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Autonomous online measurement of β-D-glucuronidase activity in surface water: is it suitable for rapid *E. coli* monitoring?

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ABSTRACT

Microbiological water quality is traditionally assessed using culture-based enumeration of faecal indicator bacteria such as *Escherichia coli*. Despite their relative ease of use, these methods require a minimal 18-24 h-incubation step before the results are obtained. This study aimed to assess the suitability of an autonomous online fluorescence-based technology measuring β-glucuronidase (GLUC) activity for rapid near-real time monitoring of *E. coli* in water. The analytical precision was determined and compared to an automated microbial detection system, two culture-based assays and quantitative real-time PCR (qPCR). Using replicate measurements of grab samples containing *E. coli* concentrations between 50 and 2330 CFU.100 mL^{-1}, the autonomous GLUC activity measurement technology displayed an average coefficient of variation (CV) of less than 5% that was 4–8-fold lower than other methods tested. Comparable precision was observed during online *in situ* monitoring of GLUC activity at a drinking water intake using three independent instruments. GLUC activity measurements were not affected by sewage or sediments at concentrations likely to be encountered during long-term monitoring. Furthermore, significant ($p < 0.05$) correlations were obtained between GLUC activity and the other assays including defined substrate technology ($r = 0.77$), membrane filtration ($r = 0.73$), qPCR ($r = 0.55$) and the automated microbial detection system ($r = 0.50$). This study is the first to thoroughly compare the analytical performance of rapid automated detection technologies to established culture and molecular-based methods. Results show that further research is required to correlate GLUC activity to the presence of viable *E. coli* as measured in terms of CFU.100 mL^{-1}. This would allow the use of autonomous online GLUC activity measurements for rapid *E. coli* monitoring in water supplies used for drinking water production and recreation.

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1. Introduction

Autonomous devices are urgently needed for rapid (near real-time) quantification of faecal microorganisms across the drinking water supply chain and in recreational waters. Noble and Weisberg (2005) considered that such rapid assays should provide results within 4 h of time to enable same-day decision for recreational water safety. Given the high cost and complexity of direct pathogen monitoring, faecal indicator bacteria (FIB) such as *Escherichia coli* or Enterococci are used to routinely monitor microbiological water quality worldwide (EC, 2006; USEPA, 2012a; WHO, 2016). Faecal indicator bacteria are traditionally enumerated using culture-based methods involving an 18–24-h incubation period in liquid or solid culture media. This is a considerable limitation given that microbiological water quality can fluctuate over short (daily or even hourly) time intervals (Boehm, 2007; Ekklesia et al., 2015; Enns et al., 2012). Intermittent short-term contamination peaks can

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occur following rainfall episodes, combined sewer overflows or accidental spills (Madoux-Humery et al., 2016, 2013; Passerat et al., 2011; Traister and Anisfeld, 2006), resulting in potential exposures to faecal pathogens.

To address this limitation, alternative rapid and user-friendly assays were developed during the past two decades. Molecular assays such as real-time PCR (qPCR) that quantifies *E. coli* DNA (ex. Chern et al., 2011; Frahm and Obst, 2003) were shown to hold promise for rapid enumeration of *E. coli* in wastewater and surface water (Lavender and Kinzelman, 2009; Noble et al., 2010). Nevertheless, the need for standardization and specialized laboratory equipment limits the generalized implementation of qPCR for rapid microbial water quality testing.

Enzymatic methods have also been proposed for rapid assays without cultivation step (Farnleitner et al., 2001; Fiksdal et al., 1994; Garcia-Armisen et al., 2005; George et al., 2000). Considering the high specificity of the β-D-glucuronidase (GLUC) enzyme to *E. coli*, these assays consist in direct measurement of GLUC activity using the 4-methylumbelliferyl-β-D-glucuronide (MUG) substrate by defining the enzymatic activity as the rate of production of fluorescent methylumbelliferone (MUF) that results from MUG hydrolysis. Several studies conducted in freshwater and marine water showed good correlations between GLUC activity and culture-based enumeration of *E. coli* (Farnleitner et al., 2001; Garcia-Armisen et al., 2005; Lebaron et al., 2005). The direct enzymatic technique is based on the same enzymatic reaction as USEPA-approved culture-based methods already in use for over 2 decades and for which epidemiological data has been gathered (USEPA, 2012a; Wade et al., 2003).

In recent years, efforts were made to automate the detection of the fluorescence generated by the enzymatic reaction in combination with a culture step (Angelescu et al., 2018; Briciu-Burghina et al., 2015; Brown et al., 2013; Heery et al., 2016; Tryland et al., 2015) or not (Koschelnik et al., 2015; Ryzinska-Paier et al., 2014). Some of these enzyme-based technologies can be organised in remote and/or low resource settings, either for grab sample testing (Wildeboer et al., 2010; Wu et al., 2018) or for autonomous online monitoring of *E. coli* at high temporal frequencies (<1 h) (Ender et al., 2017; Stadler et al., 2016). Although they offer promising perspectives towards near real-time monitoring of faecal pollution, there is a lack of information on their precision and robustness during normal operation. Also, thorough comparison of the results from these technologies to established *E. coli* enumeration assays is limited. As any microbiological method including enzymatic assays displays inherent analytical uncertainty and can be impacted by matrix-related interferences (Servais et al., 2005; Togo et al., 2006), defining the application and use of autonomous measurement of GLUC activity requires a thorough understanding of its technical performance in comparison to widely used (standard) methods.

The objective of the present study was to assess the suitability of an autonomous online GLUC activity measurement technology for rapid online monitoring of *E. coli* concentrations in water. The analytical precision of the online technology was first determined and compared to an array of other microbiological assays including an automated microbial detection system, two culture-based assays (membrane filtration and defined substrate technology) and real-time PCR (qPCR). Using samples collected during various hydro-meteorological conditions, the relationships between GLUC activity and *E. coli* concentrations were then assessed. Finally, considering the potential of the autonomous online technology to achieve long-term monitoring of GLUC activity in surface water, its measurement performance was challenged against a variety of surface water samples to identify potential matrix-related interferences. Implications for rapid autonomous online monitoring of microbiological water quality are discussed.

2. Material and methods

2.1. Study site and sample collection

Raw water samples were collected in autoclaved bottles from a drinking water treatment plant (DWTP) intake serving almost 100,000 people in the Greater Montreal Area, Quebec, Canada. For the multi-method comparison between GLUC activity and *E. coli*, samples were collected from November 2016 to July 2017, encompassing a range of hydro-meteorological conditions. Samples were refrigerated until further processing in the laboratory within 24 h after collection. During the same period, intensive event sampling was performed on three occasions following snowmelt and/or rainfall episodes using an ISCO6712 auto-sampler (Teledyne ISCO, Lincoln, NE, USA). Additional samples were collected routinely (Monday to Thursday) and during event sampling at the intake by the DWTP staff from January 2017 to April 2018 and they were used for comparisons between online GLUC activity measurements and a membrane filtration method (see Section 2 for method description).

To evaluate the measurement performance of the autonomous online technology under various controlled turbidity conditions, untreated sewage as well as treated effluent samples were collected from a nearby water resources recovery facility (WRRF) discharging treated effluents from >40,000 people. The treatment includes degritting, settling, biofiltration and UV disinfection. River sediments were sampled with a sediment collector as previously described (Yarahmadi et al., 2018) and immediately brought back to the laboratory for further processing. WRRF and sediment samples were refrigerated at 4 °C until used in matrix spikes within 6 h of collection.

2.2. Microbiological analyses

2.2.1. Autonomous online measurement of GLUC activity

The autonomous ColiMinder® (VWM Solutions GmbH, Austria) technology (Table 1) measures the β-D-glucuronidase (GLUC) activity based on the optical detection of highly fluorescent 4-methylumbelliferone (MUF). The latter is formed upon breakdown of 4-methlyumbelliferyl-β-D-glucuronide (MUG) that is catalysed by the GLUC enzyme. For each 6.5 mL sample pumped into the measurement chamber, the instrument injects pre-defined volumes of fluorogenic substrate solution and buffer that are mixed to the water sample. Incubation temperature of the chamber is set at 44 ± 0.1 °C to maximise GLUC activity and subsequent emission of fluorescence (George et al., 2000). Sampling, addition of reagents and heating phases are fully automated, and the measurement of fluorescence (excitation and emission wavelengths of 365 and 455 nm, respectively) starts automatically when the incubation temperature is stable (after ~8 min). As the fluorescence signal increases with enzymatic activity over time, high-resolution analysis of the emitted fluorescence is continuously performed in the flow-through photometric measurement chamber (patent: PCT/AT2011/000497) during the measurement phase (approximately 6 min). The rate of fluorescence emission is expressed in volts per second and converted into Modified Fishman Units per 100 mL (MFU.100 mL^{-1}). Following the standard Sigma Quality Control Test Procedure, one MFU of GLUC activity from *E. coli* generates 1.0 μg of phenolphthalein from phenolphthalein-glucuronide per hour at pH 6.8 and at a temperature of 37 °C (Sigma Aldrich, 1998). The device is equipped with a data correction algorithm (patent: PCT/AT2014/050036) to account for the influence of sample turbidity on the fluorescence intensity. Upon completion of the measurement phase, the sample is automatically discarded into a waste container and the measurement chamber and tubes are cleaned with a

Table 1
Microbiological methods used in this study and their analytical characteristics. *if target (DNA or GLUC) remains detectable; **reproducible signal exceeding background noise as determined by the manufacturer; CFUeq.r^{-1}, colony-forming unit equivalents per PCR reaction.

Method	Type	Target	Limit of quantification	Description
Autonomous GLUC activity measurement technology	Enzymatic	Culturable, VBNC, dead* *E. coli*, free enzyme	0.8 mMFU.100 mL^{-1}**	Fully automated, no culture step needed
Automated microbial detection system	Culture	Culturable *E. coli*	1 CFU.100 mL^{-1}	Shortened culture step, no dilution needed
Membrane filtration (MF)	Culture	Culturable *E. coli*	1 CFU.100 mL^{-1}	Standardized (USEPA method 1604)
Defined substrate technology (DST)	Culture	Culturable *E. coli*	1 MPN.100 mL^{-1}	Most probable number format
qPCR	Molecular	Culturable, VBNC, dead* *E. coli*	5 CFUeq.r^{-1}	No culture step needed

washing solution composed of CMQuick Clean I (VWM Solutions GmbH, Austria) and 2.5% hydrochloric acid. When the device collects a new sample, part of it is used to flush the internal tubing system and remove any remaining washing solution. The analysis phase (sampling, heating and measurement steps) only takes 15 min and the entire cycle including the cleaning step takes about 30 min. Further information on the incubation time and pH adjustment as well as on calibration and quality control is provided by Koschelnik et al. (2015) and in the supplementary material.

Data from laboratory and field measurements were continuously recorded by the device, transmitted via a wireless modem and accessed remotely through a secured Internet connection.

2.2.2. Automated microbial detection system

The automated TECTA™ B16 technology (ENDETEC, Canada) is a fibre-optic coupled fluorescence multi-wavelength sensor that shortens the culture time of *E. coli* through a patented technology involving a fibre-optic probe and a miniature spectrometer (Brown et al., 2013). Measurement cartridges contain proprietary growth media with metabolic substrates for the specific detection of *E. coli* and total coliforms through their glucuronidase and galactosidase activities, respectively. Cartridges are incubated at 35 °C into a benchtop instrument and in presence of *E. coli*, a hydrophobic fluorescent metabolite is released from GLUC-mediated breakdown of the substrate (a glucuronic acid conjugate) and detected by the instrument. The fluorescence is continuously monitored by an optical fibre coupled to the instrument miniature spectrometer and any background turbidity and matrix effects are reduced through absorption of the fluorescent metabolite to a siloxane polymer bead integrated at the bottom of each cartridge (Brown et al., 2013). The software converts the time at which a given fluorescence intensity is detected (time-to-detection, TTD) into a concentration of bacteria (CFU.100 mL^{-1}) using a default calibration curve provided by the manufacturer and based on water samples from Lake Ontario.

The kinetics approach has the advantage to use a wider dynamic range (six orders of magnitude) than conventional most probable number or membrane filtration methods without need for sample dilution. The manufacturer recommends performing a custom calibration if overall TECTA counts are consistently lower or higher than those provided by an alternate quantitative method (ex. membrane filtration). The semi-log linear relationship between TTD obtained by the TECTA and the $\log_{10}$ of *E. coli* enumeration data from the alternate method can thus be used to convert subsequent TECTA measurements into *E. coli* concentrations. The instrument can be connected to internet and configured to send an email at the start of a test, upon detection of *E. coli* and at the end of a test.

All samples were analysed without dilution by following the instructions from the manufacturer. Water samples (100 mL) were poured into a TECTA cartridge, which was gently mixed to completely dissolve the substrate and inserted into the instrument for incubation at 35 °C for 24 h. The optics of the wells were preliminarily checked using the dedicated validation cartridge. TTD as well as *E. coli* and total coliform concentrations (CFU.100 mL^{-1}) were automatically generated by the instrument.

2.2.3. Enumeration of E. coli by culture

2.2.3.1. Membrane filtration (USEPA method 1604). The membrane filtration method using MI agar was performed according to USEPA method 1604 (USEPA, 2002). One hundred millilitres (100 mL) of raw or diluted sample were filtered through a sterile 47-mm, 0.45 μm pore size cellulose ester membrane filter (Millipore) and placed on a 5-mL plate of MI agar (BD Biosciences) containing 5 μg/mL cefsulodine (Sigma Aldrich). Plates were incubated at 35 °C for up to 24 h. Identified *E. coli* colonies were counted, and concentrations expressed in colony-forming units (CFU) per 100 mL (CFU.100 mL^{-1}).

2.2.3.2. Defined substrate technology. For the defined substrate technology Colilert Quanti-Tray/2000 (IDEXX), all samples were processed (with or without dilution) by following the manufacturer instructions. Colilert reagents were mixed with the samples and poured into Quanti-Trays, which were heat-sealed and incubated at 35 ± 0.5 °C for 24 h. After the incubation period, positive wells (yellow for total coliforms, yellow and fluorescent for *E. coli*) were counted and transformed into MPN.100 mL^{-1} using the IDEXX MPN conversion table.

2.2.4. Enumeration of E. coli by real-time PCR

Samples (100–300 mL) were filtered through a sterile 47-mm, 0.45 μm pore size cellulose ester membrane filter (Millipore) and stored into a sterile microcentrifuge tube at −20 °C until further processing. DNA extraction was performed using the FastDNA® SPIN kit (MP Biomedicals). Filter samples were homogenized with Lysis matrix A on a FastPrep® instrument at a speed of 6.0 m s^{-1} for 40 s during 2 successive cycles and were cooled down on ice for 5 min between cycles to avoid overheating. Lysates were centrifuged, and DNA was purified on SPIN Filter columns according to the instructions of the manufacturer before storage at −20 °C. Real-time PCR was performed according to Chern et al. (2011). Final concentrations of primers and TaqMan probe were optimal at 300 nmol.L^{-1} and 100 nmol.L^{-1}, respectively. Samples for qPCR were prepared in 25 μL reactions containing 5 μL of DNA template, 12.5 μL TaqMan Universal Mastermix II (Thermo Fisher Scientific), 0.75 μL of each primer, 0.25 μL of probe and DNase-free water. PCR was run on a Rotorgene-6000 instrument (Qiagen) under the following thermocycling conditions: 10 min hold at 95 °C followed by 45 cycles including annealing at 95 °C for 15 s and hybridization/elongation at 60 °C for 1 min. Quality control was ensured through extraction blanks and no template controls (NTC). Duplicate PCR reactions were performed for each sample (Fig. S2). Standard curves were constructed using genomic DNA from a pure culture of *E. coli* (~10^7 CFU mL^{-1}) as described earlier (Burnet et al., 2017). Genomic DNA was then serially log-diluted in DNase-free water. To reduce the uncertainty associated with the estimation of *E. coli* concentrations from unknown samples, the pooled standard calibration curve approach (Sivaganesan et al., 2010) was adopted.

Following this approach, DNA standard measurements from all runs were pooled while simultaneously generating a calibration curve for each single PCR run (Fig. S1). The generated calibration curve equation was then used to convert Ct (cycle threshold) from unknown samples into CFU equivalents per PCR reaction (CFUeq.r^{-1}). Details about PCR inhibition assessment and removal can be found in the supplementary material.

2.3. Precision of autonomous GLUC activity measurements in comparison to the other assays

Evaluation of the precision (i.e. the closeness of agreement between independent test results (ASTM, 2014)) of the assays was performed both in laboratory and in *in situ* conditions.

For the laboratory experiment, 5-L samples were collected at the DWTP intake on four occasions and analysed by the autonomous GLUC activity measurement technology, the automated microbial detection system as well as by culture and qPCR. For each method, the measurements were replicated 6 times consecutively, giving special care to thoroughly mixing the sample before each new analysis. Between analyses, samples were kept refrigerated and negative controls were performed for each analytical method. The coefficient of variation (CV, expressed in percentages) was calculated for each method as an indication of their precision using the average and standard deviation obtained from the respective 6 replicate measurements.

For the assessment of precision under field conditions, three (3) autonomous GLUC activity measurement devices were installed in parallel on two occasions (January and May 2017) at the DWTP intake to perform online GLUC activity monitoring during 48–96 h at hourly frequency. In addition, 1-L samples were collected synchronously using an ISCO6712 auto-sampler and stored on-site at 4 °C. A subset was selected for triplicate analysis within 24 h using the automated microbial detection device, MF, DST, and qPCR.

2.4. Robustness of autonomous GLUC activity measurements

To determine whether matrix-related interferences could impact online GLUC activity measurements in our field settings, the technology was challenged with raw water samples spiked separately with raw sewage, treated wastewater effluent or river sediments. The volume of these matrices constituted 10, 1 and 0.1% of the final sample volume to be analysed (river water: spiking matrix ratios of 90:10, 99:1 and 99.9:0.1, respectively), to mimic a range of faecal pollution inputs to the urban river via combined sewer overflows, treated wastewater discharges or sediment resuspension. Raw sewage was first passed on a sieve to remove large debris before mixing with river water. Sediments were shaken vigorously before a subsample was added to river water to obtain an initial turbidity of 500 NTU. This suspension was then diluted again in river water to obtain the desired above-mentioned mixture ratios. Final mixtures generated turbidity values between 21 and 70 NTU, which lies in the medium to upper range of values typically observed at the DWTP intake. GLUC activity was first characterized in river water and in the respective spiking matrices (raw sewage, treated wastewater or sediments) to compare the expected GLUC activity to the measured one for all mixtures. In addition to GLUC activity, culturable *E. coli* (measured by DST) and turbidity were also characterized in river water, spiking matrices and final mixtures.

2.5. Statistical analyses

Least-squares linear regression was performed to analyse the relationships between GLUC activity and *E. coli* (enumerated by the automatic microbial detection system, MF, DST or qPCR). One-factor analysis of variance was used to test for significant differences between expected and observed GLUC activities in river water mixtures containing various amounts of raw sewage, treated wastewater or river sediments. Significance was assessed at an alpha level of 0.05. All analyses were run in Statistica v.12 (StatSoft, Inc.).

3. Results and discussion

3.1. Precision of autonomous GLUC activity measurements and *E. coli* enumeration

The analytical precision of autonomous online GLUC activity measurement and *E. coli* enumeration assays was assessed in samples ranging from 4.6 to 30.0 mMFU.100 mL^{-1} and from 50 to >2000 CFU.100 mL^{-1}, respectively. These values fall within the range typically measured at the drinking water treatment plant (DWTP) intake. As shown in Table 2, the lowest coefficients of variation (CV) were calculated for autonomous GLUC activity measurements. Although we did not perform a side-by-side comparison between autonomous and manual measurements of GLUC activity, results from the literature indicate that the CV for the manual assay lies around 10% and can increase to 50% for enzymatic activities approaching detection limits (Lebaron et al., 2005; Servais et al., 2005).

The precision of autonomous GLUC activity measurements was also on average 5 to 8 times higher than for culture and molecular-based enumeration of *E. coli* (Table 2). In a comparative study, Servais et al. (2005) similarly reported CVs between 8 and 15% for the manual GLUC activity assay compared to 31–105% and 23–28% for a miniaturized most probable number (MPN) method and membrane filtration, respectively. Coefficients of variation for MPN methods, such as the defined substrate technology (DST) used in this study, can typically exceed 30% as a direct consequence of the probability distribution for the MPN (Gronewold and Wolpert, 2008; Prats et al., 2008). Real-time PCR also displayed high CV values, particularly for *E. coli* concentrations >400 CFU.100 mL^{-1}. Variability in *E. coli* DNA quantification can result from alteration of

Table 2
Precision of autonomous GLUC activity measurements and *E. coli* enumeration for concentrations between 50 and > 2300 CFU.100 mL^{-1}. For each method and *E. coli* concentration, the measurements were repeated six times on each sample, and the coefficient of variation (expressed in %) was calculated by dividing the standard deviation by the average, multiplied by 100. * as determined by membrane filtration.

E. coli concentration (CFU.100 mL^{-1})*	Coefficient of variation (%)				
	Autonomous GLUC activity measurement technology	Automated detection system	Membrane filtration	Defined substrate technology	qPCR
50	5.8	17.9	29.5	34.0	31.8
83	7.2	34.2	34.5	23.9	32.9
435	0.9	46.2	10.7	12.4	45.3
2333	5.2	28.5	7.7	24.8	46.6

PCR sensitivity due to inhibitors affecting target DNA recovery and/or amplification (Schrader et al., 2012). Also, DNA extraction and purification involve multiple analytical steps, which increases the likelihood for imprecision. Noble et al. (2010) observed higher average CVs for qPCR-based enumeration of *E. coli* (between 25 and 67%) when comparing it to DST (between 19 and 25%), despite apparent absence of PCR inhibition. The higher relative error in qPCR results compared to culture was also reported for *Enterococcus* enumeration (Whitman et al., 2010).

Following laboratory assessment of autonomous GLUC activity measurement precision, the latter was verified under *in situ* conditions using three independent instruments performing parallel online GLUC activity monitoring at the DWTP intake. In the absence of rainfall (January 2017), the CV calculated from three independent GLUC activity measurements ranged from 1 to 8% (average 3.5%). Following a 37.2 mm cumulative rainfall episode (May 2017), it varied from 0.1 to 16.0% (average 3.6%) (Fig. 1 a, b). Overall, GLUC activity measurements were highly correlated between the three instruments (Fig. 1c) and reliably depicted the temporal dynamics of GLUC activity at the intake (Fig. 2).

Stadler et al. (2016) drew similar conclusions for a sediment-laden stream in an agricultural catchment. We further showed that direct comparison of analytical precision with the other methods resulted in lowest average CVs for MF (12%), followed by DST (18%), qPCR (24%) and the automated microbial detection system (30%). Despite these variabilities, similar temporal fluctuation patterns were observed for GLUC activity and *E. coli* (Fig. 2). GLUC activity peaked consecutively three times with maximum values of 13.5, 16.1 and 16.9 mMFU.100 mL^{-1}, and aligned with similar peaks in culturable and total *E. coli* as illustrated by culture and qPCR, respectively. It is important to note that the high precision of GLUC activity measurements even in the lower range of *E. coli* concentrations (<1000 CFU.100 mL^{-1}) does not necessarily imply that GLUC activity accurately accounted for *E. coli* concentrations within that range (Fig. 3). Since both methods target different *E. coli* populations, GLUC activity can also account for the occurrence of viable but non-culturable *E. coli* cells in water, which likely explains the discrepancy between both methods in this concentration range.

3.2. Robustness of autonomous GLUC activity measurement

Online sensors can be affected by matrix-related interferences (Zamyadi et al., 2012) and generate biased data that can ultimately influence decision-making. We therefore evaluated the robustness of online GLUC activity measurements by challenging them with water matrices likely to be encountered during long-term online monitoring and susceptible to cause interferences during enzymatic measurements. Based on the comparison between expected and measured GLUC activity in matrix spikes, raw sewage did not affect ($p > 0.05$) automatic GLUC activity measurements, even when it contributed to 10% of the water matrix (Table 3). For treated effluents, GLUC activity was slightly underestimated in presence of 10% effluents (90/10 mixture) and accurately estimated in the mixtures containing lower proportions of treated effluent (1% and 0.1% in 99/1 and 99.9/0.1 mixtures, respectively). Similarly, observed *E. coli* concentrations in the 90/10 mixture were lower than expected (Table S2), suggesting an error introduced during mixture preparation rather than a true matrix effect on GLUC activity and *E. coli* measurements. Although the river feeding the DWTP is impacted by multiple treated wastewater effluents and combined sewer overflow (CSO) discharge points (Yarahmadi et al., 2018), the highest GLUC activities did not exceed 55 mMFU.100 mL^{-1} at the intake over a 1.5-year monitoring period, which is over 5 times lower than the enzymatic activity measured in the 90/10 mixture with treated wastewater. We can therefore assume that treated wastewater effluents are unlikely to affect GLUC activity measurements in our settings.

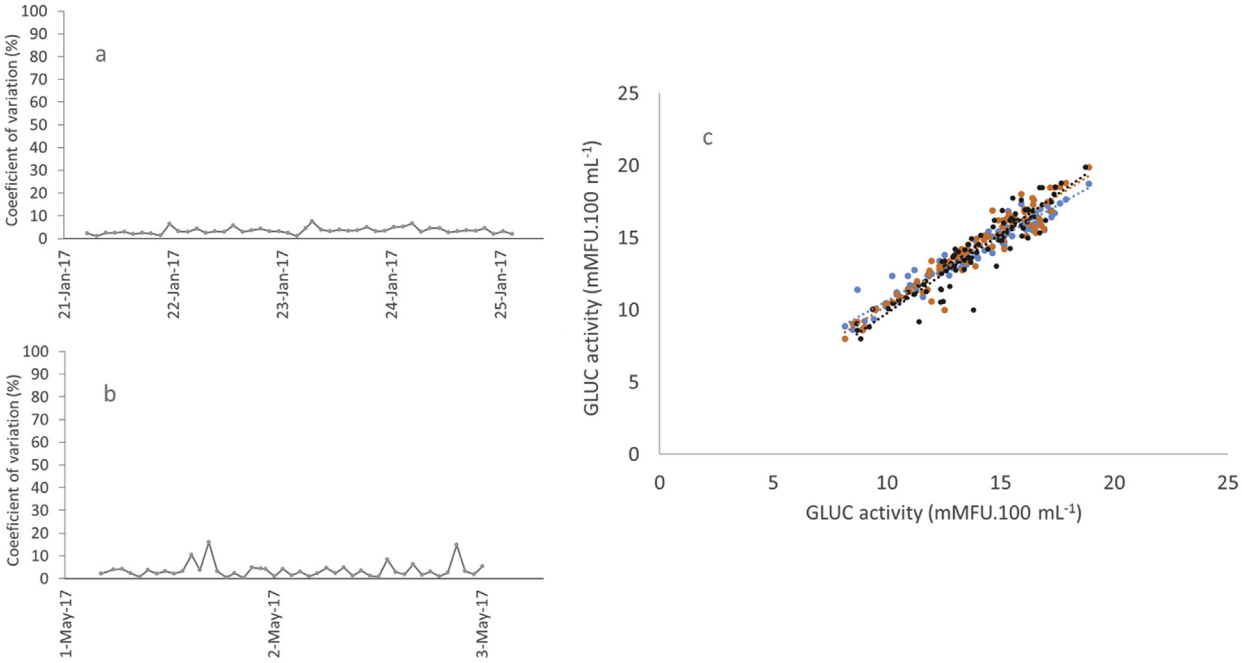

Fig. 1. Precision of autonomous GLUC activity measurements during online monitoring at the drinking water intake using three (3) independent instruments (a) in January 2017 and (b) in May 2017. (c) Pair-wise linear regression analysis of GLUC activity data from the three instruments for both monitoring periods (total of 89 measurements per instrument). Data from the three instruments (INS) were highly consistent ($R^2 > 0.9$, p-value <0.0001). INS1 vs INS2 (blue): $y = 0.84\ x + 2.5$, $R^2 = 0.91$; INS1 vs INS3 (orange): $y = 0.95\ x + 0.97$, $R^2 = 0.94$; INS2 vs INS3 (black): $y = 1.07\ x - 1.02$, $R^2 = 0.90$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

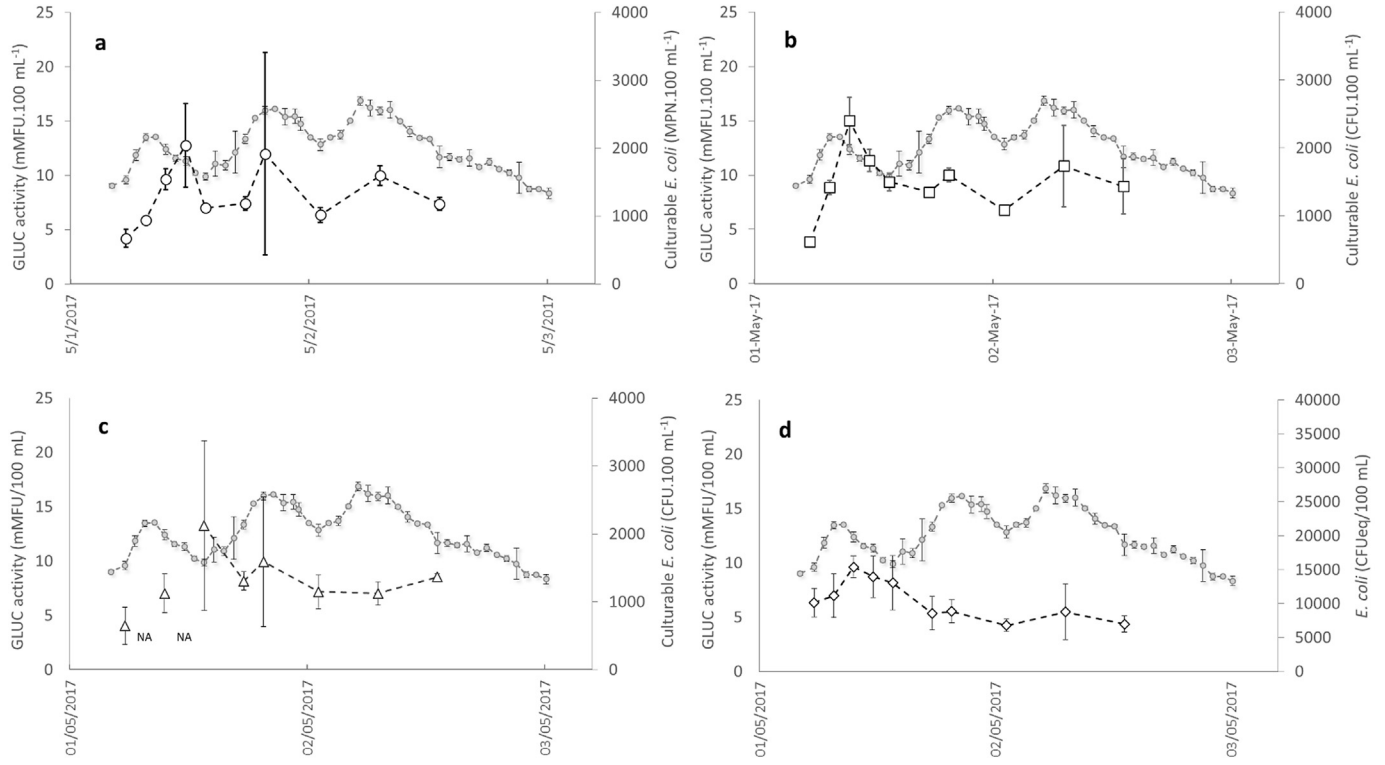

Fig. 2. Temporal series and analytical variability (average ± standard deviation, n = 3) of GLUC activity autonomously measured by three (3) independent instruments in May 2017. Comparison with each of the four methods (average ± standard deviation, n = 3) including a) membrane filtration (○), b) defined substrate technology (□), c) automated microbial detection system (Δ) and d) qPCR (◊). NA, not available. CFUeq, colony-forming unit equivalents.

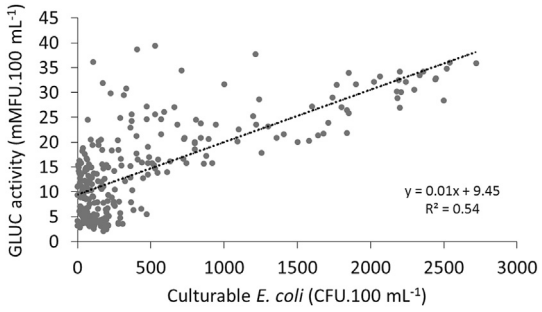

Fig. 3. Correlation between autonomous GLUC activity online measurements and culturable *E. coli* measured by membrane filtration (USEPA method 1604) during long-term monitoring at the drinking water intake. The dataset (n = 273) includes grab samples collected during this study and routine samples (3 days a week over ~1.5 year) from the municipal laboratory. $y = 0.01\ x + 9.455$, $R^2 = 0.54$, $p < 0.05$.

The addition of sediments to the river water did not influence GLUC activity measurements (Table 3), even for a turbidity of 70 NTU (90/10 mixture). The online GLUC activity measurement device is equipped with a patented technology that compensates for possible fluorescence losses in turbid samples (Koschelnik et al., 2015). Such correction likely occurred in the 90/10 mixtures with raw sewage or sediments, where river water turbidity was raised 2 and 3-fold, respectively. Overall, the results from matrix spikes demonstrated the robustness of autonomous GLUC activity measurements for testing conditions simulating the discharge of raw sewage, treated wastewater or the resuspension of river sediments. Although we did not address potential interferences by algae and humic substances on the robustness of GLUC activity measurement (Fiksdal and Tryland, 2008), it would be worth testing these compounds too.

3.3. Relationship between analytical methods

Relationships of variable patterns and strengths were reported among culture-based methods such as MF and DST (Hallas et al., 2008; Hamilton et al., 2005; Olstadt et al., 2007; Zimmerman et al., 2009; Vergine et al., 2017), or between culture and qPCR (Ahmed et al., 2012; Noble et al., 2010; Raith et al., 2013; Truchado et al., 2016). Here, we assessed the relationships between the autonomous GLUC activity measurement, culture (including an

Table 3
Assessment of potential matrix interferences on autonomous GLUC activity measurements (average ± standard deviation, n = 3) by separately spiking raw sewage, treated wastewater or river sediments into river water at fixed ratios. Before spiking of river water, GLUC activities in raw sewage, treated wastewater and river sediments averaged 4,077, 3322 and 128 mMFU.100 mL^{-1}, respectively. In river water, average GLUC activities ranged from 5.5 to 12.5 mMFU.100 mL-1 *p < 0.05.

Spiking matrix	Expected GLUC activity			Measured GLUC activity		
	River water: spiking matrix			River water: spiking matrix		
	90:10	99:1	99.9:0.1	90:10	99:1	99.9:0.1
Raw sewage	415.6 ± 40.3	52.8 ± 5.5	16.5 ± 2.0	433.5 ± 20.0	49.9 ± 0.7	14.9 ± 0.4
Treated wastewater	340.0 ± 15.8	41.8 ± 2.3	12.0 ± 0.9	283.2 ± 8.8*	35.8 ± 3.5	11.8 ± 0.3
River sediments	17.8 ± 2.5	6.7 ± 0.7	5.6 ± 0.5	15.2 ± 0.4	6.4 ± 0.1	5.8 ± 0.3

automatic microbial detection system), and qPCR.

3.3.1. Relationship between culture and qPCR-based methods

Culture-based MF and DST assays displayed the strongest correlation ($r = 0.94$) and both methods were also positively correlated with the automated microbial detection system ($r = 0.91$ and 0.92, respectively) (Table 4). Bramburger et al. (2015) reported a strong agreement between the automated detection system and MF in the Saint-Lawrence River, Canada, while Schang et al. (2016) found a weaker yet significant relationship between that system and DST in the Yarra River, Australia. Although inter-correlated, we found that the three methods differed in their relative quantification of *E. coli*. For instance, higher *E. coli* concentrations were obtained with DST compared to MF (slope of 1.2), which could result from a better recovery of stressed *E. coli* using the former assay (Hallas et al., 2008; Vergine et al., 2017). In contrast, the automated detection system consistently provided lower *E. coli* concentrations compared to MF and DST. This likely originated from the instrument default calibration used for the conversion of time to detection (TTD) into CFU.100 mL^{-1} and done by the manufacturer with samples from Lake Ontario, Canada. We performed a custom calibration of the instrument as recommended but could only use a limited set of local water samples for this purpose. This did not sufficiently correct the *E. coli* enumeration bias between the automated detection system and both MF and DST. Using a dataset that spans over a larger dynamic range of *E. coli* concentrations should remove this bias.

Because of its rapidity compared to culture-based methods, real-time quantitative PCR (qPCR) has been proposed as an alternative to achieve same-day results for more efficient decision-making. Although no current standardized qPCR exists for *E. coli*, such assay has been developed for *Enterococcus* for beach water quality monitoring (USEPA, 2012b). Our results using a published TaqMan assay targeting the 23S rRNA gene of *E. coli* (Chern et al., 2011) were marginally correlated with culture-based assays (Table 4). In a previous study targeting the same gene but using different primer sets, a moderate relationship ($r = 0.47$) was found using $\log_{10}$-transformed data (Ahmed et al., 2012). Noble et al. (2010) described a strong relationship between culture and qPCR in seawater samples, most of which were not affected by PCR inhibition. Our river samples were inhibited at various degrees as reported by the internal amplification control. Although PCR inhibition was eventually removed (Table S1), inhibitory compounds carried over during DNA extraction could have caused suboptimal DNA recovery (Schrader et al., 2012), thereby affecting the sensitivity and precision of the assay, particularly for high *E. coli* concentrations (Table 2). The qPCR-measured *E. coli* concentrations were in average 1.2 $\log_{10}$ higher than those determined by the automated microbial detection system, MF and DST. Differences of about one order of magnitude have been reported between qPCR- and culture-based enumeration of *E. coli* and *Enterococcus* (Ahmed et al., 2012; Noble et al., 2010; Raith et al., 2013; Truchado et al., 2016; Whitman et al., 2010). Culture and qPCR quantify fundamentally different targets and the latter detects not only DNA from culturable but also from viable but not culturable (VBNC) or even dying and dead cells. The comparatively higher *E. coli* concentrations estimated by qPCR are thus likely caused by the occurrence of a high proportion of non-culturable cells that partly originated from UV-disinfected wastewater discharges upstream the drinking water intake. The autonomous GLUC activity measurement technology similarly detects enzymatic activity from non-culturable *E. coli* cells. Interestingly, it was the only assay significantly correlated to qPCR (Table 4), underscoring the fact that GLUC activity and DNA measurements generate more conservative estimates of *E. coli* than culture.

3.3.2. Relationship between autonomous GLUC activity measurements and E. coli enumeration

We found linear relationships between the autonomous GLUC activity measurements and *E. coli* enumeration by four different assays (automated microbial detection system, MF, DST and qPCR) (Table 4). The strongest relationships between online GLUC activity and culturable *E. coli* were observed for DST ($r = 0.77$) and MF ($r = 0.73$). The relationships between autonomous GLUC activity measurements and *E. coli* concentrations provided by the automated microbial detection system and qPCR were weaker. This likely resulted from the higher measurement uncertainty of both assays (Table 2) but also from a smaller dataset available for method comparison. Earlier studies comparing the manual GLUC activity assay to culture reported good correlations ($r > 0.85$) for $\log_{10}$-transformed data spanning over large dynamic ranges (1–8 $\log_{10}$) (Farnleitner et al., 2001; Garcia-Armisen et al., 2005; Lebaron et al., 2005). The relationships between the autonomous GLUC activity measurement technology and DST were stronger than those reported by two recent studies comparing the same technology to DST in agricultural and mixed catchments (Ender et al., 2017; Stadler et al., 2016). Agricultural catchments are characterized by complex pollution patterns with a continuum of recent to old faecal inputs that may impact the relationship between GLUC and *E. coli* (Ryzinska-Paier et al., 2014). In contrast, the urban river investigated in this study is highly impacted by human sewage via treated wastewater and CSO discharges, which represent recent contamination inputs and are more likely to contain high proportions of culturable *E. coli*. Nevertheless, a subset of samples from routine monitoring of the drinking water intake deviated from the linear relationship between GLUC activity and MF, especially for *E. coli* concentrations <1000 CFU.100 mL^{-1} (Fig. 3). Garcia-Armisen et al. (2005) demonstrated that the fraction of non-culturable *E. coli* cells represent a higher portion of the total *E. coli* population in low-contaminated samples compared to samples highly contaminated by *E. coli*. The former usually stem from older faecal inputs where *E. coli* switched from a culturable to a non-culturable stage following prolonged exposure to nutrient scarcity and/or UV irradiation (Servais et al., 2009). The above-mentioned discrepancies were measured during and following snowmelt runoff events for samples possibly containing older faecal deposits and high

Table 4
Correlation (linear regression) matrix for the analytical methods tested in this study. Correlation coefficients (r) in bold indicate a significant relationship at $p < 0.05$. Sample number is indicated in parentheses.

	Autonomous GLUC activity measurement technology	Automated microbial detection system	Membrane filtration	Defined substrate technology	qPCR
Autonomous GLUC activity measurement technology	–	–	–	–	–
Automated microbial detection system	**0.49** (49)	–	–	–	–
Membrane filtration	**0.73** (273)	**0.91** (51)	–	–	–
Defined substrate technology	**0.77** (93)	**0.92** (51)	**0.94** (95)	–	–
qPCR	**0.55** (77)	0.23 (49)	0.31 (73)	0.39 (73)	–

amounts of non-culturable *E. coli* or interfering compounds, in turn resulting in high GLUC activities.

3.4. Implications for the use of autonomous GLUC activity measurements as surrogate of E. coli for rapid online water quality monitoring

Considering the urgent need for rapid testing of microbial water quality, autonomous near real-time measurement of GLUC activity can represent an attractive early warning tool for *E. coli* monitoring. Our results demonstrate the precision (Table 2, Fig. 2) and robustness (Table 3) of autonomous online GLUC activity measurements for a range of *E. coli* concentrations and significant correlations with culturable and total *E. coli* are reported (Table 4). These correlations appear to be weaker for *E. coli* concentrations <1000 CFU.100 mL^{-1}, likely due to a larger proportion of non-culturable cells in samples within this concentration range (Garcia-Armisen et al., 2005). The relationships between GLUC activity and *E. coli* as described in the previous section certainly merit careful attention given that culture-based enumeration of faecal indicator bacteria (FIB) prevail in most regulatory-based monitoring frameworks. However, because the goal of FIB is to reliably account for faecal pollution and point out an increased likelihood of pathogen occurrence, it is of even higher importance to determine whether GLUC activity is an equivalent or better predictor of an exposure to microbial hazards. Future work should therefore focus on the link between GLUC activity and pathogen occurrence in water and investigate the epidemiological value of autonomous near real-time monitoring of GLUC activity. While qPCR quantifies DNA from culturable and non-culturable cells, direct measurement of GLUC enzyme likewise targets a continuum of metabolically active *E. coli* cells (Garcia-Armisen et al., 2005). It remains to be tested whether GLUC activity better correlates with the occurrence of waterborne pathogens known to persist longer than culturable *E. coli* in water (Harwood et al., 2005; Jenkins et al., 2011). Such future work will be paramount for validating the usefulness of autonomous GLUC activity measurement and other rapid online monitoring technologies as early warning tools towards more efficient protection of public health.

The long sample-to-result time of traditional culture-based assays limits their usefulness in identification of intermittent hazardous events. The automated microbial detection system significantly reduces the incubation time. For the range of *E. coli* concentrations measured in our study (~1–3.5 $\log_{10}$ CFU.100 mL^{-1}) a maximum of 11.5 h and a minimum of 8.5 h, respectively, were needed to deliver quantitative results, without need of sample dilution. Furthermore, it does not require trained technicians and laboratory facilities (Table 5). Eventually though, further improvements of the automated microbial detection system should aim at reducing the analysis time (down to 4 h typically) to enable same-day decision-making.

Among the methods tested in this study, the autonomous GLUC activity measurement technology and qPCR are the most rapid. The autonomous enzymatic technology can represent an alternative user-friendly approach and generate high-resolution data on the temporal dynamics of GLUC activity as surrogate of culturable and non-culturable *E. coli* in water. The comparatively high equipment cost of the autonomous GLUC activity measurement technology may be offset in the long-term by reductions in operation costs as it does not require trained technicians and lab facilities to process samples, perform analyses and handle waste (Table 5).

Online near real-time monitoring of *E. coli* and other microbiological parameters will represent a huge leap forward for safe drinking and recreational water usage and it will ultimately lead to a paradigm shift in compliance monitoring and water resources management. Given the comparatively high equipment cost and absence of regulatory requirements though, the use of autonomous online technologies is currently limited essentially to research-oriented studies and proactive monitoring by a limited number of water utilities. Ultimately, it is expected that rapid online monitoring will provide datasets of considerable importance for water resources management, as well as risk assessment and modelling.

4. Conclusion

- The suitability of autonomous GLUC activity measurement for rapid online monitoring of *E. coli* in water was assessed. The correlation of this parameter with the presence of (culturable) *E. coli* as measured with different techniques was also evaluated.
- Laboratory and *in situ* assessment of method precision revealed that the autonomous GLUC activity measurement technology demonstrated high analytical precision with average coefficients of variation below 5%, compared to MF (16%), DST

Table 5
Costs, requirements and practical considerations for each microbiological assay tested in this study.

Method	Characteristics and costs (US dollars)						
	Cost per analysis[a]	Cost of specialist equipment[b]	Need for sterile environment (S) and/or disinfection (D)[c]	Need for trained technician[d]	Field-deployable	Automation	Time to result[e]
Autonomous GLUC activity measurement technology	3 $	44,000 $			x	x	30 min[f]
Automated microbial detection system	12 $	25,000 $	D		x	x[g]	2–18 h
Membrane filtration	3 $	2500 $	S,D	x			>24 h
Defined substrate technology	9 $	4000 $	S,D	x			>24 h
qPCR (incl. DNA extraction)	10 $[h]	25,000	S	x			4 h

[a] Cost for membrane filtration is based on 1 filter. To be multiplied accordingly with sample dilution needs.
[b] Specialist equipment for membrane filtration features a filtration ramp and vacuum pump. For defined substrate technology, it features a QuantiTray sealer and UV lamp.
[c] Addition of the cost related to standard equipment of a microbiology laboratory (incubator, autoclave, biosafety cabinet/Bunsen burner).
[d] For sample preparation, analysis and interpretation of the result. Because of large variations in hourly salaries worldwide, we did not include salary costs. Required expertise will be highest for qPCR analysis.
[e] Time to result includes sample preparation, sample analysis and data interpretation. Sample shipping may significantly extend time to result.
[f] Including the cleaning step.
[g] Automation of measurement step only, manual sample preparation required.
[h] qPCR costs per analysis and cost for specialist equipment were calculated based on the protocol of our study (Fast DNA Spin kit, MP Biomedicals and RotorGene 6000, Qiagen). It may vary according to the DNA extraction kit and qPCR platform. Costs per analysis should also be adjusted based on the number of additional reactions for standard curve and negative controls, which were not included in our calculation.

(21%), an automated microbial detection system (31%) and qPCR (31%).

- Autonomous GLUC activity measurements were not affected by raw sewage, treated wastewater or river sediments, attesting for high assay robustness.
- Although significant correlations between GLUC activity measurements and *E. coli* enumeration were observed, further work is required to link enzymatic activity with the occurrence of pathogens. This is especially warranted for *E. coli* concentrations <1000 CFU.100 mL^{-1}, for which the correlation with GLUC activity was weaker.
- Collectively, reported results illustrate that the promising potential of autonomous GLUC activity measurement for online near real-time monitoring of *E. coli* in surface water should be confirmed by further investigating the relationships between GLUC activity and the presence of viable *E. coli* cells.
- Despite cost-effectiveness of traditional culture-based assays, fully or semi-automated field-deployable instruments such as those tested in this study do not depend on state-of-art laboratory equipment nor do they require trained technicians to be operated, which would facilitate the collection of larger data sets.
- Ongoing work is examining the value of online GLUC activity monitoring for detection of peak pathogen concentrations in drinking water supplies to assist the water community with microbial risk assessment and monitoring.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2018.12.060.

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