Review

Validation, verification and comparison: Adopting new methods in water microbiology[#]

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Abstract

Until recently there has been little formal guidance on procedures for adopting new methods in water microbiology. However, the *European Union Drinking Water Directive* of 1998 specified methods that were to be used for the microbiological parameters, most being ISO methods, but allowed the use of alternative methods that were "at least as reliable". At that time, there were no published procedures for demonstrating equivalency of performance between methods. Work commissioned by the UK Drinking Water Inspectorate (DWI) developed suitable analytical and statistical protocols for comparing microbiological methods. The statistical aspects have been refined and recently published as *ISO 17994*. ISO has also recently published guidance on the validation of methods for water microbiology (*ISO/TR 13843*), which gives guidance for developers of new media on what performance information is required. These developments provide a framework for the enhancement of validation and verification procedures within a laboratory's quality system for evaluating new methods prior to their adoption. This paper overviews these developments in light of the author's experience in their use and discusses issues relating to the analytical procedures and the statistical rationale employed (including the concept of "equivalency" of performance between methods).

Keywords: water microbiology, methods, validation, verification, comparison, AQC

Introduction

Many methods used widely in water microbiology have not undergone full validation of performance, having been widely used and accepted historically, and there has been little formal guidance on demonstrating equivalent or superior performance of a new method prior to its adoption by a laboratory. Most laboratories have accepted performance claims in published scientific papers or from manufacturers. It should, however, never be assumed that a method will perform as claimed in every laboratory and for every type of matrix for which it may be used. Therefore, verification of the claimed performance, and where appropriate, demonstration of equivalent or inferior/superior performance compared to the method in current use are required. While there has been some guidance on assessment of performance characteristics for water microbiology methods (Havelaar et al., 1993; Lightfoot and Maier, 1998; ISO, 1999a) that on comparing the performance of two methods has been very limited. Typically, this has been in the nature of analysing a limited number of samples, usually 20 to 30, and using simple analysis of variance statistics (Lightfoot and Maier, 1998). This is really only sufficient to detect any gross differences between two methods. A procedure for comparing two presence/absence (P/A) methods was developed by the USEPA (1995) which required multiple analyses (20) of ten natural sources of coliform bacteria, which were then chlorine-challenged ensuring that the numbers present in the analysed test portions covered the range 1 to 10 per 100 mℓ. Similarly, Covert et al. (1992) used ten sub-samples from 22 chlorine-challenged samples containing low numbers of *E. coli* when evaluating defined-substrate P/A tests. These approaches have been successfully employed for P/A methods where the results are simply positive or negative and only a limited set of data is required for statistical analysis (typically non-parametric) to demonstrate superior or inferior performance of one method against another.

When it comes to comparing two quantitative methods, however, there are some aspects of variability in micro-organisms that have to be taken into account. Firstly, it must be borne in mind that micro-organisms are not solutes like ions, which for chemical analyses can be assumed to be homogeneously distributed. When introduced into water, micro-organisms do not form a perfect solution but a suspension, which imparts a significant degree of inherent heterogeneity (Tillett and Lightfoot, 1995; BSi, 2003). This may be exacerbated by any reactions between the target micro-organisms and any others or particles present in the sample. Additionally, there is a variability imparted by the microbial cells in a sample (even at species level) because they will be at differing stages of growth, states of stress response and metabolic status at the time of analysis (BSi, 2003; Tillett and Sartory, 2004). This will impact on the response of target bacteria in quantitative methods requiring selective growth. All these factors result in a significant natural variability in recovery of micro-organisms from water which must be taken into account when devising analytical and statistical protocols for comparing quantitative methods, particularly when the numbers that are encountered in routine samples tend to be very low, as in the case of drinking water monitoring.

The *European Union Drinking Water Directive* of 1998 (European Union, 1998) specified for the first time the methods that were to be used for the microbiological parameters for regulatory monitoring. For *E. coli*, coliform bacteria, Enterococci and heterotrophic plate counts the methods cited are the current respective ISO standards (*ISO 9308-1*, *7899-2* and *6222*) (ISO, 1999b; 2000a; 2000b), whilst for *Clostridium perfringens* mem-

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