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Detection and Persistence of Clinical *Escherichia coli* in Drinking Water Evaluated by a Rapid Enzyme Assay and qPCR

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ABSTRACT

The aims of this study were to evaluate two methods, qPCR and a chemiluminescent assay (ColiLight II), for rapid detection of *E. coli* in water, and to examine the survival and persistence of clinical *E. coli* in drinking water and biofilm using qPCR and ColiLight II. qPCR and ColiLight II were compared with a cultivation-based method (MPN), and survival and persistence of four clinical *E. coli* strains in water and biofilms on stainless steel (SS) and polyethylene (PE) surfaces were studied in a flow-through reactor with non-disinfected drinking water using ColiLight II, qPCR, ATP bioluminescence, and MPN. ColiLight II and qPCR correlated well with MPN. In drinking water, some clinical *E. coli* strains showed prolonged survival in drinking water flow-through systems, and persisted 3 - 3.4 times longer than the theoretical washout due to incorporation into biofilms. Strain specific attributes can significantly affect detection and persistence of *E. coli* in drinking water matrices.

Keywords: Biofilm; Clinical *E. coli*; Chemiluminescent Assay; Lab-Scale Drinking Water Reactor System; Drinking Water; qPCR

1. Introduction

Escherichia coli is widely used as an indicator of fecal pollution when monitoring the microbial quality of drinking water [1]. Standard methods for detection of *E. coli* in drinking water are based on cultivation which requires an analysis time of 18 - 48 h or even longer for confirmation steps. In case of contamination situations, rapid methods are needed to redress the problem [2].

Various rapid, fluorescent enzyme-based methods for detection of total and fecal coliforms based on *β*-D-galactosidase and *β*-D-glucuronidase activity, respectively, have been developed [3-7]. Additional, chemiluminescent-based detection of *E. coli* on membrane filters has been proposed [8], and later, the same type of substrate was used to detect low concentrations of stressed *E. coli* in non-contaminated drinking water [9]. Chemiluminescence is more sensitive compared to fluorescence and absorbance, and the higher sensitivity is utilized to decrease the required analysis time of the detection method.

Another rapid method to detect *E. coli* is quantitative real-time PCR (qPCR). qPCR is a highly sensitive and very specific detection method. Due to this, a cultivation step can be omitted when applying qPCR for detection of microorganisms. This is a great advantage for the use in microbial ecological studies where the majority of target organisms cannot be cultivated in the laboratory. However, prior to successful amplification, sampling strategies and DNA extraction procedures must be optimized in order to achieve representative quantification. qPCR has previously been applied to detect *E. coli* in different water types such as groundwater, sea water, freshwater, and roof-harvested rainwater [10-12]. Clinical strains of *E. coli* are rarely included in drinking water studies, but from an epidemiological and a health perspective point of view, it is more relevant to study these strains since they have caused diseases in humans.

In this study, we applied the ColiLight II method and a qPCR assay for detection of clinical *E. coli* in two different non-contaminated drinking water matrices. The ColiLight II method has been optimized and validated previously [9], and primers for detection of a part of the 1-deoxy-D-xylulose 5-phosphate synthase gene (*dxs*) in *E. coli* was described previously [13]. In our study, we optimized and validated the *dxs* qPCR assay regarding annealing temperature, primer concentration, assay representativeness, and lower level of quantification (LLOQ). The two rapid methods were applied to detect planktonic and sessile clinical *E. coli* strains in a lab-scale drinking

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water reactor system during a period of washout in order to examine the survival and persistence of clinical *E. coli* strains in non-contaminated drinking water.

2. Materials and Methods

2.1. *E. coli* Strains and Growth Conditions

Four clinical *E. coli* strains were included in this study: *E. coli* ATCC 25922, a clinical isolate originated from Seattle, USA, 1946 (American Type Culture Collection; Rockville, MD, USA), *E. coli* UTI CAB, a clinical strain isolated from blood from a Danish female hospital patient with community-acquired bacteremia (CAB) and the urinary tract as the primary site of infection, Hjørring, Denmark, 2001 (Department of Clinical Microbiology, Aalborg Hospital, Aarhus University Hospital, Denmark), *E. coli* Gall CAB, a clinical strain isolated from blood from a Danish male hospital patient with CAB and the gallbladder as the primary site of infection, Hjørring, 2005 (Aalborg Hospital, Denmark), and *E. coli* O177:H-, a clinical strain classified as attaching and effacing *E. coli* (A/EEC) isolated from feces from a Danish hospital patient (Department of Clinical Microbiology, Skejby Hospital, Aarhus University Hospital, Denmark). In addition, *E. coli* strain ED1a was included for method validation (Institut national de la santé et de la recherche médicale, Paris, France) [14]. All *E. coli* strains were grown in Fluorocult® LMX Broth (LMX; Merck, Darmstadt, Germany) for 18 h at 37˚C on a shaking table.

2.2. Drinking Water

The drinking water used in this study consisted of municipal tap water collected in the cities of Aalborg and Aarhus, Denmark (**Table 1**).

Table 1. Distribution and most recent bacteriological drinking water analyses from two Danish waterworks (Anon 2010). At Danish waterworks, the threshold values of culturable total flora [CFU·ml^{-1}] at 22 and 37˚C are 50 and 5, respectively, and coliforms may not be detected in a 100 ml water sample.

Municipality	Aalborg	Aarhus
Waterworks	Engkilden	Truelsbjergvaerket
Distribution [m^3·year^{-1}]	1,300,000	2,300,000
Disinfection	No	No
Culturable microorganisms 22˚C [CFU·ml^{-1}]	1	1
Culturable microorganisms 37˚C [CFU·ml^{-1}]	<1	<1
Coliform bacteria [CFU·100·ml^{-1}]	<1	<1
E. coli [CFU·100·ml^{-1}]	<1	<1

The tap water originates from non-contaminated groundwater, and no disinfection is used before distribution. For the persistence experiments, water from Aalborg was used, whereas both water types were used for comparison of detection methods.

2.3. Enumeration of Culturable Microorganisms in Drinking Water

Enumeration of the total amount of culturable microorganisms in drinking water was performed according to DS 6222-1 [15]. In brief, colonies formed after incubation in a nutrient agar culture medium were determined after growth at 22˚C and 37˚C for 68 ± 4 and 44 ± 4 h, respectively.

Enumeration of culturable *E. coli* was based on a most probable number (MPN) method using 96-well MasterBlocks (Greiner Bio-One GmbH, Frickenhausen, Germany). Four to seven samples were serially diluted in Colilert-18 broth (IDEXX Laboratories, Inc., Westbrooke, ME, USA). One row was used as reference, and the MasterBlocks were incubated for 18 - 22 h at 37˚C. Subsequently, the number of yellow wells that fluoresced during illumination of the MasterBlocks with UV-light (365 nm) was considered positive. Based on these counts, MPN was calculated using the Bacteriological Analytical Manual-MPN spreadsheet based on Thomas's approximation [16].

2.4. ATP Bioluminescence Assay

The total microbial biomass was determined by measuring ATP bioluminescence using a R&D Biomass Test Kit (Promicol BV, Nuth, The Netherlands) in white 24-well microplates (CulturPlates; PerkinElmer, Inc., Waltham, MA, USA). Briefly, microbial ATP was released from all cells present in 0.5 ml of sample by adding 50 µl of lysis reagent. 50 µl of a reagent containing luciferin and luciferase was added. Luciferin was hydrolyzed by luciferase in the presence of oxygen and microbial ATP. The released bioluminescence was measured for 1 sec in a Victor X2 Multilabel Plate Reader (PerkinElmer, Inc.). Autoclaved ddH_2O was used as reference.

2.5. ColiLight II

ColiLight II is a chemiluminescent assay used for detection of viable *E. coli* [9]. An outline of the ColiLight II principle is given below (**Figure 1**). 3 - 5 ml of LMX broth supplemented with 0.2 mg·ml^{-1} of methyl-*β*-D-glucuronide (MetGlu; Sigma, St. Louis, MO, USA) to induce expression of *β*-D-glucuronidase were added to each sample [17], which were incubated for 6 h at 37˚C on a shaking table. Subsequently, the samples were centrifuged for 15 min at 5000 rpm. Pellets were dissolved in 0.5 ml of supernatant, transferred to white 24-well

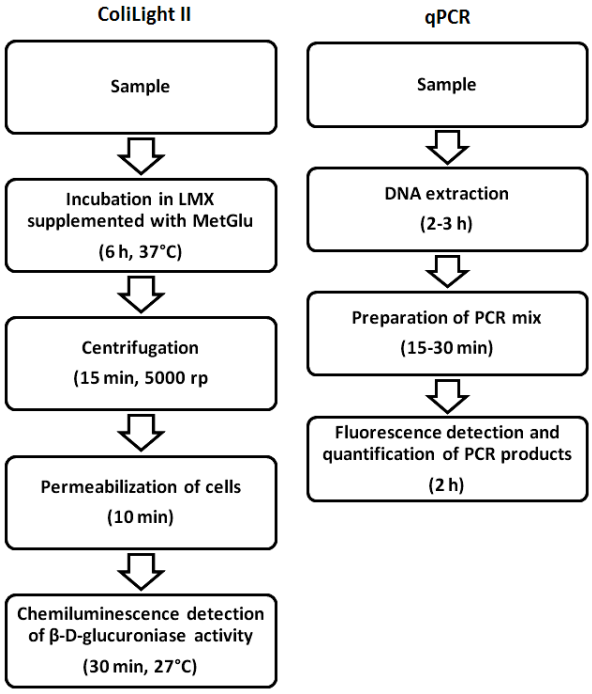


Figure 1. Outline of the rapid detection principles, ColiLight II and qPCR, for detection of *E. coli* in drinking water matrices.

CulturPlates, and incubated for 8 - 10 min at room temperature in the presence of 0.5 $mg \cdot ml^{-1}$ of protamine (from salmon; Sigma) to permeabilize cells [18]. Subsequently, 200 µl of s*β*-Glucor 102 (Michigan Diagnostics LLC, Royal Oak, MI, USA) diluted 100-fold in 0.1 $mol \cdot l^{-1}$ $NaPO_4$ buffer, pH 7.2, containing 10 $mmol \cdot l^{-1}$ EDTA and 15 $mmol \cdot l^{-1}$ glycine was added to each sample and incubated for 30 min at 27˚C. 400 µl of Triggering Reagent (Michigan Diagnostics LLC) was added to each sample to reduce quenching of chemiluminescence intensity. The plate was shaken for 10 sec, and the chemiluminescence was measured for 1 sec in a plate reader. Drinking water without coliforms was used as reference.

A standard curve was prepared using serial dilutions of *β*-D-glucuronidase purified from *E. coli* (Sigma) in 0.1 $mol \cdot l^{-1}$ $NaPO_4$ buffer, pH 7.2. S/N ratios were calculated [19], and the detection threshold (DT) was determined as $\left(\left(5SD + \text{arithmetic mean}\right)/\text{arithmetic mean}\right)$ using seven blanks [20].

2.6. qPCR

An outline of qPCR-based detection of *E. coli* based on the amplification of a part of *dxs* is given (**Figure 1**).

2.6.1. DNA Extraction

Pellet and filter samples were transferred to 2 ml bead tubes containing 0.1 mm glass beads (MOBIO Laboratories, Inc., Carlsbad, CA, USA). Drinking water without coliforms was used as extraction controls. 1 ml of DNA extraction buffer (50 $mmol \cdot l^{-1}$ NaCl, 50 $mmol \cdot l^{-1}$ Tris-HCl, 50 $mmol \cdot l^{-1}$ EDTA, 5% SDS, pH 8.0) was added to each sample together with 1 µl of 1 $mol \cdot l^{-1}$ of DTT. The samples were bead beaten for 5 min on a Vortex Genie 2 with adapter (MOBIO), and centrifuged for 1 min at 10,000 × g. Filters were removed, and DNA was extracted using half a volume of Phenol:Chloroform:Isoamyl alcohol 25:24:1, saturated with 10 $mmol \cdot l^{-1}$ Tris-HCl, pH 8.0, 1 $mmol \cdot l^{-1}$ EDTA (Sigma) followed by one volume of chloroform.

DNA was precipitated on ice for at least 1 h at –20˚C using 0.7 volume of isopropanol and a final concentration of 0.3 $mol \cdot l^{-1}$ sodium acetate in the presence of 1 µg of glycogen. The samples were centrifuged for 30 min, and DNA pellets were washed with 0.5 ml ice-cold 70% ethanol. After ethanol removal, DNA was resuspended in 25 µl of nuclease free water (Sigma). Templates were stored at –80˚C prior to use.

2.6.2. *dxs* Assay

A part of *dxs* was amplified using previously described primers [13]. The qPCR assay was optimized regarding concentration of Mg^{2+}, primer concentration, and annealing temperature. qPCRs were carried out in triplicates in an Mx3000P™ Quantitative PCR system (Stratagene, Agilent Technologies, CA, USA).

The 20 µl reaction mixtures contained 10 µl of Brilliant® II SYBR QPCR Low ROX Master Mix (Stratagene), 0.2 $\mu mol \cdot l^{-1}$ of the forward primer, 0.3 $\mu mol \cdot l^{-1}$ of the reverse primer, a final concentration of 3 $mmol \cdot l^{-1}$ of $MgCl_2$, and 4 µl of template. The PCR conditions were enzyme activation at 95˚C for 10 min followed by 35 cycles of denaturation at 95˚C for 30 sec and amplification/extension at 62˚C for 1 min (data collection). Subsequently, a melt curve analysis was done from 55˚C to 95˚C. A standard curve was prepared using serial dilutions of *E. coli* ED1a gDNA in nuclease free water (Sigma). The DNA stock concentration was based on quantification of cells using McFarland standards, viable microscopy counts using a counting chamber, and plate counts on Tryptone Bile X-glucuronide Agar (Merck), and on DNA concentrations measured on a UV-Vis nanodrop spectrophotometer ND-1000 (NanoDrop Technologies, Inc.; Wilmington, Delaware, USA). LLOQ was determined for the qPCR assay. Intraassay precision (expressed as coefficient of variation, CV) was calculated from standard deviations of genome copy numbers obtained from five replicates of nine different template concentrations run simultaneously. CV for interassay precision was calculated from standard deviations of genome copy numbers obtained from

five replicates of one template concentration at three different days.

2.7. Comparison of Detection Methods

Correlation analyses were performed in order to compare the outcome of the rapid detection methods ColiLight II and qPCR with more conventional MPN enumeration. The analyses were done using two different types of drinking water collected from Aalborg and Aarhus municipalities in Jutland, Denmark (**Table 1**). For each location, 100 ml of drinking water was transferred to sterile 250-ml BlueCap bottles and spiked with concentrations between ~10^2 and 10^7 CFU 100 ml^{-1} of *E. coli* ATCC 25922, *E. coli* UTI CAB, *E. coli* Gall CAB, and *E. coli* O177:H-. The samples were incubated for 24 h at 10˚C on a shaking table. Subsequently, 33.3 ml of each sample was filtrated through a 0.45 µm nylon membrane filter and 33.3 ml through a 0.45 µm mixed cellulose ester (MCE) membrane filter (47 mm; Frisenette, Knebel, Denmark). The nylon filter was used for qPCR analysis, and the MCE filter was used for ColiLight II analysis. In addition, 4 × 1 ml of each water sample was used for MPN analysis.

2.8. Persistence of *E. coli* in a Flow-Through Drinking Water Reactor System

Persistence and washout of *E. coli* ATCC 25922, *E. coli* UTI CAB, *E. coli* Gall CAB, and *E. coli* O177:H- was studied in a lab-scale drinking water reactor system (**Figure 2**). The system was comprised of a water reservoir (5 - l BlueCap bottle) connected to a CDC Biofilm reactor (Biosurface Technologies corp., Bozeman, MT, USA) via a peristaltic pump (U4-8R Midi 2.5 - 50 rpm; Alitea AB, Stockholm, Sweden) with PVC tubes (116-0549-19; MikroLab Aarhus A/S, Aarhus, Denmark). A PVC pump tube (Yellow-Blue, 1.52 mm; SEAL Analytical Ltd., Hampshire, UK) was used to ensure a uniform water flow of 0.4 $ml \cdot min^{-1}$. The water was pumped from the reservoir to the reactor with a total water volume of 350 ml resulting in a dilution rate, D, of 1.646 d^{-1}.

The rector was fitted with PE and SS coupons to allow studies of cell adherence to different surface materials. The bioreactor was comprised of glass, and the PE and SS coupons were inserted into coupon holders made of polypropylene (PP). The total surface area of the inner surfaces below the water surface during each experiment was 388.6 cm^2 of which 196 cm^2 was constituted by glass, 142 cm^2 by PP, 25.3 cm^2 by PE, and 25.3 cm^2 by SS. From the reactor, the water flowed through to a waste bottle. Seven removable PP coupon holders each containing three removable coupons were inserted in the reactor. Three holders contained three PE coupons, three

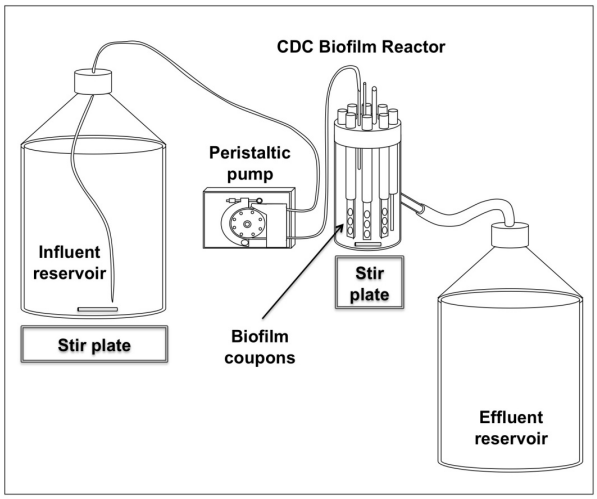


Figure 2. Experimental set-up of a flow-through drinking water reactor system used in the persistence experiments.

holders contained three SS coupons, and one holder contained one PE and one SS coupon. The coupons were below the water surface throughout the experiment. The reservoir and the reactor were placed on magnetic stirrers. Four experiments were performed, one for each *E. coli* strain. Prior to each experiment, clean drinking water was run through the system for 72 h. Subsequently, the reservoir and the reactor were inoculated with ~10^4 ml^{-1} *E. coli* cells. The reactor system was exposed to the *E. coli* cells for 24 h, followed by nine days of washout where clean drinking water without *E. coli* was run through the system. All experiments were conducted in a darkroom at 10˚C. All reactor parts and tubes were disinfected with 70% of ethanol between experiments.

Sampling of water and coupons were performed prior to *E. coli* inoculation, 24 h after *E. coli* inoculation at the time where washout was initiated (day 0), and after 3 and 9 days of washout (day 3 and day 9, respectively). Additional sampling of the water phase was also carried out after 6 days of washout (day 6). A total volume of 10 ml of water was collected at each sampling. Using a glass pipe, increments of 2 ml of water representing the vertical dimension of the water column were drawn from the reactor and pooled. One holder with three PE coupons and one holder with three SS coupons were also drawn from the reactor. Each of the three coupons from one holder were each aseptically placed in 3.33 ml of buffered peptone water in sterile 50 ml Greiner tubes, and to release attached cells from the coupons, the samples were vortexed for 10 sec followed by sonication for 10 sec (×3). The coupons were removed, and the eluates from the same coupon material were pooled. 7 × 0.2 ml of water and coupon eluates were used for MPN determinations, 3 × 0.5 ml were used for both ATP bioluminescence, 3 × 0.5 ml were used for ColiLight II analyses,

and pellets from 3 × 0.8 ml of each sample were used for DNA extraction followed by qPCR.

2.9. Data Analysis

Sample coefficients of determination, R^2, and Pearson's product moment correlation coefficients, r_P, were calculated to examine the linearity between datasets. QQ-plots were made to confirm that the data were normally distributed, and removal rates were compared using one-way ANOVA tests at 95% confidence level using Bonferroni corrections. SPSS Statistics 17.0 was used for statistical analyses (SPSS Inc, Chicago, IL, USA). Removal rates were determined as the slope of the regression line

$$\ln(C/C_0) = kt \quad (1)$$

where C is the bacteria concentration at time t, C_0 the initial concentration on day 0, and k the removal rate in d^{-1}.

3. Results

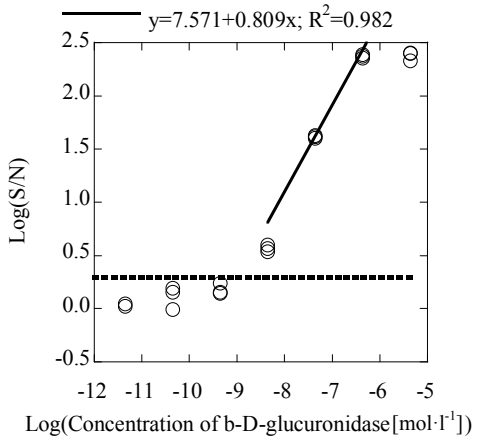
3.1. Detection of *E. coli* Using ColiLight II and qPCR

The suggested rapid detection methods showed good linear responses (**Figure 3**).

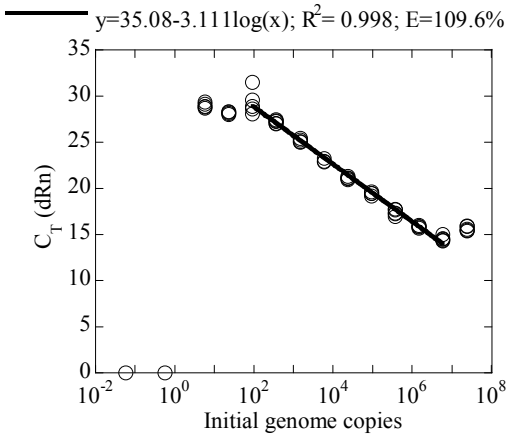
For ColiLight II, a standard curve was prepared using known concentrations of purified *β*-D-glucuronidase from *E. coli* (**Figure 3(a)**). A positive linear correlation between log transformed enzyme concentrations and log transformed S/N ratios was observed in a ~3-log interval from ca. 10^{-9} to ca. 10^{-6} mol·l^{-1} of *β*-D-glucuronidase (R^2 = 0.982). For qPCR, a standard curve using known concentrations of *E. coli* ED1a showed a positive log linear correlation of *E. coli* genome copy number and PCR threshold cycle number over a ~6-log interval from ~2 × 10^1 to ~6 × 10^6 genome copies (**Figure 3(b)**), hence LLOQ was determined to ~23 genome copies. R^2 was 0.998, and the PCR efficiency, E, was 109.6%. CV for repeatability was found to be 13.9%, and 23.2% for reproducibility. R^2 and E for all runs ranged from 0.997 to 0.998 and 92.4% to 109.6%, respectively, which was considered satisfactory [21].

3.2. Correlation between MPN and Rapid Methods

In order to examine whether ColiLight II and qPCR can be used as alternatives or supplements to traditional cultivation dependent methods, linear regression analyses were performed. All three methods were applied to detect *E. coli* ATCC 25922, *E. coli* UTI CAB, *E. coli* Gall CAB, and *E. coli* O177:H- after incubation for 24 h at 10˚C in two different types of drinking water (**Table 1**). The total



(a)



(b)

Figure 3. Standard curves for *E. coli* based on (a) ColiLight II by emission of chemiluminescence where the dashed line indicates the detection threshold (n = 3); and (b) qPCR by on amplification of a part of *dxs* (n = 5).

flora measured as culturable microorganisms in both water types were below 200 CFU ml^{-1} at 22˚C and below 50 CFU ml^{-1} at 37˚C.

Linear regression analyses between the MPN enumeration and ColiLight II and MPN and qPCR in two different water types showed positive linear relationships (**Table 2**).

From the coefficient R^2, we see that most of the variation (71% - 98%) was described for each isolate and each drinking water type using each of the two rapid methods in comparison with the MPN method.

However, when the results from each strain were compiled, the explained variance decreased (65% - 82%) indicating that the biological variation had a higher impact on the results than both the applied method and the type of drinking water used, especially for the ColiLight II method based on metabolic activity. From the correlation coefficients, we see a strong linear relationship ($r_P \geq$ 0.74) in the results obtained from MPN and ColiLight II

Table 2. Sample coefficients of determination, R^2, and Pearson's product moment correlation coefficients, r_P, between results obtained by MPN and ColiLight II and by MPN and qPCR for two drinking water types using four different strains of *E. coli*.

E. coli	ColiLight II				qPCR			
	Aalborg		Aarhus		Aalborg		Aarhus	
	R^2	r_P	R^2	r_P	R^2	r_P	R^2	r_P
ATCC 25922	0.82	0.91	0.88	0.96	0.87	0.93	-	-
UTI CAB	0.93	0.95	0.74	0.90	0.94	0.97	0.91	0.95
Gall CAB	0.71	0.78	0.92	0.89	0.93	0.96	0.72	0.85
O177: H-	0.75	0.87	0.92	0.74	0.98	0.99	0.95	0.97
All strains	0.65	0.81	0.82	0.89	0.71	0.85	0.69	0.83

and from MPN and qPCR.

The degree of correlation between MPN and ColiLight II and between MPN and qPCR are illustrated for drinking water from both Aalborg and Aarhus municipalities (**Figures 4(a)** and **(b)**, respectively). Here we see that a positive, linear relationship between MPN and the two rapid methods also exists when results from both types of drinking water are used. The correlation coefficients for MPN and ColiLight II and MPN and qPCR were 0.72 and 0.82, respectively, indicating a strong relationship between the outcomes of the two methods. The explained variations were below 70% again indicating the importance of biological variation.

3.3. Persistence of *E. coli* in a Flow-Through Drinking Water Reactor System

Persistence of *E. coli* ATCC 25922, *E. coli* UTI CAB, *E. coli* Gall CAB, and *E. coli* O177:H- in a flow-through drinking water reactor system was examined over a time period of nine days. The system was initially operated for 72 h with clean drinking water to allow initiation of a conditioning film on the inner surfaces, and then inoculated with a specific *E. coli* strain for 24 h in a flow-through mode. Subsequently, the system was run with clean drinking water to facilitate *E. coli* washout. Changes in the total microbial flora in water and biofilms were followed using an ATP assay (**Table 3**).

At day 0, accumulation of biomass was detected on the PE and SS surfaces. After 9 days of washout, biomass on both surface types had increased in terms of higher concentration of ATP compared to day 0 whereas the ATP concentration in the water phase had decreased.

3.3.1. *E. coli* in Biofilm on PE and SS Surfaces

During the 24 h of contamination, all four *E. coli* strains did accumulate on PE and SS surfaces within the biofilm reactor (**Figure 5**). At day 0, higher levels of *E. coli* were

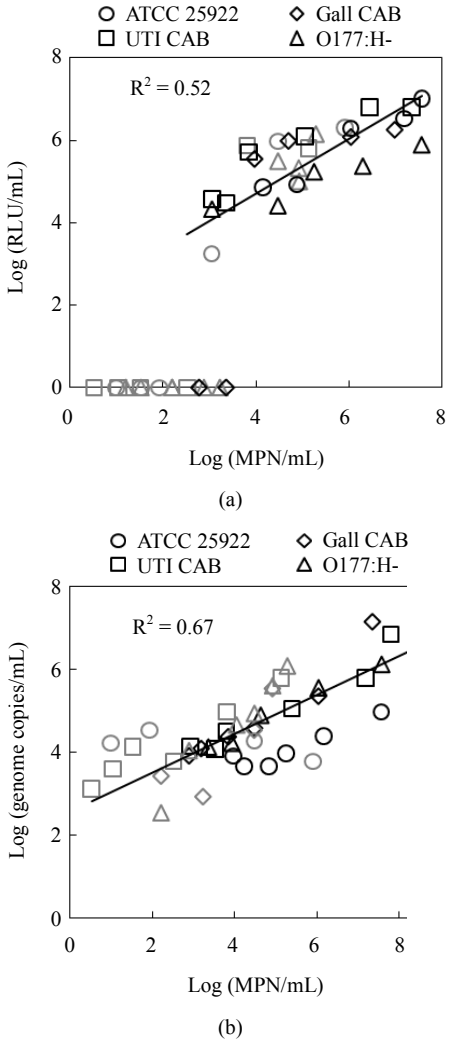


Figure 4. Correlation curves between (a) MPN and ColiLight II and between (b) MPN and qPCR for two water types. Black: Aalborg water. Grey: Aarhus water.

Table 3. Biofilm formation on PE and SS surfaces measured as ATP bioluminescence at day 0 and day 9 in three lab-scale drinking water reactor system experiments (mean RLU ± SE; n = 3).

Sample	Day 0	Day 9
Water [RLU·ml^{-1}]	822 ± 513	191 ± 64
PE coupons [RLU·cm^{-2}]	3404 ± 1288	10,917 ± 2828
SS coupons [RLU·cm^{-2}]	2089 ± 769	5842 ± 1063

detected on PE surfaces compared to SS surfaces using both the MPN method and qPCR where the latter method detected ca. 10 fold higher levels compared to MPN. The levels of total *E. coli* detected on PE and SS surfaces by qPCR were similar during the period of washout whereas a decrease in culturable *E. coli* was observed by MPN. 1.45% of the initial concentration of culturable *E. coli*

ATCC 25922 in the water phase (MPN·ml^{-1}) had accumulated in the indigenous biofilm on the PE surface (MPN·cm^{-2}) at day 0, and for the strains UTI CAB, Gall CAB, and O177:H-, these numbers were 1.79%, 0.13%, and 1.04%, respectively (**Figure 5(a)**). Less *E. coli* cells attached to the SS surface compared to the PE surface (**Figures 5(a)** and **(c)**).

0.4% of the initial concentration of culturable *E. coli* ATCC 25922 in the water phase (MPN·ml^{-1}) was found on the SS surface (MPN·cm^{-2}) at day 0, and for UTI CAB, Gall CAB, and O177:H- these numbers were 0.3%, 0.03%, and 0.45%, respectively (**Figure 5(c)**).

On SS and PE surfaces, the number of culturable *E. coli* ATCC 25922 decreased 10 to 20-folds and UTI CAB decreased 10 to 40-folds during the first three days of washout. The number of culturable O177:H- did not change significantly on either PE or SS surfaces. On PE, the number of culturable Gall CAB increased 4-fold in the same period whereas a 200-fold decrease was observed on SS. After 9 days of washout, low numbers of all four *E. coli* strains could be detected on the PE surfaces using MPN whereas only *E. coli* UTI CAB and O177:H- could be detected on SS.

β-D-Glucuronidase activity could be detected in all strains at day 0 on both PE and SS surfaces. After three days of washout, 16% of the initial enzyme activity was detected in Gall CAB on PE and below 0.3% in UTI CAB and O177:H-. On SS, only *E. coli* Gall CAB could be detected at day 3. At day 9, no *β*-D-glucuronidase activity could be detected in any of the four strains on either PE or SS surfaces. In general, the measured activity was 2 to 8 fold higher on the PE surfaces compared to the SS surfaces. The lowest *β*-D-glucuronidase activity was detected in *E. coli* ATCC 25922, and the highest

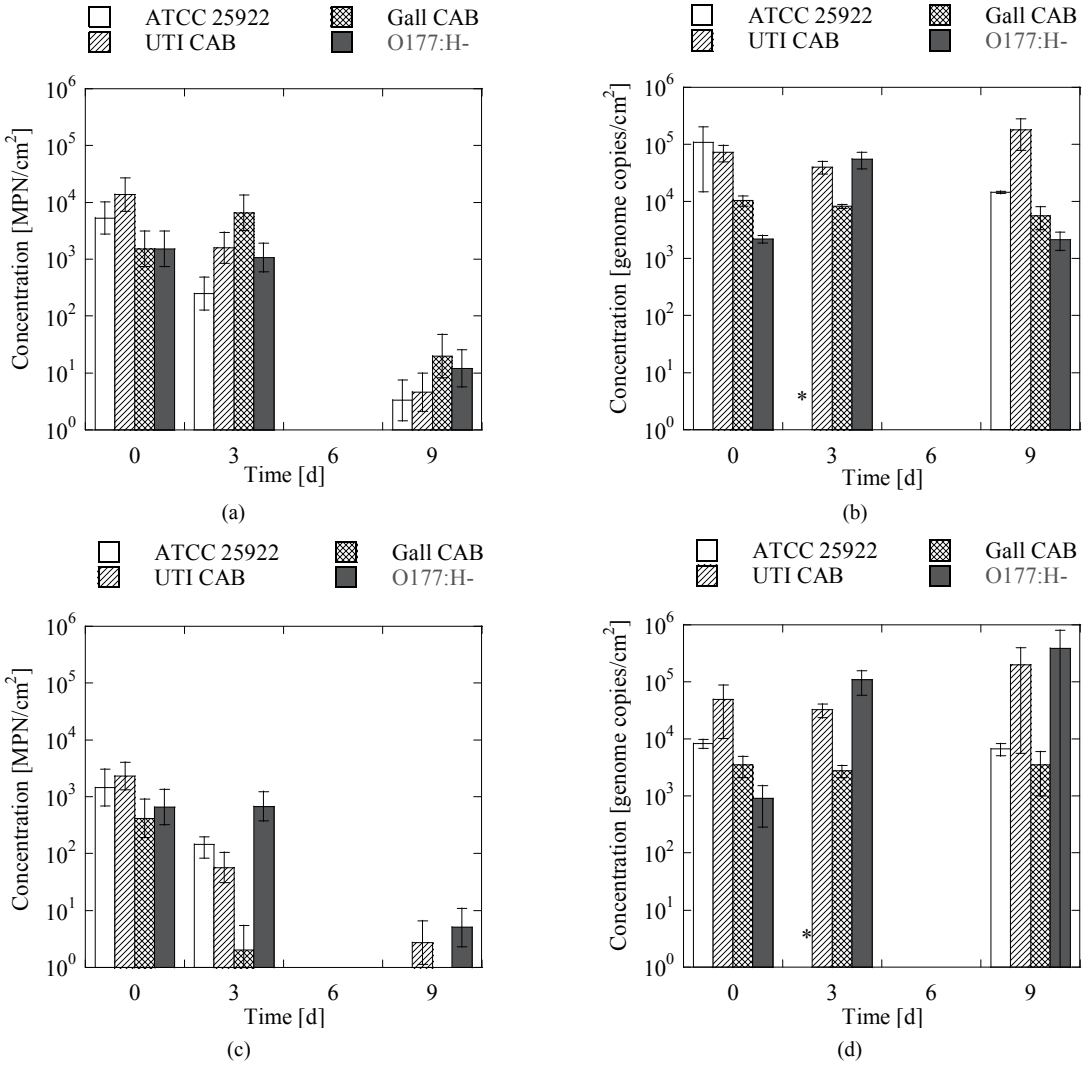


Figure 5. Concentration of *E. coli* in biofilms on PE ((a) and (b)) and SS ((c) and (d)) coupons in a flow-through drinking water reactor system during washout. Concentration of ((a) and (c)) culturable *E. coli* (MPN·cm^{-2} ± 95% CI; n = 7) and ((b) and (d)) *E. coli* measured by qPCR (genome·copies·cm^{-2} ± SE; n = 3). *: not sampled.

activity was detected in *E. coli* O177:H-.

The total amount of *E. coli* quantified by qPCR did not change significantly on the PE and SS surfaces during the washout procedure except for a 25-fold and a 100-fold increase, respectively, in the number of O177:H- at day 3, and a 4-fold increase in the number of UTI CAB at day 9 compared to day 0. At day 3 and day 9, qPCR for *E. coli* Gall CAB and *E. coli* O177:H- (only day 9) was conducted both with and without a 4-h enrichment step in a 5:1 solution of mineral modified glutamate agar and brain heart infusion broth at 37˚C on a shaking table. This was done in an attempt to examine whether cells detected by qPCR could still multiply or existed in a dead or non-culturable state. At day 3, we saw a 1951 ± 1259 (mean ± SE; n = 3) fold increase in the total number of *E. coli* Gall CAB in water, a 381 ± 42 fold increase on PE surfaces, and a 504 ± 47 fold increase on SS surfaces. At day 9, the 4-h enrichment did not result in any increase in amplifiable DNA for either *E. coli* Gall CAB or *E. coli* O177:H-. Hence, the cells were either more stressed and required a longer enrichment step or the cells were non-culturable or had died.

3.3.2. Concentration of *E. coli* in the Water Phase

The concentration of culturable *E. coli* in the water phase of the flow-through drinking water reaction system decreased exponentially over time as a consequence of washout, dead, and adhering to surfaces (**Figure 6(a)**). At day 9, 0.0021% and 0.0069% of the initial concentrations of the strains Gall CAB and O177:H-, respectively, could be detected.

β-D-Glucuronidase activity of planktonic *E. coli* in the water could be detected in all strains at day 0. After 3 days, 0.6% of the initial activity at day 0 could be detected in Gall CAB. No activity was detected in the other strains. The qPCR results showed that the amount of total planktonic *E. coli* was rather constant over the 9 days (**Figure 6(b)**). However, the levels were lower in the water phase compared to on PE and SS surfaces.

3.3.3. Removal and Washout of *E. coli* from the Flow-Through Drinking Water Reaction System

To compare the removal of *E. coli* from the water phase and the surfaces, removal rates, k, was calculated (**Table 4**). The theoretical washout was the dilution rate, D, based on the flow rate and reactor volume. Culturable *E. coli* was shown to adhere to biofilm on PE surfaces and this interaction had an effect on the washout procedure as removal of adhered cells were slower (k ranged from –0.482 to –0.888) compared to the theoretical removal (k = –1.646). On SS, the strains UTI CAB and O177:H- had slower removal rates (k = –0.749 and k = –0.541, respectively) compared to ATCC 25922 and Gall CAB (k = –1.576 and k = –1.438, respectively). *E. coli* O177:H-

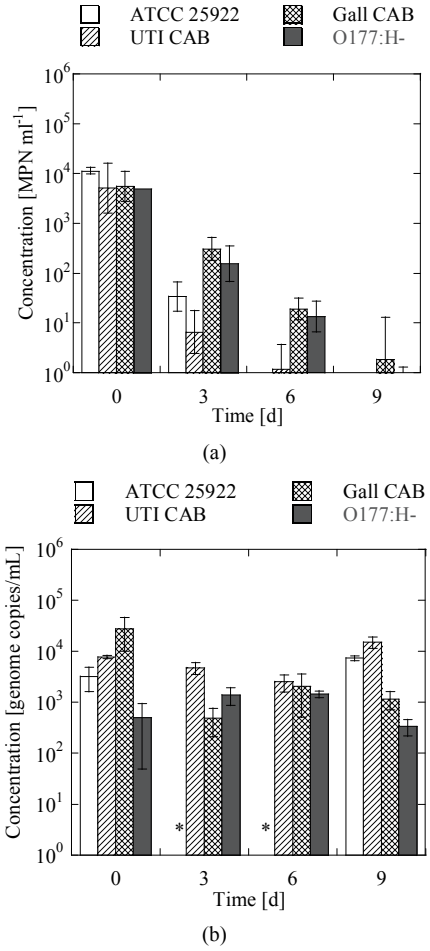


Figure 6. Concentration of *E. coli* in the water phase in a flow-through drinking water reactor system during washout. Concentration of a) culturable *E. coli* (MPN·ml^{-1} ± 95% CI; n = 7) and b) *E. coli* measured by qPCR (genome·copies ml^{-1} ± SE; n = 3). *: not sampled.

Table 4. Removal rates for decrease in *E. coli* culturable cell numbers. R^2 was >0.7 for all rates.

	Water	PE	SS
	[(MPN·ml^{-1}) d^{-1}]	[(MPN·cm^{-2}) d^{-1}]	[(MPN·cm^{-2}) d^{-1}]
Theoretical washout	–1.646		
ATCC 25922	–1.372*	–0.805	–1.171*
UTI CAB	–1.212*	–0.901	–0.714
Gall CAB	–0.892	–0.551	–0.950*
O177:H-	–0.962	–0.568	–0.581

*No cultivable *E. coli* was detected at day 9; hence MPN was determined as <0.05 MPN ml^{-1} which is just below the minimum detection limit of *E. coli* in Colilert18 [22].

were capable of adhering to and persist in biofilms for up to nine days on both SS and PE surfaces.

In the water phase, the removal rates of the two clinical strains *E. coli* Gall CAB and O177:H- (k = –0.888

and k = –0.979, respectively) were slower than the removal rates of *E. coli* UTI CAB and ATCC 25922 (k = –1.393 and k = –1.662, respectively) which were similar to the theoretical washout rate. In both water and biofilm, the clinical *E. coli* strain O177:H- persisted three times longer than the culture collection strain *E. coli* ATCC 25922.

4. Discussion

The results from this study showed that the two rapid methods ColiLight II and qPCR could be applied to detect *E. coli* in drinking water and to evaluate the survival and persistence of *E. coli* in drinking water systems. We found that clinical *E. coli* strains were able to persist in a flow-through lab-scale drinking water reactor system for at least nine days.

In the lab-scale reactor system, the relative amount of accumulated biofilm on the PE and SS surfaces determined by measuring the ATP content was not significantly different from each other during the time period. This corresponds to the findings of Yu *et al*. who studied biofilm formation on different water distribution pipe materials in disinfected tap water [23]. Yu *et al*. found similar biomass levels on PE and SS surfaces after 90 days of incubation in drinking water.

A relatively low metabolic activity by means of *β*-D-glucuronidase activity was found on both PE and SS surfaces and in the water phase after 3 days. This can be explained by the fact that the concentration of culturable and active *E. coli* cells present on the surfaces and in the water phase at day 3 were ca. or below that of the analytical limit of detection (10^2 - 10^3 cells) of the ColiLight II method [9].

From the qPCR results we see, that the concentration of nucleic acids was rather stabile over time both in the water phase and on surfaces. This indicates that *E. coli* was released from surfaces, mainly glass and PP surfaces since the levels on PE and SS were rather constant, over time to the water phase, and that the released cells were dead, had entered a VBNC state, or a combination of both. Furthermore, *E. coli* was not released from SS and PE surfaces to an extensive degree, but had entered a VBNC state or had died. These persistent levels of *E. coli* DNA have also been found in other studies. Cell integrity and respiratory activity together with loss of culturability over time was found in a study by Cook and Bolster, who tested the survival of *Camp. jejuni* and *E. coli* in groundwater microcosms during starvation at 4˚C [10]. Lothigius *et al*., who examined the survival and gene expression of enterotoxigenic *E. coli* in sea water and freshwater, also found high degree of cell integrity for up to 12 weeks in both water types together with expression of genes involved in metabolic pathways and genes encoding enterotoxins [12]. In our study, however, the cellular integrity did not correlate with the metabolic activity by means of *β*-D-glucuronidase activity as no activity could be detected at day 9 where the DNA levels were high. However, this may be explained by a very low activity in the cells in combination with the sensitivity of the ColiLight II method.

Our results indicated the existence of strain-differentiated removal of *E. coli* from both water and surfaces in a lab-scale drinking water reactor system due to incorporation into biofilms. The initial concentrations of *E. coli* did not vary significantly between experiments and cannot explain the differences in the measured concentrations over time. These differences indicated a differentiated washout where the strains Gall CAB and O177:H- could persist longer in the system compared to ATCC 25922 and UTI CAB. Especially the strain O177:H- showed high persistence in the system. It is known that curli production by non-pathogenic and pathogenic *E. coli* enhances the attachment of cells to SS surfaces [24] and glass surfaces [25,26]. As an A/EEC strain, *E. coli* O177:H- used in this study are likely to be able to produce curli which increases the ability of the strain to form biofilm [26].

Our findings point towards differentiated strain-specific survival of *E. coli* in drinking water matrices. The survival of different *E. coli* strains has been examined in other types of freshwaters such as river water [27] and well water [28], and both studies suggest strain differential survival of *E. coli*.

The ColiLight II method measures *β*-D-glucuronidase activity in viable cells with a greater sensitivity than methods based on fluorescent and chromogenic substrates [9]. This method can be applied to detect metabolic active *E. coli* in drinking water when present in adequate concentrations. Using qPCR, the total amount of extracted nucleic acids in a sample is measured with a high specificity and sensitivity.

Overall, the two rapid detection methods, qPCR and ColiLight II, are suitable for detection of *E. coli* in drinking water and biofilms. Both methods can complement standard cultivation-based methods for detection of *E. coli* in drinking water and can provide results within one work day.

Clinical *E. coli* strains persisted longer in drinking water than a culture collection strain, and strain specific attributes can significantly affect detection and persistence of *E. coli* in drinking water matrices.

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