Executive Summary

Introduction
Monitoring of microbiological contaminants in water supplies requires fast and sensitive methods for the specific detection of indicator organisms or pathogens. The standard cultivation methods are too time-consuming to match the requirements of modern water safety management, i.e. approaching the equivalent of online measurements. Within the TECHNEAU project a protocol for the simultaneous detection of *E. coli* and coliform bacteria based on the Fluorescence *in situ* Hybridization (FISH) technology was developed by vermicon and TZW (TECHNEAU project deliverable 3.4.1). The developed FISH protocol consists of two different approaches. One approach allows the direct detection of single *E. coli* and coliform bacterial cells on the filter membranes. The second approach includes incubation of the filter membranes on a nutrient agar plate and the subsequent detection of the grown micro-colonies.

Importance
Standard cultivation methods for the detection of indicator organisms or pathogens are on the one hand laborious and on the other hand too time-consuming to match the requirements of modern water safety management, i.e. coming close to on-line monitoring technologies. To be able to use the developed faster FISH-protocols in drinking water samples it has to be assured that also samples from the end of treatment for instance after disinfection procedures can be analysed reliably. This was tested by TZW and vermicon within this study.

Approach
In this study, both approaches were validated using drinking water samples spiked with pure cultures (both approaches) and naturally contaminated water samples (approach 2). The effects of heat, chlorine and UV disinfection on the FISH based detection of *E. coli* and coliform bacteria were investigated.

Result
During this study some limitations became visible for the single cell approach. The method can not be applied for water samples which have been disinfected by UV irradiation. Cells inactivated by UV irradiation apparently can still be stained with the FISH probes. In addition, the results indicated that the green fluorescent dyes are not suitable to be used with chlorine disinfected samples. As all cells (FISH stained and not stained, target as well as non-target cells) emitted an unspecific green fluorescence at the excision wavelength of the green fluorescent dyes after chlorine treatment, false-positive results can be obtained. Furthermore, without the implementation of an appropriate automation procedure for cell detection and counting, this approach can become very labour-intensive, especially for samples with low numbers of *E. coli* or coliform bacterial cells.

In contrast, the micro-colony approach yielded very good results for all different samples and conditions tested, and thus can be thoroughly recommended for usage as an alternative method to detect *E. coli* and coliform bacteria in water samples.

More information

DVGW-Technologiezentrum Wasser
Dr. Beate Hambsch
Karlsruher Str. 84
76139 Karlsruhe, Germany

email: beate.hambsch@tzw.de