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**Enhancing Environmental Health: Rapid Bacterial Identification by combining Reverse Purification Nucleic Acid Extraction and Nanopore Sequencing**

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**Abstract:**

**Background/Objective:** Microorganisms existing in wastewater pose a threat to human and animal populations. Surveillance of wastewater represents an ideal early warning system to monitor emerging and re-emerging pathogens circulating in a society and the surrounding environment. Molecular detection methods offer a highly sensitive and specific option for the detection of microbial DNA. However, they are highly dependent of an effective extraction method. Ideally, those methods should be rapid, low-cost, field-deployable, and easy operable. In this study, a rapid DNA extraction protocol was developed for deployment outside of the laboratory and combined with real-time PCR and Nanopore Sequencing to identify microbial wastewater composition.

**Methods:** Different pre-treatment and buffer options combined with Reverse Purification principle were evaluated. Extraction efficacy was assessed by *S. aureus* and *E. coli* spiked wastewater and in crude samples from different collection points at the treatment plant. All results were compared to a spin column-based reference method.

**Results:** The highest DNA amount in both detection methods was achieved using Buffer TLS combined with Bead Beating and demonstrated adequate sensitivity with down to 103 and 100 bacterial cells per reaction using Nanopore Sequencing and real-time PCR, respectively. Nanopore Sequencing allowed the identification of up to eight different bacteria species. A three-fold increase of average length as well as an increase of quality score and a 15-fold decrease of total reads could be achieved compared to the spin-column method.

**Conclusion:** With a duration of less than 3.5 hours the developed extraction protocol represents a practical tool and could contribute to global public health monitoring from wastewater.

**Keywords:** DNA extraction, Nanopore Sequencing, Wastewater-based epidemiology,

molecular wastewater monitoring, Point-of-Need diagnostics