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ABSTRACTS

POSTER

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In this study, a simple and rapid high-throughput method for the detection of *Enterococcus spp.* in water resources by loop-mediated isothermal amplification (LAMP) is described. A set of six specific LAMP primers was designed to amplify a diagnostic fragment of the *Enterococcus* 23S rDNA, which is present in several enterococcal species targeted by US EPA quantitative PCR (qPCR) Method 1609, but cannot be found in any relevant non-enterococcal species. The specificity tests were performed with a group of 30 reference strains, consisting of 15 target and 15 non-target bacterial species, where LAMP showed equal performance compared to the previously mentioned reference qPCR method. A sensitivity test with a 10-fold dilution series of *Enterococcus faecalis* reference strain DSM 20478 DNA revealed that the LAMP method is capable of reliably detecting less than 100 DNA target copies per reaction within 30 minutes. Results were confirmed by real-time fluorescence monitoring using a thermal cycler as well as by gel electrophoresis and SYBR Green I staining. Additionally, a set of samples covering drinking water, karst water and surface water, as well as *Enterococcus* isolates were tested. In conclusion, this method has great potential to be performed on a simple heating block and is therefore a good candidate for the efficient screening and testing of water samples in less sophisticated laboratories and by non-professional personnel.

P 16 Pitfalls in DNA-based diagnostics in environmental water samples - tools for validation and optimisation of filtration/extraction procedures

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Contamination of water with fecal pollutants originating from human and animal sources puts a strong constraint on its use for drinking water, recreational purposes or the irrigation of cultural crops. To improve target-oriented catchment protection and management of water quality state-of-the-art molecular diagnostic tools, such as quantitative PCR (qPCR), are used to identify and quantify contamination sources. However, analysis of fecal markers by qPCR requires the extraction of DNA from environmental water samples, which regularly contain varying background matrices challenging DNA extraction. If no internal control (defined cell standard) is used during extraction, potential losses of DNA due to background matrices remain undetected. For example, the strong adherence of microbial cells and DNA to surface-reactive sediment fractions makes DNA recovery highly dependent on the sample type and often very inefficient. To overcome this bottleneck certain molecules, e.g. phosphate, dNTPs, DNA or RNA, can be added as adsorption site competitors prior to lysis of the microorganisms to increase the yield of DNA extraction. The aim of the present study was to develop a method for efficient DNA extraction from samples containing high amounts of total suspended solids (TSS) by subsequently controlling the efficiency of the extraction by adding a defined cell standard (DeTaCS, defined genetic target number cell standard) as internal control. In order to cover a broad range of TSS values different sampling sites in and around Neusiedler See, Eastern Austria were chosen. As adsorption site competitors we added different amounts of sodium pyrophosphate and salmon sperm DNA during DNA extraction.

From these experiments we proofed that the addition of salmon sperm DNA prior to cell lysis improved the DNA recovery to near to control levels and we could show that the use of an internal control is inevitable for a correct interpretation of qPCR results.

P 17 Quantification of *Campylobacter* in drinking water with a cell based approach

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Drinking water may be contaminated by bacteria, viruses and parasites of which some can be health threatening pathogens. The World Health Organisation reports around 330.000 cases of water-related diseases in Europe per year. One of the most important water-transmitted pathogens is *Campylobacter* that causes severe human gastroenteritis. Detection of specific pathogens is usually based on cultivation on microbiological media. However, cultivation needs a long time to result and may overlook pathogens in the viable but non-culturable state. Specific and sensitive detection and quantification of *Campylobacter* is an essential tool for assessing and improving drinking water safety. New, culture-independent techniques concentrate on the development of methods for rapid detection of pathogens in water that have a very low infection dose of a few hundred cells and are usually present in drinking water at low concentrations. This study reports a cell-based *Campylobacter sp*. detection method (CARD-FISH) which can identify thermo-tolerant *Campylobacter* species and clearly differentiate between single *Campylobacter* species. The advantages of the established *Campylobacter* detection method are culture-independency, high specificity and sensitivity and pathogen detection within a few hours. So far, campylobacter concentrations between ~10^3 and ~10^7 cells per ml can be detected with the CARD-FISH method with the use of epifluorescence microscopy. Furthermore, it can be distinguished between *C. jejuni* and other thermo-tolerant *Campylobacter* species such as *C. coli*, *C. lari* and *C. upsaliensis*. Additional work requires the implementation of *Campylobacter* CARD-FISH with a solid-phase-cytometer (Chem*Scan RDI*, bioMérieux) that enables reliable quantification of approx. 10 bacterial cells in 500 ml of drinking water.

P 18 Weltweites Auftreten von human-assoziierten genetischen Fäkalmarkern in hohen Konzentrationen in ungereinigtem und mechanisch-biologisch gereinigtem kommunalen Abwasser

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Fäkale Einträge in Grund- und Oberflächengewässer stellen eine weltweite Gefährdung der Wasserqualität dar. Zum sensitiven Nachweis fäkaler Einträge stehen für Fäkalbakterien (SFIB) seit langer Zeit standardisierte Methoden zur Verfügung. SFIB können jedoch die Herkunft fäkaler Einträge nicht eruieren. Um Fäkalkontaminationen in Wasserkörpern Verursachern zuordnen zu können, werden neu entwickelte Nukleinsäure-basierte Nachweissysteme eingesetzt. Diese Verfahren sind bis jetzt jedoch nicht ausreichend für einen weltweiten Einsatz evaluiert worden.