforms in water distribution systems.

This study has demonstrated that a polymer added as a coagulation aid has bactericidal properties and its presence in treated water can lead to inaccurate microbiological results. Utilities that use such polymers should do their utmost to ensure that there is no residual polymer in the final water or, that if this is unavoidable, that they are able to demonstrate that it has no impact on microbiological data.

Overall the data generated during this study is largely in agreement with many of the other studies that have previously been reported. It is clear that methods based upon detection of the expression of β-D-galactosidase for detecting total coliforms detect more target organisms than those based upon lactose fermentation. In general, the "newer" methods for total coliforms and *E. coli* out-performed the traditional lactose-based methods, which is in keeping with the majority of studies described in the literature.