A sensitive and robust method for automated on-line monitoring of enzymatic activities in water and water resources

G. Ryzinska-Paier, T. Lendenfeld, K. Correa, P. Stadler, A. P. Blaschke, R. L. Mach, H. Stadler, A. K. T. Kirschner and A. H. Farnleitner

ABSTRACT

The realisation of a novel concept for automated on-line monitoring of enzymatic activities in water was successfully demonstrated by long-term field testing at two remote Austrian ground water resources. The β-D-glucuronidase (GLUC) activity was selected as a representative enzymatic model parameter for the on-line determination. But the device can be adapted to any enzymatic reaction with diagnostic relevance for microbial water quality monitoring, as demonstrated for the β-D-galactosidase activity. Automated filtration of volumes up to 5 litres supports sensitive quantification of enzymatic activities. Internet-based data transfer, using internal control parameters for verification and a dynamic determination of the limit of quantification, enabled robust enzymatic on-line monitoring during a 2-year period. A proportion of 5,313 out of 5,506 GLUC activity measurements (96.5%) could be positively verified. Hydrological (discharge, gauge, turbidity, temperature, pH, electric conductivity, spectral absorbance coefficient at 254 nm) as well as microbiological parameters (Escherichia coli, coliforms) were concurrently determined to characterise the different hydrological conditions and contamination patterns of the test sites. Contrary to expectations, GLUC did not qualify as a proxy-parameter for the occurrence of cultivation-based E. coli contamination and warrants further detailed investigations on its indication capacity as a rapid means for microbial faecal pollution detection in such aquatic habitats. Microbial on-line monitoring is likely to become more important in the future, complementing existing surveillance strategies for water safety management. Further perspectives on the application of such analytical on-line technologies, such as their connection with event-triggered sampling and standardised diagnostics, are discussed.

Key words | enzymatic on-line monitoring, beta-glucuronidase, beta-galactosidase, faecal pollution, E. coli, water safety plan

INTRODUCTION

The determination of microbial water quality is a key requirement for water safety management. Standard determination procedures still rely on cultivation-based methods. Unfortunately, these methods are laborious and time consuming and are not suitable for rapid water quality assessment (ISO 2000). In contrast, online monitoring of chemo-physical and hydrological water quality parameters has been applied frequently during the last decade. There is a significant lack of automated on-line systems for the detection of microbial parameters. Enzymatic activity determination has the potential for rapid measurements. For example, several publications from the last two decades have suggested the use of direct β-D-glucuronidase (GLUC) determination for monitoring faecal pollution in various water sources (Fiksdal et al. 1994; Farnleitner et al. 2002; Servais et al. 2003; Fiksdal & Tryland 2008). The measurement of enzymatic activities relies on fluorometric (Hoppe 1993; Bukh & Roslev 2010), voltammetric (Wutor et al. 2007) or
amperometric (Togo et al. 2007) quantification. However, conventional determination of enzymatic substrate hydrolysis requires a specialised laboratory structure as well as laborious sampling, manual sample handling and further processing procedures.

In this study, a novel automated device that is capable of measuring enzymatic activity in water by applying fluorogenic model substrates was rigorously tested. The device supports automated sampling of adjustable sampling volumes (100 ml up to 5,000 ml) and allows online quantification of enzymatic activities. Internet-based transfer to the user of the generated activity data, including internal quality control parameters, enables remote monitoring, with the final goal of supporting near-real-time water quality management. The device was permanently installed at the measuring site to allow on-line data generation. The GLUC activity was selected as a representative model parameter, but the device can be adapted for any enzymatic determination for which substrates are available.

The main focus of the study was a rigorous field evaluation of the following: (i) the operational applicability, (ii) the stability of measurement, and (iii) the reliability of data transfer of the newly developed measuring device. Over a period of 2 years (2010 to 2011), continuous GLUC data were automatically generated and analysed together with concurrently recorded physicochemical, hydrological and microbiological parameters. Laboratory testing of the biochemical measuring principle was also applied to verify comparability to standard enzymatic determination. The possibility to adapt the device to other enzymatic activities was verified by application of the β-D-galactosidase assay. Because the Austrian water supply relies on almost 100% ground water, two representative but hydro-geologically different ground water sources were chosen as test sites.

MATERIALS AND METHODS

The automated enzymatic measuring device: the fluidic system and the determination process

The central element of the automated measuring device (Coliguard EChs prototype, mbOnline, Austria) is the reaction chamber, where the investigated water sample is concentrated and the enzymatic activity in the water sample is estimated. The whole fluidic system is outlined in Figure 1.

The measuring process consists of several consecutive steps and starts with the initialisation of the instrument (checking the electrical components and flushing the sample and reagent lines). The sample is pumped through the reactor and the filter. Programmable sample volumes range from 100 ml to 5,000 ml (we used 1,000 ml). The filtration pressure is monitored. For incubation, a buffer is injected, and the pH is adjusted during continuous stirring of the chemical enzyme substrate. For GLUC activity determination, methylumbelliferyl-β-D-glucuronide (MUG) is added until a final concentration of 200 mg l⁻¹ and 0.025% Triton-X-100 (w/v) is reached; the temperature is set to 44 ± 0.1 °C according to George et al. (2000). The total

![Figure 1](http://iwaponline.com/wst/article-pdf/69/6/1349/472402/1349.pdf)

**Figure 1** | The fluidic system of the automated measuring system for GLUC determination. The sample is aspirated through the input tubing (1) by the sample pump (3), which also measures the sampled volume and is calibrated for flow rates from 2–20 ml min⁻¹. During forward pumping, the sample is pumped through the reactor (5). The pressure is monitored by a sensor (4), which also regulates the flow rate. The reactor consists of a reaction chamber and a filtrate chamber, separated by a ceramic filter with a 0.45 μm pore size (7). The reaction chamber of the reactor comprises a heating unit, a temperature sensor, an agitation unit and a window for the fluorescence optic measurement. Capillaries for reagents (6) are used to support the reaction chamber. The system valves (2) direct the flow in the system. A launching cleaning procedure is performed using heat incubation and a cleaning solution (8). The resulting waste is discharged through the waste line (9).
incubation time was set to 75 min (default). Degassing of the reactor limits the formation of air bubbles and reduces the risk of failure during detection. The subsequent enzymatic reaction in the agitated reaction chamber is tightly controlled based on the control parameters, as given in Figure 2. The increase of fluorescence is permanently monitored, and the slope of the signal in the steady state phase is used to calculate the enzymatic activity by least square linear regression analysis (see also Figure 1add, additional material; available online at http://www.iwaponline.com/wst/069/032.pdf). The enzymatic activity is expressed as methylumbelliferyl (MUF) production per time and volume (pmol MUF min$^{-1}$ 100 ml$^{-1}$); GLUC activity directly corresponds to the hydrolysis rate of the MUG substrate (Fiksdal et al. 1994; Farnleitner et al. 2001). Further technical details can be found in the respective patent description of the automated measuring device (AT505306, Lendenfeld 2008; Austrian Patent Office).

Each measurement is concluded with a cleaning procedure, which uses a cleaning solution and heat incubation of the reaction unit to eliminate all enzymatic activity of the fluidic components and tubes. Blank values are typically determined once daily to capture minor technical and methodological changes, and are performed using the same procedure used for standard measurements except that the filtration volume is set to a minimum of 7 ml.

The instrument communicates the enzymatic activity data and numerous instrument control parameters (Figure 2(b)) in predefined time intervals via General Packet Radio Service (GPRS) using a virtual private network (VPN) protocol to a server application, where data are stored. A user connects to the server application to view measurements and instrument data and to set schedules or to change instrument settings (Figure 2(a)). Data can be extracted from the database by external programs for further use.

As an additional microbiological parameter, the activity of the galactosidase enzyme (GAL) was also determined using a second automated enzymatic measuring device to complement the GLUC activity measurement. GAL activity was estimated as the rate of hydrolysis of the substrate 4-methylumbelliferyl-$\beta$-D-galactopyranoside (MUGal) (Sigma-Aldrich, St Louis, USA) due to an increase of the product MUF similarly to how the GLUC measurement was performed. The biochemical conditions during the measurements were optimised according to George et al. (2000).

Laboratory experiments were also performed to assess the comparability of the enzymatic assays as applied in the automated device using a standard enzymatic assay.

Comparison of the GLUC activity assay was performed at 44 °C (EC 3.2.1.31 Sigma Aldrich, G7646). The sample was simulated by a GLUC solution with an activity of 5 U ml$^{-1}$. The Sigma Aldrich assay was conducted according to the manufacturer’s instructions except that MUG was used as the substrate instead of phenolphthalein glucuronide. The released MUF was determined using high performance liquid chromatography (HPLC) (Agilent 1100 System, Vienna, Austria).

Establishing a limit of quantification

The principle of the limit of quantification (LOQ) is based on a statistical approach that determines when the measured enzymatic reaction becomes significant compared to the background signal at the start of the measurement (time zero). The approach follows the basic philosophy established for the field of chemical analysis (Mocak et al. 1997) but includes the aspects of continuous and linear signal increase (based on zero-order enzyme kinetics). By the least square method, a linear regression curve and its confidence limits are fitted to the enzymatic measurements. The measured enzymatic reaction (or the corresponding slope of the regression line) at a certain point in time is regarded as statistically significant when the average measurement exceeds threefold the standard deviation (3$\sigma$) in relation to the theoretical zero line of the reaction (as exemplified by $k_2$, Figure 2add (additional material; available online at http://www.iwaponline.com/wst/069/032.pdf)). Regression curves with slopes below 3$\sigma$ (as exemplified by $k_1$, Figure 2add, additional material) are considered non-significant. The threshold slope ($k_t$) is defined as the slope of the regression curve that equals 3$\sigma$. Threshold slopes are calculated by the software of the automated enzymatic measuring device for each enzymatic activity determination and are considered to be the LOQ. It follows from the continuous increase of the signal, that, in theory, any measured enzymatic reaction will eventually become significant. However, the measuring time is usually fixed at a maximum incubation time of 75 min, although this can be adjusted to longer time intervals if needed.

Chemo-physical, hydrological and microbiological field parameters

All hydrological and chemo-physical data were recovered by in-field on-line sensors directly installed at both test sites. At the limestone karst aquifer spring 2 (LKAS2) site, conductivity, water temperature and electrical conductivity were
registered with the data collecting system GEALOG-S from Logotronic (Vienna, Austria). Turbidity, SAC254 (spectral absorption coefficient at 254 nm) and pH were measured with a Sigrist and an HL2200 device (for detailed information see Farnleitner et al., 2005). At the porous ground water aquifer well 1 (PGAW1) site, turbidity and SAC254 were measured with a spectro::lyser (scan Measuring Systems, Vienna, Austria). The values for ground water levels were estimated with a CTD-Diver (Van Essen, Delft, The Netherlands), conductivity and temperature were measured with an SC1000.
(Hach Lange, Düsseldorf, Germany), and the abstraction rate was determined using pressure sensors (Keller, Winterthur, Switzerland). Water samples were analysed twice per week for the bacterial faecal indicators *Escherichia coli* and coliforms by Colilert-18 (IDEXX Laboratories, USA), a cultivation-based method, according to the manufacturer’s procedures (http://www.idexx.com/water).

**Description of the test sites: LKAS2 and PGAW1**

The investigated LKAS2 is located in the Northern Calcareous Alps in Austria, reaching altitudes of up to approx. 2,300 m, with a total catchment area of approx. 100 km². The hydrological regime is highly dynamic and is characterised by frequent surface runoff during precipitation events. Because this aquifer has been the focus of several studies, further detailed background information can be found in other publications (Reischer et al. 2008; Stadler et al. 2008, Wilhartitz et al. 2009). The PGAW1 is located in an alluvial back-water area downstream of Vienna (Vierheilig et al. 2013). The water quality is quite stable throughout the whole year. Contamination only tends to occur during flood events of the Danube River (Kirschner et al. 2009, in press).

Physicochemical, hydrological and microbiological parameters were recorded at both locations during the 2-year investigation period (Table 1add; additional material, available online at http://www.iwaponline.com/wst/069/032.pdf). Although median values for the SAC254 and the turbidity were quite similar, the LKAS2 site showed much higher variation. Microbial indicators accurately demonstrated this difference in the frequency and extent of surface contamination. For LKAS2, *E. coli* counts exhibited a broad range from undetectable to 435 MPN 100 ml⁻¹ (in total 95 (46%) positive detections), whereas at the PGAW1 site, *E. coli* was not detected in 100 ml throughout the period of the investigation. Additionally, coliforms could only be detected on very rare occasions (n = 6) at the PGAW1 site, with a maximum of 3 MPN (most probable number) 100 ml⁻¹. In contrast, concentrations of up to 1,100 MPN 100 ml⁻¹ were detected at the LKAS2 site (in total 153 (74%) positive detections). Recovered data clearly highlight the contrasting contamination characteristics of the selected ground water sources, providing a basis for the rigorous field testing of the automated measuring device.

**Statistical analysis and software**

Time corresponding data were extracted from data material using relevant functions of Excel (MS Office 2007). Statistical analysis was performed with SPSS Version 17.0. Correlation analysis was performed using Spearman’s rank correlation analysis.

**RESULTS**

**Laboratory testing: comparability to standard biochemical assays**

Laboratory testing demonstrated that the automated enzymatic measuring device yields comparable results to those determined using the standard Sigma assay. For the short incubation time, the Sigma assay revealed a hydrolysis rate of 2.1 mg MUF l⁻¹ (n = 10; coefficient of variation (CV) = 3.9%, HPLC). The automated measuring device also yielded 2.1 MUF mg l⁻¹ (n = 10; CV = 4.6%). The measurements taken after 75 min of incubation time showed a concentration of 5.2 MUF mg l⁻¹ (n = 10; CV = 7.6%; HPLC) for the Sigma assay and compared to the automated measuring device, which detected 4.8 MUF mg l⁻¹ (n = 10; CV = 3.6%).

**Field testing**

**Basic performance characteristics of the automated measuring device**

The automated enzymatic measuring device was able to generate and transmit GLUC activity data approximately four times per day from both locations during 2010 and 2011. The device at the LKAS2 site registered 2711 GLUC activity measurements throughout the whole period, among which 2603 (96%) could be verified based on the internally defined control parameters (e.g., filtration pressure, incubation temperature and signal increase after adding substrate). The majority of the verified data (n = 1804 corresponding to 69%) was also above the LOQ and was subjected to further analysis. At the PGAW1 site, 2795 measurements for GLUC activity were registered, among which 2710 (98%) of the measured data) could be verified based on the respective control parameters, but only 468 (17% of the verified) measures exceeded the LOQ and could be used for further evaluation. The LKAS2 site showed a broad range of GLUC activities, ranging from <0.1 to 8.4 pmol MUF min⁻¹ 100 ml⁻¹. The PGAW1 site was quite constant, ranging from <0.1 to 0.7 pmol MUF min⁻¹ 100 ml⁻¹ over the 2 years of testing.

As many as 1404 GAL activity measurements were generated at the LKAS2 site for 2011 (not possible for 2010),
and 1340 (95%) of these could be verified by the respective control parameters. Of these, 1193 (89% of the verified) were above the LOQ, ranging from <0.1 to 3.7 pmol MUF min⁻¹ 100 ml⁻¹. At the PGAW1 site, 2741 measurements of GAL activity were registered for 2010 and 2011. Of these, 2650 (97%) could be verified, and 799 (30% of the valid) were higher than the LOQ.

**Dynamics of enzymatic on-line data**

Automated measurements revealed remarkable temporal GLUC activity patterns for the test sites at LKAS2 and PGAW1, as demonstrated by the representative time series for the winter 2010/2011 and summer 2010 seasons (Figure 3). In winter, the GLUC activity at the PGAW1 site showed almost no variation and was at approximately the same level as that of the LOQ. The GLUC activity determined at the LKAS2 site during winter 2010/2011 was also comparable with the activity at the PGAW1 site. However, one flooding event, which was in accordance with increased discharge, SAC254 and turbidity (data not shown), reflected the scenario of aquifer contamination by surface runoff (up to 2.5 pmol MUF min⁻¹ 100 ml⁻¹). During the summer months, the situation at the PGAW1 site did not change significantly, showing only slight variations in the GLUC activity. In contrast, the GLUC activity measurements at the LKAS2 site during the summer reflected the highly dynamic regime of this aquifer, with pronounced variations and maxima of up to 6.0 pmol MUF min⁻¹ 100 ml⁻¹.

**Correlating enzymatic activities with environmental data**

For the LKAS2 site, a pronounced correlation between the GLUC data and the SAC254 (n = 1804; ρ = 0.64; P < 0.001), turbidity (n = 1804; ρ = 0.69; P < 0.001) and discharge (n = 1804; ρ = 0.77; P < 0.01) could be observed; GLUC and GAL activity also revealed a high correlation (n = 666, ρ = 0.72; P < 0.001; data only available for 2011). Correlations observed between GLUC activity and concentrations of cultivation-based E. coli (n = 113; ρ = 0.53; P < 0.001) and coliforms (n = 113; ρ = 0.65; P < 0.001) were below the correlation levels observed amongst the other environmental parameters (i.e., SAC254, turbidity) and...
GLUC. The PGAW1 site showed no correlation or very low correlations between GLUC activity and the physicochemical or hydrological parameters ($p < 0.3$). Remarkably, GAL activity measurements at the PGAW1 site significantly correlated with GLUC activity ($n = 113; r = 0.42; P < 0.001$; data obtained for 2010). As $E. coli$ and coliforms were not detectable except on very rare occasions ($n = 6$) at the PGAW1 site, these data were not used for further statistical analysis.

**DISCUSSION**

Long-term testing during 2010 and 2011 demonstrated that the new measuring device is a robust and qualified method for automated enzymatic determinations under field conditions. Comparison of the results with available literature data indicates that such rigorous testing has not been performed so far, entering a completely new field of automated long-term monitoring of enzymatic activities in water (Noble & Weisberg 2005; Morikawa et al. 2006; Togo et al. 2007; Wutor et al. 2007; Wildeboer et al. 2010; Connelly & Baeumner 2012). Continuous series of automatically generated GLUC activity values, measured four times per day, could be determined and transferred online using the automated enzymatic measuring device. As much as 96.5% of the single measurements (5313 out of 5506) could be positively verified by the control parameters (such as filtration pressure and reaction temperature). Remarkably, the automated enzymatic measuring device could be successfully used under highly fluctuating hydrological conditions, as observed at the dynamic LKAS2 site. Automated enzymatic measurements were robust, even with high variations in suspended and dissolved materials and with turbidity levels of up to 4.5 FNU (formazin turbidity unit) and SAC254 values of up to 6.4 (abs m$^{-1}$). A monthly service interval (to change the filter and refill the reagent) appeared to be sufficient to maintain successful long-term operation at these ground water sites. Successful detection of GAL activity demonstrated that the enzymatic range is not limited to GLUC and may be extended to other enzymatic activities of interest for which fluorogenic model substrates are available (Hoppe 1995; Noble & Weisberg 2005).

Reliable detection of enzymatic GLUC activity as low as approximately 0.01 pmol MUF min$^{-1}$ 100 ml$^{-1}$ and up to 8.4 pmol MUF min$^{-1}$ 100 ml$^{-1}$ proved to be achievable under the differing ground water test site conditions. The range of the observed GLUC activity appeared low compared to previously reported ranges, from a few up to $10^6$ pmol MUF min$^{-1}$ 100 ml$^{-1}$ (George et al. 2000; Farnleitner et al. 2002; Lebaron et al. 2005; Ouattara et al. 2011). However, the determined GLUC activity levels were reasonable, considering the very low to moderate faecal pollution occurring in the investigated ground water sources (Reischer et al. 2008; Kirschner et al. in press). The automated GLUC activity measurements accurately reflected the different hydrological situations and contamination patterns of the ground water test sites, as revealed by correlation analysis with concurrently determined environmental parameters. Water quality dynamics in the LKAS2 system were driven by discharge and surface runoff conditions and were reflected by bulk parameter changes, as demonstrated by SAK254 (used as a surrogate for dissolved organic carbon input), turbidity (used as a surrogate for particle input) and GLUC activity (used as a surrogate of particle-bound enzyme input). The strong effect of the discharge conditions on the spring water quality of the LKAS2 gas already been demonstrated (Farnleitner et al. 2005, 2011; Reischer et al. 2008). Unlike the pronounced water quality dynamics at the karstic LKAS2 site, the alluvial porous PGAW1 site revealed very constant water quality, which was only extremely rarely influenced from the surface, as demonstrated by the measured physicochemical parameters as well as by applied microbiology standards. The situation at the PGAW1 was also accurately reflected by the automated GLUC activity determination, yielding very low and constant values, frequently below the LOQ. No relevant correlations were discernible amongst any of the physicochemical and hydrological parameters at these constant conditions. Only particle-associated enzymatic GAL and GLUC activities revealed a significant association. Although cross-interference between the GAL and GLUC substrate cannot be completely ruled out, the statistical relationship suggests a capacity of particle-associated enzymatic activities to sensitively monitor for surface influence at alluvial porous ground water sites. Automated filter enrichment before performing the enzymatic measurements supports highly sensitive detection for any subtle changes in microbial abundance or biomass in ground water. In this sense, GLUC and GAL activity (or that of any other microbial enzyme, such as esterase or phosphatase) may be regarded as a sensitive process parameter for on-line monitoring of microbial surface influence, complementing the traditional physicochemical parameters (such as SAC254 and turbidity). However, further investigations must be undertaken to evaluate this promising approach in more detail in such habitats.
The observed level of correlation between E. coli and GLUC activity ($r = 0.53$) at the LKAS2 site was far below the expected level. The results do not indicate that GLUC activity can serve as a surrogate for cultivation-based bacterial standard faecal indicators in such habitats. This observation is in contrast to previously published studies on the indication capacity of GLUC activity, reporting tight associations between E. coli or thermotolerant coliforms and GLUC (Fiksdal et al. 1994; George et al. 2001; Farnleitner et al. 2002). However, it should be noted that previous studies were focused on catchments with recent influences from municipal sewage (human origin). The investigated alpine LKAS2 catchment shows a completely different faecal pollution characteristic because it is mainly influenced by ruminant faecal sources (Reischer et al. 2008; Farnleitner et al. 2011). Furthermore, a complex pollution pattern, ranging from old to recent faecal excreta input is expected, according to the prevailing sources and the hydrological runoff situation. The recovered data highlight the strong need for further detailed investigations on the actual faecal indication capacity of GLUC activity at complex catchments, such as the LKAS2 site, in order to evaluate whether GLUC adds useful information for water abstraction management. GLUC activity may be considered a conservative biochemical indicator that can detect culturable, viable but non-culturable (VBNC) and dead bacterial cells of faecal origin (Garcia-Armisen et al. 2005), leading to low correlations with bacterial standard indicators, especially with respect to aged faecal pollution. However, GLUC activity may also show associations with non-faecal sources, such as with particles from the soil matrix. Indeed, previous investigations reported the existence of non-faecal-associated GLUC activity such as that caused by algae interference (Davies et al. 1994).

Given the protected ground water habitat at the PGAW1 site, a correlation of GLUC or GAL activities with bacterial standard indicators was not expected. Most of the enzymatic measurements resulted in levels below or close to the LOQ and may be regarded as natural enzymatic background activities in alluvial ground water. Clearly, further basic knowledge on the occurrence and origin of very low-level enzymatic background activities in porous alluvial aquifers is needed to substantiate future monitoring applications.

CONCLUSION AND PERSPECTIVES

The results of this study highlight that automated on-line monitoring devices for microbial or biochemical parameters to support water quality monitoring is a realistic task. The application of such automated field systems will likely become increasingly important for sustainable and proactive water management in the near future (Noble & Weisberg 2005; Liu & Lay 2007; Hammes & Egli 2010; Connelly & Baeumner 2012). However, one of the basic requirements for such automated systems is its sufficient sensitivity and robustness (Noble & Weisberg 2005), which is still a major obstacle for many potential on-line sensor and detectors (Liu & Lay 2007; Connelly & Baeumner 2012). Nonetheless, increasingly advanced microbial on-line systems will become available, combining automated sampling procedures with efficient target concentration, purification and detection mechanisms (Noble & Weisberg 2005). It is important to note that many of the developed on-line technologies will be complementary to existing microbiological standard parameters; they are not designed to replace any of the proven surveillance practices. High-resolution on-line information on microbial water quality aspects can be considered a type of ‘process parameter’ that continuously monitors a water source or supply system’s behaviour for any subtle changes. Such continuous on-line information will also offer the unique opportunity to include ‘event-triggered’ automated sampling activities. In the case of water quality changes, whether detected by physical, chemical or microbiological probing, samples will then be automatically taken for further standard microbiological analysis in the laboratory. We are thus anticipating an exciting future in which the realisation of water safety plans is based on ‘intelligent’ systems with a high level of interdisciplinary interaction.

ACKNOWLEDGEMENTS

This paper was supported by the Austrian Science Fund (FWF) as part of the “Vienna Doctoral Program on Water Resource Systems” (W1219-N22), the FP7 KBBE EU project (AQUAVALENS), and the research project “Groundwater Resource Systems Vienna”, in cooperation with the Vienna Waterworks as part of the “(New) Danube–Lower Lobau Network Project” (Gewässervernetzung (Neue) Donau–Untere Lobau (Nationalpark Donau-Auen)) funded by the Government of Austria (Federal Ministry of Agriculture, Forestry, Environment & Water Management), the Government of Vienna, and the European Agricultural Fund for Rural Development (project LE 07-13). This is a joint investigation of the Interuniversity Cooperation Centre for Water & Health (http://www.waterandhealth.at).
REFERENCES

Bukh, A. & Roslev, P. 2010 Characterization and validation of a chemiluminescent assay based on 1,2-dioxetanes for rapid detection of viable Escherichia coli. Applied Microbiology and Biotechnology 86, 1947–1957.

Connelly, J. T. & Baemumer, A. J. 2012 Biosensors for the detection of waterborne pathogens. Analytical and Bioanalytical Chemistry 402 (1), 117–127.

Davies, C. M., Apte, S. C., Peterson, S. M. & Stauber, J. L. 1994 Plant and algal interference in bacterial beta-D-galactosidase and beta-D-glucuronidase assays. Applied Environment Microbiology 60, 3959–3964.

Farnleitner, A. H., Hocke, L., Beiwl, C., Kavka, G. G., Zechmeister, T., Kirschner, A. K. T. & Mach, R. L. 2001 Rapid enzymatic detection of Escherichia coli contamination in polluted river water. Letters in Applied Microbiology 33, 246–250.

Farnleitner, A. H., Hocke, L., Beiwl, C., Kavka, G. G. & Mach, R. L. 2002 Hydrolysis of 4-methylumbelliferyl-beta-D-glucuronide in differing sample fractions of river waters and its implication for the detection of fecal pollution. Water Research 36, 975–981.

Farnleitner, A. H., Willhartitz, I., Ryzinska, G., Kirschner, A. K. T., Stadler, H., Burtscher, M. M., Hornek, R., Szweczyk, U., Herndl, G. & Mach, R. L. 2005 Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities. Environmental Microbiology 7, 1248–1259.

Farnleitner, A. H., Reischer, G. H., Stadler, H., Kollanur, D., Sommer, R., Zerobin, W., Blöschl, G., Barrera, K. M., Truesdale, J. A., Casarez, E. A. & Giovanni, G. D. 2011 Agricultural and rural watersheds, In: Microbial Source Tracking: Methods, Applications, and Case Studies (C. Hagedorn, A. R. Blanch & V. J. Harwood, eds). Springer, New York, pp. 399–431.

Flaksdal, L. & Tryland, I. 2008 Application of rapid enzyme assay techniques for monitoring of microbial water quality. Current Opinion Biotechnology 19, 289–294.

Flaksdal, L., Pommeup, M., Caprais, M. P. & Midttun, I. 1994 Monitoring of fecal pollution in coastal waters by use of rapid enzymatic techniques. Applied and Environmental Microbiology 60, 1581–1584.

Garcia-Armisen, T., Lebaron, P. & Servais, P. 2005 beta-glucuronidase activity assay to assess viable Escherichia coli abundance in freshwaters. Letters in Applied Microbiology 40, 278–282.

George, I., Petit, M. & Servais, P. 2000 Use of enzymatic methods for rapid enumeration of coliforms in freshwaters. Journal of Applied Microbiology 88, 404–413.

George, I., Crop, P. & Servais, P. 2001 Use of beta-n-galactosidase and beta-n-glucuronidase activities for quantitative detection of total and fecal coliforms in wastewater. Canadian Journal of Microbiology 47, 670–675.

Hammes, F. & Egli, T. 2010 Cytometric methods for measuring bacteria in water: advantages, pitfalls and applications. Analytical and Bioanalytical Chemistry 397 (5), 1083–1095.

Hoppe, H. 1993 Use of model substrates for extracellular enzyme activity measurement of bacteria. In: Handbook of Methods in Aquatic Microbial Ecology (P. Kemp, B. Sherr, E. Sherr & J. Cole, eds). Lewis Publishers, Boca Raton, pp. 423–432.

ISO 2000 Water Quality – Detection and Enumeration of Escherichia Coli and Coliform Bacteria – Part 1: Membrane Filtration Method (ISO 9308–1:2000). International Organization for Standardization, Geneva, Switzerland.

Kirschner, A. K. T., Kavka, G. G., Velimirov, B., Mach, R. L., Sommer, R. & Farnleitner, A. H. 2009 Microbiological water quality along the Danube River: integrating data from two whole-river surveys and a transnational monitoring network. Water Research 43, 3673–3684.

Kirschner, A. K. T., Kavka, G., Reischer, G. H., Sommer, R., Paul Blaschke, A., Vierheilig, J., Stevenson, M., Mach, R. L. & Farnleitner, A. H. in press Microbiological quality of the River Danube: status quo and future perspectives. In: Handbook of Environmental Chemistry 'The Danube River Basin' (I. Liska & J. Slobodnik, eds). Springer Verlag, Berlin (in press).

Lebaron, P., Henry, A., Lepeuple, A.-S., Pena, G. & Servais, P. 2005 An operational method for the real-time monitoring of E. coli numbers in bathing waters. Marine Pollution Bulletin 50, 652–659.

Lendenfeld, T. D. S., A-3100 St. Pölten, AT 2008 Apparatus for monitoring water for microbial germs, mbOnline Gmbh (Steiner Landstrasse 27a, 3500 Krems, AT).

Liu, W. T. & Lay, C. 2007 Lab-on-a-chip devices for microbial monitoring and detection in water. H. H. P. Fung and J. H. W. Lee. Water Science and Technology: Water Supply 7 (2), 165–172.

Mocak, J., Bond, A. M., Mitchell, S. & Scollary, G. 1997 A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: application to voltammetric and stripping techniques. Pure & Applied Chemistry 69, 297–328.

Morikawa, A., Hirashiki, I. & Furukawa, S. 2006 Development of a coliforms monitoring system using an enzymatic fluorescence method. Water Science and Technology 53 (4–5), 523–532.

Noble, R. T. & Weisberg, S. B. 2005 A review of technologies for rapid detection of bacteria in recreational waters. Journal of Water and Health 3 (4), 381–392.

Ouattara, N. K., Passerat, J. & Servais, P. 2011 Faecal contamination of water and sediment in the rivers of the Scheldt drainage network. Environmental Monitoring and Assessment. 183, 243–257.

Reischer, G. H., Haider, J. M., Sommer, R., Stadler, H., Keiblinger, K. M., Hornek, R., Zerobin, W., Mach, R. L. & Farnleitner, A. H. 2008 Quantitative microbial faecal source tracking with sampling guided by hydrological catchment dynamics. Environmental Microbiology 10, 2598–2608.

Servais, P., Garcia-Armisen, T., Lepeuple, A. S. & Lebaron, P. 2005 An early warning method to detect faecal contamination of river waters. Annals of Microbiology 55, 151–156.

Stadler, H., Skrteke, P., Sommer, R., Mach, R. L., Zerobin, W. & Farnleitner, A. H. 2008 Microbiological monitoring
and automated event sampling at karst springs using LEO-satellites. Water Science and Technology 58, 899–909.

Togo, C., Wutor, V., Limson, J. & Pletschke, B. 2007 Novel detection of Escherichia coli β-D-glucuronidase activity using a microbially-modified glassy carbon electrode and its potential for faecal pollution monitoring. Biotechnology Letters 29, 531–537.

Vierheilig, J., Frick, C., Mayer, R., Kirschner, A. K. T., Reischer, G. H., Derx, J., Mach, R. L., Sommer, R. & Farnleitner, A. H. 2015 Clostridium perfringens is not a suitable indicator for fecal pollution from ruminant wildlife but is associated with non-herbivorous excreta and human sewage. Applied and Environmental Microbiology 79 (16), 5089–5092.

Wildeboer, D., Amirat, L., Price, R. G. & Abuknesha, R. A. 2010 Rapid detection of Escherichia coli in water using a hand-held fluorescence detector. Water Research 44 (8), 2621–2628.

Wilhartitz, I., Kirschner, A. K. T., Stadler, H., Herndl, G., Dietzel, M., Latal, C., Mach, R. L. M. & Farnleitner, A. H. 2009 Heterotrophic prokaryotic production in ultraoligotrophic alpine karst aquifers and ecological implications. FEMS Microbiology Ecology 68, 287–299.

Wutor, V. C., Togo, C. A., Limson, J. L. & Pletschke, B. I. 2007 A novel biosensor for the detection and monitoring of β-D-galactosidase of faecal origin in water. Enzyme and Microbial Technology 40, 1512–1517.

First received 29 August 2013; accepted in revised form 8 January 2014. Available online 24 January 2014.