COLILERT - WHAT'S AL THE FUSS ABOUT?

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ABSTRACT

Assessment of the microbiological quality of potable waters by water utilities relies on the detection of *E.coli* and coliforms. In water microbiology, there have been relatively few changes in methods since the inception of water testing. Recent method updates have resulted in significant alteration to the methods as they have incorporated changes to coliform and *E.coli* definitions. Defined substrate technology describes the technology whereby the presence of microorganisms possessing a particular enzyme may be detected. Colilert is an example of such a test. This paper describes the new generation of tests and their impact on microbiological results. In particular, the impact of the use of Colilert on recent coliform data is discussed.

KEYWORDS

Defined Substrate Technology, Colilert, Microbiological water quality, Coliforms

1.0 INTRODUCTION

1.1 Principle of Microbiological Water Quality Monitoring

Assessment of the microbiological quality of potable waters by water utilities relies on the detection of *E.coli* and coliforms. The tests associated with the screening of waters for *E.coli* and coliforms are accepted as relatively simple, cheap and rapid. This is in contrast to methods associated with monitoring for specific individual pathogens. *E.coli* and coliforms are intestinal bacteria, easy to isolate and quantify because of their abundance in the faeces of warm-blooded animals, including humans. The presence of indicator organisms in potable water may indicate the possible presence of pathogens.

1.2 Traditional Membrane Filtration (MF) and Most Probable Number (MPN) Methods

Traditionally, the two accepted methods for the isolation and enumeration of coliforms and *E.coli* are the membrane filtration (MF) and the most probable number (MPN) methods. The membrane filtration method requires filtration of the sample through a sterile membrane, whilst the most probable number method involves inoculation of aliquots of samples into a selective enrichment medium.

Up until the present time in routine laboratory practice, the MF method is that most frequently employed for water supply monitoring. This is because there is considerable saving in technical labour and equipment; presumptive results for coliforms and *E.coli* may be available within 18 hours and this method provides a direct count of organisms. The MPN method is employed less frequently and in specific circumstances. It is employed in those instances where the membrane will become blocked before sufficient water can be filtered and where the accumulated deposit on the membrane may inhibit the growth of indicator organisms. The MPN method is also used for waters containing only small numbers of the indicator organisms sought in the presence of many other bacteria capable of growth on the media used.

Both MF and MPN methods involve primary recognition of a presumptive positive result followed by a multi-step process of confirmation.

Often the presumptive result may be made available within 18 hours of sample receipt, depending on the method employed, however the confirmed result may take up to three days or more for an MF test and up to six days for the MPN result. Both methods generate the *E.coli* result prior to that of the coliform. In the absence of any bacterial growth on membranes, final results are available within 18 hours. With MPN, final results are available within 48 hours where presumptive results are negative *ie* no positive reactions.

In the MF method, presumptive indicator (*E.coli* and coliform) results are obtained by counting the colonies of characteristic morphology and colour and taking into account any dilutions made. For the MPN method, an estimate of coliform results (presumptive result) is obtained from the number of tubes showing positive reactions and by reference to probability tables.

Confirmed indicator results are obtained in the MF method by subculturing all or a representative number of colonies formed, into a medium containing lactose (for coliforms and *E.coli*) and a medium containing tryptophan (for *E.coli* only). In the MPN method, confirmation of positive tubes is likewise performed by subculture to tubes of the same confirmatory media.

An elevated incubation temperature (44°C) is used to differentiate *E.coli* and faecal coliforms from the total coliform population.

1.3 History of Methods Employed for the Determination of Coliforms in Water

In water microbiology, there have been relatively few changes in methodology since the inception of water testing. Initially, an MPN technique was employed to be later superseded (but not totally) by a membrane filtration technique, made possible through the availability of membrane filters composed of cellulose esters with uniform pore sizes. Whilst a variety of bacteriological media have been employed in the execution of the membrane filtration (MF) technique they may be broadly classified into those used in the USA by the American Public Health Association (APHA) and those used in the United Kingdom (Report 71). APHA methods specify that M-FC and m-Endo agar/broth be employed in the MF method as the primary isolation medium for enumeration of *E.coli* and coliforms respectively. In contrast, Report 71 methods since 1982 have specified the use of sodium lauryl sulphate agar/broth.

Despite the different recovery efficiency of m-Endo agar/broth and sodium lauryl sulphate agar/broth for *E.coli* and coliforms (m-Endo is the more inhibitory medium of the two), the APHA and Report 71 MF methods until recently have employed the same *E.coli* and coliform definitions. Consequently the only variables in these two methods employed for *E.coli* and coliform determinations, aside from the media employed, have related to procedural aspects, such as incubation temperature and duration of incubation.

Recent method updates have resulted in significant alteration to the methods as they have incorporated changes to coliform and *E.coli* definitions. In the latest version of Report 71 (*ie* 1994) the definition of a coliform has been broadened to come more in line with a molecular one rather than a methods-based one.

1.4 Defined Substrate Technology including Colilert-18Ô/Quanti-TrayÔ

Defined substrate technology describes the technology whereby the presence of microorganisms possessing a specific enzyme(s) may be detected. Each enzyme is able to metabolise a specific substrate. For example, the enzyme β-D-galactosidase metabolizes β-galactopyranoside and the enzyme β-glucuronidase metabolizes β-D-glucuronide.

When specific substrates are attached to a chromogen and are included in a growth medium, microorganisms possessing the target enzyme (where present) will metabolise the substrate, resulting in release of the chromogen. The release of chromogen manifests itself in a colour change of the substrate. Microorganisms other than the target cannot grow and metabolise and do not affect the test. A major characteristic of this technology is that more than one enzyme can be assayed at the same time providing that each is attached to a chromogen of a different colour.

The Colilert system, among other systems (e.g. those using chromogenic media where membrane filtration is employed and colonies of a certain colour are counted and confirmed as coliforms), uses defined substrate technology. In the Colilert system targeted microbes metabolise the indicator nutrients in the Colilert-18 medium. Coliforms (including *E.coli*) will metabolise ortho-nitrophenyl galactopyranoside using the enzyme β-galactosidase to produce ortho-nitrophenyl. This results in a yellow colouration of the test well. *E.coli* will also metabolise 4-methyl-umbelliferyl glucoronide using the enzyme β-glucuronidase to produce 4-methyl-umbelliferone which fluoresces under long wave (365 nm) ultra violet light.

The Colilert system simultaneously detects and enumerates total coliforms and *E.coli* directly from water samples without the requirements of confirmation.

Quantification of numbers of total coliform and *E.coli* organisms is achieved through the use of a "Quanti-Tray". One hundred millilitres of water sample mixed with Colilert-18 medium is dispensed into a Quanti-Tray with either 51 or 97 wells depending upon the resolution of result required (up to 200 orgs/100 mL or 2,000 orgs/100 mL respectively). After the appropriate incubation period (18-22 hours for Colilert-18) the number of wells positive for total coliforms and *E.coli* are counted. Results are then calculated from relevant MPN probability tables.

Employment of Colilert-18 and Quanti-Tray technology has some parallels with traditional MPN methodology. As for the traditional MPN methods, Colilert-18 and Quanti-Tray can be employed for waters with high turbidity and in those instances where water contains only small numbers of indicator organisms sought in the presence of many other bacteria. As for traditional MPN methods the confirmed result obtained is an estimate or "most probable number". One obvious advantage of the Colilert-18 and Quanti-Tray utilisation of 52 and 97 wells in the determination of the most probable number is that the confidence interval associated with each is smaller than for traditional MPN methods where sets of tubes are generally fifteen or less.

The definition of a coliform and *E.coli* are as follows with each based upon their ability to metabolise a particular substrate:

Coliform organisms are capable of metabolising ortho-nitrophenyl galactopyranoside using the enzyme β-galactosidase to produce ortho-nitrophenyl.

E.coli are capable of metabolising 4-methyl-umbelliferyl glucuronide using the enzyme β-glucuronidase to produce 4-methyl-umbelliferone.

The Colilert system ***does not*** produce a faecal coliform result. Traditional MF or MPN methodology must be used for faecal coliform determination, as the definition of such is a methodology-based one associated with lactose fermentation at 44°C.

The manufacturer of Colilert, IDEXX, recommends an incubation time of between 18 and 22 hours for this product.

2.0 RESULTS

Comparisons of conventional microbiological (MF and MPN) methods and Colilert for the detection and enumeration of coliforms have been conducted by many investigators. Findings of such studies, (including those conducted in Victoria) have included the following observations:

A good correlation between Colilert and conventional methods with respect to final confirmed *E.coli* results .

"Colilert" method is more sensitive than traditional MF/MPN methods.

"Colilert" result differs from Report 71 methods with respect to recovery efficiency and the coliform (including *E.coli*) definition.

A faecal coliform result cannot be obtained using "Colilert".

Confirmed "Colilert" results are available within 24 hours

The ability to track coliforms, *Aeromonas*, *Pseudomonas* and background colonies visually through a water supply is lost with the employment of "Colilert".

Identification of organisms responsible for discrepant results showed them to predominantly belong to the genera *Enterobacter* and *Serratia*.

"Colilert" method allows greater resuscitation of coliform organisms

MF coliform results reported for some supplies have historically been falsely low given the degree of interference of background microorganims

The subjective reading of coliform membranes (MF method) leads in some instances to some operators overlooking some coliform populations.

The "Colilert" definition of a coliform is more encompassing and includes those organisms which do not appear as orange or orange yellow colonies on membranes and thus are overlooked by membrane filter readers.

3.0 DISCUSSION

The impact of the use of alternative methods with enhanced sensitivity has elicited responses from water authorities on previous occasions. One example, which elicited concern in the USA and from which parallels can be drawn is the use of m-T7 medium for coliform detection. In particular, m-T7 medium results in enhanced detection of injured coliforms and thus significantly higher coliform results compared to m-Endo medium. It should be noted in the context of the use of more sensitive methods that use of such methods does not undermine the goal of improved water quality. Rather, the detection of the entire population of coliforms, including injured organisms (enhanced resuscitation of coliforms is indicated in Colilert studies) provides an increased margin of safety by increasing analytical sensitivity and providing a greater capability to detect developing problems within the water supply system. This increased safety margin therefore allows for a more timely response. In addition, increased method sensitivity is useful in epidemiological investigations and might show that coliforms presently undetected or detected in low numbers by traditional and less sensitive techniques are in fact present.

The parallels relating to the introduction of Colilert as of July 2000 in Victoria and to the use of m-T7 medium in USA in 1990 (Mc Feters, 1990) are striking. There are a number of justifications for the enumeration of injured (and other) coliforms and recognition of their significance. In particular (and as also demonstrated for the use of m-T7) drinking water systems with high quality drinking water showed little change in coliform recoveries with the use of Colilert. As more data is collected relating to the adoption of Colilert on microbiological data for Victorian non-metropolitan urban supplies the full impact of the introduction of a more sensitive technology will become clearer.

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