**IDEXX Summary**

**Topic**
Review of studies in France leading to AFNOR Certification Validation mark for Colilert®-18 / Quanti-Tray® for the testing of drinking water samples

**Title**
Improved methods for detecting *E. coli* and coliforms in drinking water: AFNOR validation of Colilert®-18/Quanti-Tray®

**Authors**
D P Sartory and C Allaert Vandevenne

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**Highlights**

- In France, in order to be granted the use of the AFNOR Validation mark, Colilert-18 / Quanti-Tray underwent rigorous studies focused on the comparison of the IDEXX method with the standardized reference method (ISO 9308-1)

- Based on the results of these studies, AFNOR Certification granted IDEXX the use of the AFNOR Validation mark as discussed and described in the attached paper

- Per AFNOR Certification guidelines, a preliminary study was conducted where the relative accuracy, linearity, detection and quantification limits, selectivity (inclusivity and exclusivity) and practicability of the Colilert-18/Quanti-Tray method were assessed using samples of distribution water and fountain water spiked with strains of both coliform bacteria and *E. coli*. All the assessed performance characteristics of the Colilert-18/Quanti-Tray system were found to be satisfactory

- Subsequent to the preliminary study, an inter-laboratory study was conducted; 13 laboratories were sent samples inoculated at various levels of contamination with a strain of *E. coli* originally isolated from water. Samples were analyzed by both NF EN ISO 9308-1 method and Colilert-18/Quanti-Tray. Results from each method were then statistically compared and were found to be statistically equivalent

- Official AFNOR Certificate can viewed here or viewed on the IDEXX website here [AFNOR-Validation mark for Colilert-18/Quanti-Tray](#)
Improved methods for detecting *E. coli* and coliforms in drinking water: AFNOR validation of Colilert®-18/Quanti-Tray®

D P Sartory¹ and C Allaert Vandevenne²

¹ SWM Consulting, Little Ness, Shrewsbury, UK  
E-mail: david.sartory@tesco.net  
² IDEXX Laboratories, Av. Acàcies, nº4, Alella 08328, Spain  
E-mail: corrie-allaert@idexx.com

Summary

*Escherichia coli* (*E. coli*) and the related coliform bacteria are the most important parameters in the testing of drinking water. In France the detection and enumeration of these bacteria has for many years been based on growth on TTC-Tergitol agar (NF EN ISO 9308-1). Recent developments in the detection of enzymes specific for *E. coli* and coliforms have led to the development of more accurate, rapid methods. One method, the IDEXX Colilert®-18/Quanti-Tray® system, has recently been validated in accordance with an AFNOR certification protocol. The method was found to be statistically equivalent to NF EN ISO 9308-1, and gave accurate confirmed results within 18 hours, instead of 48–72 hours.

*E. coli* and coliform testing of drinking water

In 1885 Theodor Escherich described the bacteria *Bacterium coli commune* and *Bacterium lactis commune* which had been isolated from faeces. Shortly afterwards, in 1892, Franz Schardinger, in Vienna, proposed the testing of water for *Bacterium coli* (now *Escherichia coli*), since it was found abundantly in faeces and its absence in water would, therefore, indicate the absence of faecal contamination and, hence, the absence of enteric pathogens. It was soon realised that there was a whole group of related bacteria similar to *E. coli* that could be found in human and animal faeces and contaminated water. This group of bacteria, which includes *E. coli*, was given the name “coliform bacteria”. So started the long history of testing for *E. coli* and coliform bacteria as the principal indicators in assessing the sanitary safety of water.

For over 100 years now *E. coli* and the related coliform bacteria have been the key bacterial indicators for assessing the microbiological quality of drinking water and environmental waters. *E. coli* is regarded as the primary indicator of faecal contamination and, hence, a potential health risk with consumption of contaminated water. Standards for drinking water based on the absence of *E. coli* and coliform bacteria were soon adopted in several countries in Europe and North America, typically based on their absence in a 100 ml test volume of sample. This is still the case with current legislation being based on the European Drinking Water Directive (Council Directive 98/83/EC) which has been incorporated into national legislation by the Member States of the European Union. This Directive additionally specified for the first time the methods that were to be used for the microbiological parameters for regulatory monitoring. For *E. coli* and coliform bacteria the method cited is the current ISO/CEN standard NF EN ISO 9308-1. Although this method is not widely used in Europe, in France it is the only method allowed to be used for compliance testing of drinking water. The Directive additionally states that alternative methods “may be used, provided that the results obtained are at least as reliable as those produced by the methods specified”. Also, recent developments in the detection of enzymes specific for *E. coli* and coliforms have led to a change in the definitions for these bacteria and this has led to the development of more accurate, easy to use and rapid methods. Since 2000 a number of studies have been undertaken in several European countries comparing the performance of alternative methods with the NF EN ISO 9308-1. Acceptance of the performance of several alternative methods has been agreed upon by the Expert Group on Microbiology which advises the European Commission on microbiological aspects of the Drinking Water Directive. These include the Colilert®-18/Quanti-Tray® system (for example in Germany, Spain and the UK), membrane-lactose glucuronide agar (in the UK) and lauryl sulphate agar (in the Netherlands). Until now there have been no approved alternative methods for the detection and enumeration of *E. coli* and coliform bacteria from drinking water in France.
Methods for detecting *E. coli* and coliforms in drinking water

Traditionally, *E. coli* and coliform bacteria have been detected in water using media that allow demonstration of the fermentation of lactose. This is the basis of the NF EN ISO 9308-1 method with the TTC-Tergitol agar medium. This medium contains lactose which when fermented produces acid that is detected as a change in colour around the colony from red to yellow. In France the tests are incubated for up to 48 hours, and if colonies are present they are termed “presumptive” as they must be tested by a confirmation test. For *E. coli* this involves the demonstration of production of indole from tryptophan and takes a further 24 hours. A confirmed result for *E. coli* can take up to 72 hours to obtain.

Today the approach for detecting *E. coli* and coliforms is more often based on the detection of specific enzymes: β-galactosidase (involved in the fermentation of lactose) for the detection of coliform bacteria and β-glucuronidase for the specific detection of *E. coli*. This approach has been adopted by the ISO working group responsible for deriving standards for the detection and enumeration of *E. coli* and coliforms in water.

The first step in lactose fermentation is the splitting of the molecule into glucose and galactose by β-galactosidase, an enzyme expressed by the great majority of coliform bacteria. As it is simple to test for the presence of β-galactosidase using chromogenic or fluorogenic substrates that are readily taken up by cells, a number of methods based on this approach have been developed over the last 20 years. A widely used chromogenic substrate for β-galactosidase is o-nitrophenyl-β-D-galactopyranoside (ONPG) which produces a yellow colour when split by the enzyme (Figure 1). This is the basis of a number of methods for coliform bacteria, including the IDEXX Colilert®-18/Quanti-Tray® system. Basing the definition of coliforms on a single diagnostic enzyme that is indicative of the actual or potential ability of the bacterium to ferment lactose results in a definition that is microbiologically acceptable. The ISO working group on methods for *E. coli* and coliforms has agreed on a definition for a coliform as being a “member of the Enterobacteriaceae that expresses β-galactosidase” and this will be the basis for the development of any future standards.

![Figure 1: Detection of coliform bacteria β-galactosidase using ONPG](image)

In addition to β-galactosidase, *E. coli* expresses the enzyme β-glucuronidase which is very rarely expressed by other coliform bacteria. Detection of this diagnostic enzyme for *E. coli* has become well established for food and water testing, particularly using the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) (Figure 2). The ISO working group has agreed that *E. coli* be simply defined as a “member of the Enterobacteriaceae that expresses β-galactosidase and β-glucuronidase”. This is a more microbiologically correct definition for *E. coli* than the one based on lactose fermentation and production of indole from tryptophan. ISO’s new definition is also the basis for *E. coli* detection by the Colilert®-18/Quanti-Tray® system.

There are two basic approaches to processing of samples for analysis of *E. coli* and coliforms. The first is membrane filtration, which is the basis of NF EN ISO 9308-1, where the bacteria are trapped on a membrane filter which is then placed on medium for selective growth of the target bacteria. The alternative approach is the multiple tube most probable number (MPN) method where aliquots of sample are dispensed into a series of tubes containing media and incubated. The number of positive
tubes is statistically related to the number of coliforms or \textit{E. coli} present. This is also the basis of Quanti-Tray® where the tubes have been replaced with a reaction pouch, that divides the sample into a series of wells (Figure 3). For drinking water, the pouch is divided into 51 wells, giving a much more accurate MPN count than the traditional multiple tube method of ISO 9308-2.

**Figure 2:** Detection of \textit{E. coli} $\beta$-glucuronidase using MUG

**Figure 3:** Examples of modern MPN format methods for \textit{E. coli} and coliform bacteria – the 51-well Colilert®-18/Quanti-Tray® (foreground) and the 97-well Colilert®-18/Quanti-Tray® (background).

**Validation of Colilert®-18/Quanti-Tray® in France**

New alternative methods must be validated prior to adoption. Validation is when the performance characteristics of the alternative method are defined. If the performance characteristics are satisfactory then a comparative study is undertaken where the performance of an alternative method is compared to that of a reference method. The reference method for drinking water is NF EN ISO 9308-1. The Colilert®-18/Quanti-Tray® system has been validated against the reference method in a study using a recently developed AFNOR protocol based on FD ENV ISO 13843 (on validation of microbiological methods for water analysis) and NF EN ISO 16140 (on validation of alternative methods for food analysis). AFNOR protocols have been developed for validating food methods for
several years, but this is the first time that a new validation protocol has been applied to drinking water samples with the goal of certifying an alternative method for drinking water testing.

A preliminary study was conducted where the relative accuracy, linearity, detection and quantification limits, selectivity (inclusivity and exclusivity) and practicability of the Colilert®-18/Quanti-Tray® system were assessed using several samples of distribution water and fountain water spiked with strains of both coliform bacteria and *E. coli*. All the assessed performance characteristics of the Colilert®-18/Quanti-Tray® system were found to be satisfactory.

On completion of the preliminary study an interlaboratory study was conducted where participating laboratories were sent samples inoculated at various levels of contamination with a strain of *E. coli* originally isolated from water. These samples were analysed by both the NF EN ISO 9308-1 method and Colilert®-18/Quanti-Tray®. The results from each method were then statistically compared. The Colilert®-18/Quanti-Tray® method was found to be statistically equivalent to the NF EN ISO 9308-1 method, particularly for low to medium counts that could be expected from drinking water samples. Colilert®-18/Quanti-Tray®, however, did on average give slightly higher counts, indicating greater accuracy.

The validation study has been reviewed and passed by the AFNOR Technical Board which is composed of a president, a secretary, a representative of AFNOR standards, and representatives of manufacturers, users and public authorities.

Conclusions

The AFNOR validation study demonstrated that Colilert®-18/Quanti-Tray® is a suitable alternative method to the current NF EN ISO 9308-1 method. As this method is based on the revised ISO definitions of *E. coli* and coliform bacteria, it is by definition more accurate, since it finds all the bacteria that are included in the new definitions. In addition, it has the advantage of not requiring a confirmation test, like NF EN ISO 9308-1. Consequently, confirmed results are available after 18 hours incubation compared to the 48 – 72 hours needed for the reference method NF EN ISO 9308-1. Its widespread use under acceptance for compliance drinking water testing in 16 European and 38 countries worldwide has shown it to be much more convenient and easy-to-use than the reference method.

Colilert®-18/Quanti-Tray® is produced in a ISO 9001:2000 certified facility that has been recently inspected by an AFNOR auditor, who passed it with high praise. Colilert®-18/Quanti-Tray® has been accepted for compliance testing of drinking water in several countries, including the USA, UK, Germany, Italy, Spain and Denmark.

References


AFNOR (2009) Validation project – revision 29.05.2009 – Application à l’analyse microbiologique de l’eau – Protocole de validation d’une méthode alternative commerciale par rapport à une méthode de référence.
