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Fast coliform detection in portable microbe enrichment unit (PMEU) with Colilert® medium and bubbling

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Abstract

Laboratory strains of coliforms *Escherichia coli* and *Klebsiella mobilis* were used to artificially contaminate water samples in two different cultivation and detection systems, without and with bubble flow. Samples were collected with an automated system (ASCS). The positive coliform signal caused the color change into yellow (at 550–570 nm). This signal could also be transmitted on-line to cell phones. *E. coli* containing samples emitted UV fluorescence at 480–560 nm when activated by UV light. If cultivation was started with inocula varying from 10,000 to 1 cfu/ml, the positive detection was obtained between 2 and 18 h, respectively, in Colilert medium using Coline PMEU device without gas bubbling. Accordingly, a single *K. mobilis* cell produced detectable growth in 18 h. Various clinical *E. coli* strains were compared to each other with equal inoculum sizes, and they showed slight variations in the initiation and speed of growth. The gas bubble flow in PMEU Spectrion promoted the mixing and interaction of bacteria and indicator media and speeded the onset of growth. Carbon dioxide also accelerated bacterial growth. In the presence of vancomycin, the onset of *E. coli* culture growth was speeded up by the volatile outlet flow from previous cultures. In the last cultivation syringe in a series of five, the lag phase disappeared and the growth of the inoculum continued without major interruption. In conclusion: the stimulation of the cultures by the gas flow turned out to be a useful means for improving the detection of indicator bacteria. It could also be used in combination with antibiotic selection in the broth medium.

Keywords: Hygiene monitoring; Indicator bacteria; Coliforms; *Escherichia coli*; Rapid microbial detection; Colilert; PMEU; Coliline; Antibiotic selection; Carbon dioxide

1. Introduction

Human feces contain huge amounts of microbes, some of which can be pathogenic, such as *Salmonella*, different viruses, protozoa or parasites [1,2]. These pathogens may also be found in the feces of warm-blooded animal. It is very important for human health to know if there is any risk for fecal contamination in the environment. Since pathogens need all their own methodology for identification, the concept of indicator bacteria has been widely accepted in environmental monitoring [3]. Ashbolt et al. [4] have described the early development of the concept of hygiene indicator bacteria.

Coliforms are commonly used bacterial indicators of sanitary quality of water and foods. They are rod-shaped Gram-negative non-spore forming bacteria which ferment lactose into acid and gas at 35–37 °C. Coliforms are common inhabitants of the gut of the warm-blooded animals, but they can be found in the environment, on vegetation and in soil. Their presence indicates the potential presence of pathogenic organisms. *Escherichia coli* is a facultative mixed-acid fermenting member of the coliform group being capable of fermenting lactose at 44 °C. Presence of *E. coli* is considered as an almost sure sign of fecal contamination [5,6].

The Colilert® method is based on specific bacterial enzymes. Coliforms and *E. coli* possess the enzyme β-D-galactosidase, giving them the ability to degrade *ortho*-nitrophenyl-β-D-galactopyranoside (ONPG), and produce yellow-color due to *o*-nitrophenol. *E. coli* also has the

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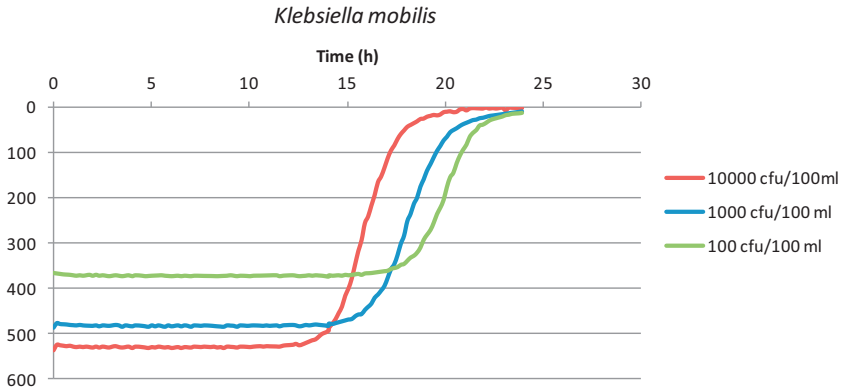

Fig. 1. *Klebsiella mobilis* cultivation in PMEU Coliline™ using Colilert® as the growth medium without gas bubbling. The growth was initiated in 13–18 h depending on the inocula.

ability to cleave methylumbelliferyl-β-glucuronide (MUG), which results in release of the fluorescent product 4-methylumbelliferone [7,8]. One widely used rapid method for the detecting hygiene indicator bacteria, Colilert® was developed by IDEXX. It simultaneously detects coliforms and *E. coli* in water in 24 h (Colilert®) and within 18 h for Colilert-18® [9]. This method produces results with improved sensitivity and specificity when compared with the earlier standard methods based on multiple-tube lactose fermentation or membrane filtration method. However, the method still suffers from the drawbacks caused by false positives [10]. The detection of contaminations in PMEU Spectrion® is based on readings of transmitted IR or visible light, or on emitted UV fluorescence [11]. Fischer et al. [12] have been successfully using the latter principle to detect *E. coli* in the biofilms in Baltic Sea down to levels of 4000 cells per cm^2.

PMEU method has been validated for the detection of coliforms and *E. coli* using a medium equivalent to the Colilert® where the cleavage of ONPG and that of MUG were the foundations for detection. These indicators can be detected in 10 h or less in normal laboratory conditions [13]. The PMEU technology is based on accelerated cultivation of water samples in the enrichment unit with optical detection of *o*-nitrophenol and 4-methylumbellifore. PMEU equipments have been equipped with automated sample collection system (ASCS) which also transfers data in real-time from the water monitoring PMEU Coliline™ units [14]. Nearly 30 alarms were obtained in one month, whereas the standard Colilert® approach without pre-enrichment with the PMEU gave only a couple of positive indications for household water contamination from the same samples [15]. In this study, the tap water was also artificially contaminated with salmonellas. Then the verification of a single pathogen cell was achieved in about 10 h when selective RVS broth was used as the enrichment medium. This result was obtained with a PMEU Scentrion® prototype capable of detecting the volatile compounds liberated from a bacterial culture [16].

In a wide follow up study in the Lake Kallavesi, surrounding Kuopio City, Finland, the results from PMEU procedure and a reference method were compared. Number of different *E.* coli phenotypes in PMEU cultivations have been determined by PhenePlate™ method [17]. According to this analysis, 81 phenotypes were detected from six sample stations at Lake Kallavesi, outside the city of Kuopio with PMEU cultivation only, whereas 16 phenotypes were found by traditional methods, three of these phenotypes being identical with both methods [3]. In testing the water distribution networks, the contaminating strains can sometimes be residing in some parts of the system. During the survey in Kuopio City water department, the results from the PMEU monitoring experiments along the distribution network were used to focus the renovation works to those parts of the system which clearly needed the hygienic improvement.[15].

The aim of this present study was to search for methods to further speed up the demonstration of coliformic presence and growth in enrichment cultures.

2. Materials and methods

2.1. PMEU technology with Colilert® medium

PMEU technologies comprise of different strategies in monitoring hygiene in water and other samples. Enhanced microbial growth and metabolism options in the PMEU have been developed for hospital, industrial and environmental samples [11,14,18]. The essential point is the option to boost the microbes with gas bubble flow. Various standard media can be used for cultivations [18].

Colilert® medium has been used for the monitoring of fecal coliforms and *E. coli* simultaneously [9]. Growing coliformic cultures produce yellow color when incubated in Colilert® medium and simultaneous UV fluorescence is produced by *E. coli* if it is present in the sample. These reactions can be measured by the PMEU Spectrion® and the PMEU Coliline™ automated version. Positive signal caused by coliformic bacteria is detected within wavelength 550–570 nm. In the case of samples contain *E. coli* they emitted UV fluorescence within a wavelength area of 480–560 nm, when