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Development and validation of a field-compatible workflow for molecular diagnostics of microbial water quality

Short title: Field-compatible molecular workflow for microbial water diagnostics

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Abstract

Molecular diagnostics provide advantages in many areas of water quality analysis compared to cultivation-based methods, yet their implementation remains limited mainly due to reliance on specialized instruments, trained personnel and regulatory barriers. To bridge this gap, we developed and evaluated a rapid and field-compatible workflow for the assessment of microbiological water quality. The approach combines simple, ionic liquid-based DNA extraction directly from water filters with detection by loop-mediated isothermal amplification (LAMP). Performance was assessed using three representative bacterial targets (*Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis*) and the human-associated faecal marker HF183 in environmental water samples to determine the applicability in resource-limited settings. Each step of the workflow was carefully benchmarked against state-of-the-art molecular diagnostics. First, our sample processing method was evaluated using dilution series of target cells, demonstrating strong linearity and reliable detection down to very low cell numbers. In spiked environmental water matrices, the method performed competitively with a commercial extraction kit and phenol-chloroform-based extraction both of which require bead-beating steps. The evaluated sample processing method was then coupled with LAMP for on-site target detection. This integrated workflow was highly competitive with reference workflows across the entire concentration range of the chosen targets and showed similar process limits of detection. Validation with environmental water samples demonstrated robust performance, rapid detection times, and minimal variability across matrices. Overall, our workflow offers simplicity without compromising applicability and reliability compared to state-of-the-art molecular methods. The results indicate strong potential for rapid on-site molecular testing in resource-limited settings.

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Keywords

— Faecal pollution

— Molecular diagnostics

— On-site water quality testing

— Loop-mediated isothermal amplification (LAMP)

— Microbial Source Tracking (MST)

— Resource-limited settings

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1. Introduction

Access to clean and safe water is a foundation of human health and development. Water used for drinking and personal hygiene that is contaminated with harmful microorganisms can have immediate and profound effects on health, contributing to disease transmission, either by waterborne diseases or indirectly by foodborne diseases. These diseases disproportionately affect low- and middle-income countries, where infrastructure for water and sanitation remains limited [1]. The ability to accurately and rapidly assess microbial water quality is central for the protection of public health. Cultivation-based approaches remain the gold standard in microbial water quality assessment. These methods are inexpensive, simple, well standardized and have long been embedded in regulatory frameworks, routine monitoring and standardized protocols [2]. However, they suffer significant limitations, including low specificity, if no additional specific analyses are performed, and long incubation times [2–4]. By contrast**,** molecular diagnostic methods, including quantitative PCR (qPCR), isothermal amplification and high-throughput sequencing offer rapid, sensitive, and specific detection with added potential for comprehensive microbial community profiling. These techniques enable identification of both culturable and non-culturable pathogens, including viruses and protozoa that cannot be detected by conventional culture [4,5]. In contrast to PCR methods, isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) do not require complex thermal cyclers, making them a promising alternative for field-testing in resource-limited settings [6]. In the context of faecal contamination of environmental waters, molecular detection methods also have the advantage of allowing source determination by detecting host-associated source tracking markers, such as HF183 (Human Faecal Marker) or BacR (Ruminant Faecal Marker) [7,8].

Despite these advantages, molecular diagnostics remain underutilized in water quality monitoring worldwide [9]. Factors contributing to this slow rate of implementation are:

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Regulatory frameworks rarely mandate molecular methods, favouring standardized cultivation assays that are well-established in legislation [10]. Moreover, molecular diagnostics are often methodologically complex, requiring specialized laboratories and highly trained personnel. They also tend to be more expensive compared with traditional approaches [11]. Also, usually molecular diagnostics do not provide information about cell viability or pathogen infectivity [5]. Before implementing a molecular diagnostic assay, it is important to evaluate the assay characteristics not only with pure cultures of the target, but also with environmental samples containing variable sample matrices and a diverse background microbiota [3]. For qPCR methods it is essential and often challenging to establish calibration standards and standardized protocols to ensure accuracy and consistency [5]. Additionally, to enable detection of specific DNA sequences with qPCR or isothermal amplification, the genetic material must first be released from cells and separated from interfering components. This generally involves disrupting cells, removing proteins, lipids, inhibitors, and nucleases, and isolating the purified DNA. Cell disruption can be achieved through thermal, chemical, enzymatic, mechanical, or combined treatments [12]. Subsequent purification steps commonly employ hazardous reagents like phenol–chloroform or expensive commercial extraction kits. While these approaches yield high-quality DNA, they tend to be labour-intensive, time-consuming, and costly, and in some matrices can produce inconsistent DNA recovery [13]. These limitations become particularly problematic in low-resource environments, where complex sample processing remains a major barrier to implementing molecular diagnostics. As a result, the use of molecular diagnostics for water quality is largely confined to research contexts, specialized government laboratories, and commercial testing facilities, rather than being widely deployed in municipal or community-based monitoring systems [14]. This disconnect highlights a pressing need to translate laboratory-based innovations into practical tools that can be implemented at scale. One of the avenues of implementation could be the availability of fast, low-cost methods, that can be performed on-site by non-specialists.

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The present study seeks to address this gap by developing and evaluating a rapid and simplified workflow for the assessment of microbiological water quality integrating all the necessary steps of sample concentration, DNA extraction, DNA amplification and evaluation of the results. To this end, we developed a rapid sample processing method relying on minimal equipment and coupled it with isothermal amplification protocols and an easy visualisation of amplified products by eye to enable rapid on-site detection. We benchmarked this simplified workflow against state-of-the-art lab-based molecular diagnostics using a set of three representative bacterial target organisms (*E. coli*, *P. aeruginosa* and *E. faecalis*) and the human-associated faecal marker HF183. An in-depth comparison was conducted with environmental water samples, aiming to determine the applicability of the developed workflow for reliable microbial monitoring in resource-limited and point-of-care settings.

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2. Material and Methods

2.1 Bacterial reference strains

As bacterial reference strains we selected *Escherichia coli* ATCC 11775, *Enterococcus faecalis* ATCC 19433 and *Pseudomonas aeruginosa* ATCC 10145. *E. coli* and *P. aeruginosa* were grown overnight in lysogeny broth (Merck, Darmstadt, Germany) and *E. faecalis* in tryptic soy broth (Merck) at 37°C and 180 rpm. On the next day, cultures were diluted to an OD_{600} of 0.1 and grown until reaching OD_{600} of 1. Cells were harvested by centrifugation (5 min, 4000 × g), washed, and resuspended in isotonic saline solution. For the experiments described in section 3.3, each bacterial suspension was diluted with isotonic saline solution in serial 10-fold dilutions, whereby "dilution 0" represents the undiluted bacterial suspension. For the experiments described in section 3.4, the resuspended cells were used for spiking different types of water samples. To quantitatively determine total bacterial cell counts, epifluorescence microscopy was used (S4 Table, S3 Fig). Bacterial cell suspensions were fixed with paraformaldehyde (final concentration 0.8%) overnight at 4 °C and filtered through 0.2-µm Anodisc 25 filters (Whatman, Germany) as previously described [15].

2.2 Filtration

Polycarbonate membrane filters with a pore size of 0.22 µm and a diameter of 25 mm (Merck) were used for filtration of cells. 1 mL of each sample was filtered in duplicates (3.1) and triplicates (3.2 and 3.3). For the spiking experiments in section 3.4, 1 mL of wastewater influent, 50 mL of surface water and 100 mL of groundwater were filtered, each in duplicates.

2.3 DNA extraction

The extraction of genomic DNA was performed using one of the following methods: an ionic liquid-based extraction protocol, a phenol-chloroform extraction protocol and QIAamp DNA Mini Kit as well as Qiagen PowerSoil Kit (Qiagen, Hilden, Germany). Extraction controls were always carried out for each DNA extraction method in order to rule out contamination during the extraction process.

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When directly extracting DNA from cell suspensions in section 3.1 and 3.2 employing ionic liquids (IL) and magnetic beads (MB) [15], 10 µL of the cell suspension was mixed with 90 µL of 90% w/w 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) in 10 mM Tris pH 8 buffer and incubated at room temperature for 5 min. Afterwards, the undiluted [C2mim][OAc] lysate was mixed with 150 µL of SeraSil-Mag™ 400 silica coated superparamagnetic beads (Cytiva, Vienna, Austria), vortexed and diluted with 765 µL of 10 mM Tris pH 8 buffer. The solution was incubated (24°C, 10 min, 1400 rpm) to aid binding of nucleic acids to the beads. The beads were collected by placing the tube onto a magnetic separation rack for 30 to 60 s and the supernatant was discarded. 500 µL of 70% ethanol in 10 mM Tris pH 8 buffer was used for washing of the beads, before letting them air-dry for 10 to 15 min. DNA was eluted with 50 µL of TE buffer and incubation (65°C, 3 min, 1400 rpm).

Fig 1 shows the adjusted method for DNA extraction from filters with ionic liquids and magnetic beads (F+IL+MB). For this, 90 µL of 90% w/w [C2mim][OAc] with 10 mM Tris pH 8 buffer was mixed with the filter and vortexed at room temperature for 5 min. Afterwards, the filter was washed with 765 µL of 10 mM Tris pH 8 buffer. The resulting mixture was transferred into a clean reaction tube, and the filter was discarded. Then, the solution was mixed with 150 µL of SeraSil-Mag™ 400 silica coated superparamagnetic beads and the standard protocol was followed as described above.

<<Fig 1>>

Fig 1: Optimized IL-based extraction method enabling recovery of DNA, with the complete procedure taking roughly 30 minutes and requiring only about 5 minutes of actual hands-on work; Figure created in BioRender.com;

The extraction with Qiagen kits was performed following the manufacturer's instructions. For the Qiagen PowerSoil Kit, filters were directly put into the "Power Bead Tubes". In the last step, DNA was eluted in 50 µL of the respective buffer.

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For phenol-chloroform extraction, the protocol by [16] was followed, with DNA precipitation using isopropanol instead of polyethylene glycol. Recovered DNA was dissolved in 50 µL of TE buffer. All isolated DNA was stored at -20°C until further use.

2.4 Quantitative PCR targeting *E. coli, P. aeruginosa, E. faecalis* and HF183

The following quantitative PCR (qPCR) assays were used for the quantification of target strains: *E. coli* [17], *P. aeruginosa* [18], *E. faecalis* [19,20] and HF183 [7]. Reactions were carried out in a total reaction volume of 15 µL containing 2.5 µL DNA sample and performed on a qTOWER³ G real-time thermocycler (Analytik Jena, Jena, Germany). qPCR reactions were carried out in triplicates, and no-template controls (NTCs) were included in each run. A plasmid dilution series containing a known number of the target gene was used to generate a calibration curve. Potential qPCR inhibition associated with residual IL was assessed by analysing different dilutions (1:4, 1:8 and 1:16) of the same F+IL+MB DNA extracts. The resulting Cq values were compared across dilution steps to determine whether dilution of the extracts resulted in an unexpected improvement in amplification, which would indicate the presence of inhibitory compounds. All reaction compositions and oligonucleotides used are listed in S1 and S2 Table, respectively.

2.5 LAMP reactions targeting *E. coli, P. aeruginosa, E. faecalis* and HF183

All LAMP reaction compositions and conditions as well as the oligonucleotides for *E. coli* [21], *P. aeruginosa* [22], *E. faecalis* [23] and HF183 [24] are listed in S1 and S2 Table, respectively. The LAMP reactions were either performed on a heating block or in a qTOWER³ G real-time thermocycler. The real-time fluorescence was considered as positive, if a threshold time (t_t) value could be detected in 60 minutes. The automatic manufacturer settings were used to set the thresholds to ensure that there was no bias in the data analysis. Since LAMP is generally considered to be a qualitative method, the results were evaluated solely based on the presence or absence of a signal above the threshold value. Additionally, LAMP reactions were carried out on a heating block for 60 min at their respective temperature, followed by 10 min at 80°C

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to stop the reaction. For the visualization of results, 9 µL aliquot of the products from finished LAMP reactions were mixed with 1 µL of a 1000 × SYBR® Green I nucleic acid stain (Thermo Fisher Scientific, Waltham, USA) solution. When the LAMP reaction was successful, the originally orange dye shifted to a bright light-green colour as it intercalated into resulting double-stranded DNA, which altered its visible-light emission profile. By comparing this visualisation with the t_t values of the analysis in the real-time thermocycler, we could estimate how long the amplification time needs to be to reach a positive signal even at low sample concentrations. LAMP reactions were carried out in triplicates and NTCs were included in each run.

2.6 Water Samples

Influent samples were collected from a municipal wastewater treatment plant serving approximately 280.000 population equivalents. Sampling was conducted over a 24-hour period using a flow-proportional composite sampling approach. Surface water samples were collected as grab samples from the Danube River in Krems, Austria in sterile 1 L glass bottles. Groundwater samples were obtained from a large karstic spring in the Northern Calcareous Alps, which is used for drinking water production, using Nalgene sampling bottles (4.2 L; Nalgene Europe, Hereford, UK) that had been rinsed with acid and ddH_2O and subsequently autoclaved. All samples were kept at 4°C in cooling boxes during transport and processed within six hours of collection.

2.7 Spike Calculations

For the spiking experiments in section 3.4, different water types were spiked with overnight cultures of *E. coli*, *P. aeruginosa*, *E. faecalis* and, as a source for HF183, wastewater influent. The target concentration was 10^3 cells/reaction or 10^3 marker equivalents/reaction in the final assay reaction. Therefore, 1.4×10^7 cells/filter of *E. coli*, 2×10^6 cells/filter of *P. aeruginosa*, 8×10^6 cells/filter of *E. faecalis* and 2×10^6 marker equivalents/filter of HF183 were spiked into/added to the different water samples. Each organism was spiked and analysed separately.

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Since different volumes were filtered for each specific water type, the results were evaluated per eluate to ensure better comparability. All un-spiked water samples were assessed with specific qPCR and LAMP assays, to obtain background values for the sample matrix. A background contribution below 5% of the corresponding spiked sample signal was considered negligible, as such a contribution was not expected to materially affect the comparison between spiked samples or extraction methods. In the two samples exceeding this threshold, background signals remained substantially lower than the corresponding spiked signals and did not affect the interpretation of the results.

2.8 Software used

Calculations, tables and diagrams were generated in Microsoft Excel (2025). The graphical abstract and Fig 1 were generated in BioRender under academic license (Farnleitner, A.) (biorender.com).

2.9 Data availability

The raw data of the qPCR and LAMP analyses can be found on the TU Wien Research Data repository under the DOI: 10.48436/gje3c-e3x80 (https://doi.org/10.48436/gje3c-e3x80).

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3. Results

Our simplified workflow was designed to enable rapid, on-site molecular detection of bacterial indicators and pathogens and comprises three major steps: sample filtration, DNA extraction, and isothermal amplification with read out of results. Membrane filtration was performed using polycarbonate filters (0.2 µm pore size, 25 mm diameter). The DNA extraction method using hydrophilic ionic liquids (ILs) combined with magnetic bead (MBs) purification was specifically adapted to the prior filtration step to ensure simple, rapid and efficient recovery of nucleic acids. This sample processing method for extracting DNA directly from the filter (F+IL+MB) was evaluated using three representative bacterial targets. To assess analytical performance, dilution series of selected bacterial indicators and pathogens were analysed by qPCR and compared to current standard DNA extraction protocols. To further examine applicability under realistic conditions, environmental water samples were included in the evaluation. Finally, the complete workflow was validated by analysing both the dilution series and the spiked environmental samples with isothermal amplification assays, allowing direct comparison between the simplified workflow and the state-of-the-art laboratory-based molecular detection methods.

3.1 Establishment of filter-based DNA extraction method for bacteria

In the present study, we developed and optimized a novel filter-based sample processing method specifically for application in microbial water quality analysis by integrating membrane filtration with an ionic liquid- and magnetic bead-based DNA extraction protocol (IL+MB). Its extraction chemistry was based on a previously established protocol for direct DNA extraction from liquid bacterial cell suspensions, primarily in the context of clinical samples [15]. In contrast to the previous protocol, the sample processing method developed in this study enables direct processing of filter-retained microorganisms and was specifically optimized and validated for water samples.

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To evaluate this approach, *E. coli* was chosen as the model due to its relevance as a common water quality indicator. For method establishment, detection was performed using an *E. coli*-specific qPCR assay, while direct extraction of the cell spike using the QIAamp DNA Mini Kit (Qiagen) served as a reference. To optimize extraction efficiency, different volumes of 90% w/w [C2mim][OAc] were tested (500 µL, 250 µL, 90 µL) and added to the filter, which has been transferred to a clean reaction tube after filtration. Likewise, different mixing approaches were evaluated for ensuring contact between filter and IL in the reaction tube, including incubation of filter+IL with shaking and with vortexing at room temperature for 5 min. The resulting mixture was transferred into a clean reaction tube, and the filter was discarded. Then, the solution was mixed with 150 µL of SeraSil-Mag™ 400 silica coated superparamagnetic beads and the standard protocol was followed as described in Section 2.3. These pre-experiments showed that vortexing the filter with 90 µL IL for 5 min was sufficient to lyse bacterial cells retained on the filter (S1 Fig). Also, the effect of additional filter rinsing was evaluated, because the viscous IL partially adheres to the wall of the reaction tube after vortexing, which could result in a lower DNA yield. For this, 90µL IL was added to the filter and the vial was vortexed at room temperature for 5 min. Before mixing the solution with MB, the filter was rinsed either one time with 765 µL, two times with 383µL or three times with 255µL of 10 mM Tris pH 8 buffer. Results showed that one additional washing/elution step of the filter with 765 µL of Tris buffer improved the extraction efficiency by a factor of 2.46 (S2 Fig). Although the protocol was initially established using *E. coli*, subsequent optimization with *P. aeruginosa* and the Gram-positive bacterium *E. faecalis* showed that the same extraction conditions performed best for all three species. This optimized sample processing method was used as the simple extraction procedure for the subsequent experiments (F+IL+MB). To assess whether residual IL affected downstream qPCR amplification, different dilutions of the same F+IL+MB DNA extracts were analysed. Amplification followed the expected pattern across the tested dilutions, with no indication of improved amplification upon sample dilution. These

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results provided no evidence of relevant qPCR inhibition by residual IL under the tested conditions.

3.2 Assessment of sample processing method with selected target bacteria

To evaluate efficiency and applicability of the newly developed sample processing method (F+IL+MB), *P. aeruginosa* and *E. faecalis* were added as target organisms. Cell suspensions of all three bacterial target strains were processed and quantified using species-specific qPCR assays to assess extraction efficiency. As reference methods, the ionic liquid-based extraction method (IL+MB) [15] and the QIAamp DNA Mini Kit (QIAamp) were used. Both methods were applied directly on the cell suspension, without prior filtration. The results of the QIAamp Kit were defined as the 100% recovery benchmark. In Fig 2 all results are summarized. Absolute values of measured genome copies/µL are provided in S3 Table. For *E. coli*, all workflows yielded comparable results, with the F+IL+MB method yielding DNA concentrations only 0.2 $\log_{10}$ genome copies/µL less compared to the QIAamp Kit. For *P. aeruginosa*, similar trends were observed with high recovery across methods, although IL+MB and F+IL+MB performed slightly better than the QIAamp Kit. In the case of *E. faecalis*, more pronounced differences emerged. The IL+MB approach resulted in lower and more variable recovery, approximately 1.4 $\log_{10}$ genome copies/µL lower than those obtained with the QIAamp Kit. By contrast, the combined F+IL+MB method achieved yields that were only 0.9 $\log_{10}$ genome copies/µL lower compared to the QIAamp Kit, thereby improving extraction efficiency and reducing variability compared to direct IL+MB.

<<Fig 2>>

Fig 2: Comparison of DNA extraction efficiency for three target bacteria E. coli, P. aeruginosa and E. faecalis using different extraction workflows; The field-compatible sample processing method (F+IL+MB) (grey) was compared with the commercial QIAamp DNA Mini Kit (QIAamp) (crosses) and the previous published ionic liquid-based extraction protocol (IL+MB) (lined) without upstream filtration of cell suspensions; Boxes cover the 25th to 75th percentile; Line within the boxes: median; Whiskers: min and max; Values shown are mean values from three biological replicates;

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3.3 Validation of sample processing method with dilution series of selected target bacteria

To systematically validate performance and robustness of the developed sample processing method, dilution series of *E. coli*, *P. aeruginosa*, *E. faecalis* cells and wastewater containing the HF183 marker were analysed to assess quantitative consistency across decreasing target concentrations. The dilution series was designed to extend to complete dilution of the organism, with the lowest dilution representing a condition in which the presence of the target organism is no longer statistically expected. As water quality analysis workflows inherently rely on filtration as an initial concentration step, the simplified sample processing method for DNA extraction from filters (F+IL+MB) was benchmarked against two commonly used laboratory reference protocols for filter-based DNA extraction: phenol–chloroform extraction (P/C) and the Qiagen PowerSoil Kit (QPS). The use of filtration-based reference methods ensures methodological comparability and reflects standard practices. Both reference methods use an initial bead-beating step for mechanical lysis. All samples were processed in parallel and analysed with their specific qPCR assays. Cell counts of bacterial cultures used for the dilution series can be found in S4 Table, and representative pictures of the cultures imaged and quantified under an epifluorescence microscope are shown in S3 Fig.

All three workflows showed strong linear relationships between cell numbers and detected genome copies or marker equivalents for each target over the whole range of the dilution series (Fig 3). For *E. coli*, only minor differences in recovery were observed between extraction protocols. Overall, the F+IL+MB method demonstrated reliable linearity across the dilution range ($R^2 = 0.996$). In the case of *P. aeruginosa*, the F+IL+MB method yielded a regression of $y = 0.914x + 0.314$, which closely matched the slopes obtained for P/C (0.915) and QPS (0.924). The lower intercept of the F+IL+MB method reflects that the absolute DNA yield per cell is reduced compared to the reference methods (P/C and QPS). Despite this shift, the near-identical slopes confirm that our novel sample processing method maintained a high degree of linearity and quantitative consistency across the entire dilution series. For *E. faecalis*, our method

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demonstrated reliable linearity between cell count and detected genome copies ($R^2 = 0.975$). Nevertheless, a slightly lower extraction efficiency of about 1-2 $\log_{10}$ genome copies at higher input levels was found. For the source tracking marker HF183, similar regressions were found along the dilution series and between the different methods. However, as the starting solution had lower input values, only 4 dilution steps were detected for P/C and 5 steps for both QPS and the F+IL+MB method. The clear linearity of all workflows further supports the estimation of the range of quantification in the absence of background matrix interference, indicating reliable detection at even low input values of 1-2 $\log_{10}$ genome copies/reaction. This underlines the applicability of our workflow for sensitive monitoring of drinking water quality.

<<Fig 3>>

Fig 3: Validation of sample processing method (F+IL+MB) with dilution series of selected target bacteria: E. coli (A), P. aeruginosa (B), E. faecalis (C) and HF183 (D); Comparison of F+IL+MB (rhombus, blue) with phenol-chloroform extraction protocol (P/C; circle, black) and commercial Qiagen PowerSoil Kit (QPS; triangle, grey); Values shown are mean values from two biological replicates;

3.4 Validation of sample processing method with various environmental water matrices

To determine the applicability of the developed sample processing method for reliable microbial monitoring in resource-limited settings, target detection in environmental water samples was assessed. Wastewater, river water and groundwater were used in this experiment. These water samples were spiked separately with known concentrations of *E. coli, P. aeruginosa, E. faecalis* and HF183, respectively. DNA was extracted with F+IL+MB, P/C and QPS and analysed with their respective qPCR assays.

Table 1 shows the results of qPCR analysis of all three water types with the four different targets and three different DNA extraction methods. For *E. coli* spiked in wastewater, our sample processing method reached similar values as QPS, and approximately 0.5 $\log_{10}$ genome copies more than P/C. Similar trends were observed in surface and groundwater. For *P. aeruginosa*, QPS and F+IL+MB performed comparably in wastewater, both surpassing P/C by a factor of roughly 2. In surface water, our sample processing method reached values of 0.3 – 0.4 $\log_{10}$

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genome copies less than QPS, being similar to the results from P/C. In groundwater, the three methods produced variable results, with F+IL+MB yielding 1.1 – 1.5 $\log_{10}$ higher genome copy numbers than QPS. The greatest extraction variability between methods occurred in the case of *E. faecalis*. In wastewater, F+IL+MB resulted in substantially lower yields of 1.1 – 2 $\log_{10}$ genome copies less compared to the reference methods. As for surface water, it achieved considerably less than QPS, however our method achieved approximately 2 $\log_{10}$ genome copies more than P/C. Similar to the other water samples with *E. faecalis*, our sample processing method reached about 1.7 – 1.8 $\log_{10}$ genome copies lower yields compared to QPS in the case of groundwater. For the human-associated marker HF183, both QPS and F+IL+MB produced comparably high recoveries in wastewater with F+IL+MB reaching 83-151% of the values achieved by QPS. Both methods were outperforming P/C by several fold. In surface water, all three methods yielded similar values, with the largest deviation being 0.25 $\log_{10}$ genome copies. In the case of groundwater, F+IL+MB resulted in comparable values to QPS, with P/C delivering lower extraction efficiencies. Target recoveries obtained with F+IL+MB were comparable across wastewater, surface water and groundwater, with discernible effect of the water matrix consistently on target yield. This indicates that no substantial matrix-associated effect on workflow performance was observed under the applied conditions.

Table 1: Validation of sample processing method with diverse environmental water matrices (wastewater, surface water, groundwater) spiked with E. coli (turquoise), P. aeruginosa (yellow), E. faecalis (blue) or HF183 (brown), respectively; Comparison of three extraction methods: phenol-chloroform protocol (P/C), Qiagen Kit (QPS), developed sample processing method (F+IL+MB); Shade of colour corresponds to target concentration in relation to the min and the max for the respective organism; Results are in $\log_{10}$ (genome copies +1) / eluate;

Organism	Water sample	P/C	QPS	F+IL+MB
E. coli	Wastewater	7.32	8.06	7.86
		7.29	7.93	7.98
	Surface water	5.16	8.34	8.33
		8.15	8.63	8.12
	Groundwater	7.77	8.38	7.92
		7.57	8.66	7.88

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P. aeruginosa	Wastewater	6.92	7.09	7.10
		6.74	7.17	6.96
	Surface water	6.92	7.14	6.72
		6.91	7.25	6.99
	Groundwater	6.83	5.56	7.07
		6.94	5.78	6.91
E. faecalis	Wastewater	8.59	8.91	6.94
		7.78	8.69	6.96
	Surface water	5.29	8.89	7.35
		5.21	9.25	7.54
	Groundwater	8.14	9.00	7.20
		7.76	9.09	7.35
HF183	Wastewater	6.26	7.10	7.28
		6.76	7.14	7.06
	Surface water	7.13	7.32	7.13
		7.19	7.45	7.33
	Groundwater	6.80	7.34	7.15
		6.69	7.41	7.09

3.5 Validation of integrated simplified workflow – combining sample processing with LAMP detection

For the validation of our complete workflow, the established sample processing method was combined with isothermal amplification to enable rapid, on-site detection. To directly assess whether the extraction procedure affected subsequent LAMP performance, DNA obtained from the same dilution series of the selected targets using P/C, QPS and F+IL+MB was analysed with the respective target-specific LAMP assays under identical amplification conditions for each extraction method. Amplification was achieved both in a real-time thermocycler and on a standard heating block with subsequent SYBR Green I addition for rapid and simple visual read-out of results by the naked eye. Absolute threshold times for all samples can be found in S5 Table.

The evaluation with LAMP revealed distinct detection patterns across dilution levels and extraction methods for all four targets (Table 2). For *E. coli*, *P. aeruginosa* and *E. faecalis* all three extraction approaches (P/C, QPS, F+IL+MB) showed consistent detection (3/3 replicates)

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starting from a theoretically equivalent DNA content of 6.0, 7.0 and 6.2 $\log_{10}$ cell counts/reaction (dilution 0_A and B, Table 2) down to 1.0, 2.0 and 1.2 $\log_{10}$ cell counts/reaction, respectively (dilution 5_A and B, Table 2). For samples with lower concentrations, only partial detection was observed with 2/3 or 1/3 positive replicates depending on the extraction method. In line with qPCR results, no signal was obtained at the highest dilution step, in which no target cells are expected to be present (dilution 7_A and B, Table 2), for all three bacterial targets. The comparable detection ranges obtained for F+IL+MB, P/C and QPS also was an indication that the IL-based extraction procedure did not adversely affect subsequent LAMP amplification under the tested conditions.

Table 2: TOP: Validation of workflow with dilution series of selected target bacteria E. coli, P. aeruginosa, E. faecalis and marker HF183 analysed with their respective LAMP assay; Comparison of F+IL+MB with phenol-chloroform extraction protocol (P/C) and Qiagen PowerSoil Kit (QPS); BOTTOM: Validation with diverse environmental water matrices (wastewater, surface water, groundwater) spiked with E. coli, P. aeruginosa, E. faecalis or HF183, respectively; Comparison of three extraction methods: P/C, QPS, F+IL+MB measured with specific LAMP assays;

	E. coli			***P. aeruginosa***			***E. faecalis***			**HF183**		
	P/C	QPS	F+IL +MB	P/C	QPS	F+IL +MB	P/C	QPS	F+IL +MB	P/C	QPS	F+IL +MB
Section 3.5.: Dilution series analysed with LAMP												
0_A	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
0_B	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
1_A	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
1_B	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
2_A	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/3	3/3	3/3
2_B	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
3_A	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	3/3
3_B	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	3/3	3/3
4_A	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	1/3	2/3
4_B	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3
5_A	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3
5_B	2/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	0/3
6_A	2/3	2/3	3/3	2/3	3/3	2/3	3/3	2/3	3/3	0/3	0/3	0/3
6_B	1/3	2/3	3/3	1/3	3/3	1/3	3/3	3/3	3/3	0/3	0/3	0/3
7_A	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
7_B	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Section 3.6.: Spiked water samples analysed with LAMP												
Wastewater	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

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Surface water	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Groundwater	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

For *E. coli*, our F+IL+MB method maintained positive replicates even at dilution level 6 (dilution 6_A and B, Table 2), whereas not all replicates of P/C and QPS were detected. In the case of *P. aeruginosa*, only 2/3 and 1/3 replicates of the F+IL+MB method at 1.1 $\log_{10}$ cell counts/reaction (dilution 6_A and B, Table 2) were detected, although all replicates of QPS were confirmed. For *E. faecalis*, no clear differences between extraction methods were found. As no cell counts can be obtained for the HF183 marker, results were analysed based on dilution steps. Samples until dilution 2 were generally positive across methods. Consistent with qPCR results, the detection rate dropped below that and from dilution 3 onwards, replicate losses were frequent. However, the F+IL+MB method was able to maintain detection of all replicates of dilution 3 and also partially of dilution 4. In comparison, no signal was obtained after dilution 2 for P/C, and for QPS only one biological replicate of dilution 3 was detected. As a representative example of visualization of amplified products by eye, the dilution series of *E. coli* extracted with F+IL+MB was amplified on a heating block and stained with SYBR Green I (S4 Fig). All dilution levels, except for the highest dilution 7, exhibited the characteristic colour change from orange to green. The lowest detected concentration (dilution 6) had a threshold time of 23.3 minutes and corresponded to 27.4 genome copies/reaction as determined by qPCR (Table 1). Overall, the simplified workflow showed performance levels highly competitive with lab-based methods and the findings of fluorescence staining were in perfect accordance with those obtained from real-time thermocycler analysis, which is a promising result in view of the intended application.

3.6 Validation of integrated simplified workflow with environmental water matrices

For the validation of our integrated workflow in realistic resource-limited settings, the spiked environmental water samples were also assessed with their respective LAMP assays.

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Qualitative results are shown in Table 2, while absolute threshold times for all samples can be found in S6 Table. In summary, all spiked water samples were reliably detected (3/3 replicates) for all four targets. Across all tested water matrices and target organisms, our sample processing method consistently yielded the shortest detection times and predominantly the lowest variability between replicates. For *E. coli*, the F+IL+MB method reduced detection time by several minutes (3-8 minutes) compared to P/C, with the most pronounced difference observed in wastewater samples. Similar trends were observed for *P. aeruginosa*, where notably, F+IL+MB showed the lowest variability across replicates and water types, suggesting more robust performance in complex matrices. Although the differences were smaller for *E. faecalis*, our sample processing method maintained consistent performance across all water matrices, even though the extraction efficiency was two orders of magnitude lower than for QPS, as seen in the qPCR results (Table 1). The human-associated marker HF183 exhibited similar method-dependent patterns, with F+IL+MB improving detection speed, particularly in surface and groundwater samples. No systematic increase in threshold times or loss of detection was observed across the tested water matrices, indicating that matrix-associated components did not substantially interfere with amplification under the applied conditions. All spiked water samples exhibited the characteristic colour change from orange to green (indicating positive detection) after the amplification on a heating block and staining with SYBR Green I (S5 Fig).

3.7 Additional fluorescence-based characterization of LAMP reveals semi-quantitative behaviour

To further characterize the analytical behaviour of the LAMP assays, the real-time fluorescence data were additionally analysed for a potential relationship between amplification kinetics and template concentration. This evaluation served as a complementary characterization of LAMP and was independent of the field-compatible visual readout with SYBR Green I. When comparing absolute threshold times of LAMP analyses from the dilution series as well as spiked water samples (S5 and S6 Tables), we observed that the isothermal amplification method,

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463 although generally considered a qualitative analysis, exhibited a consistent correlation between
464 amplification time and template concentration. As shown in Fig 4 for the F+IL+MB samples,
465 the time to positive signal (minutes) correlated linearly with the logarithm of the genome copy
466 numbers or marker equivalents determined by qPCR. Linear regression analysis revealed strong
467 coefficients of determination for all tested targets, indicating that LAMP signal dynamics reflect
468 target abundance over a wide dynamic range. Similar relationships were confirmed for P/C and
469 QPS extraction samples (S6 Fig). These findings therefore highlight the semi-quantitative
470 potential of fluorescence-monitored LAMP, while the simplified field-compatible workflow
471 described in this study relies on visual endpoint readout rather than real-time instrumentation.

472 <<Fig 4>>

473 *Fig 4: Comparison of results from LAMP (minutes) and qPCR (log_{10} (genome copies +1) / reaction or log_{10} (marker equivalents*
474 *+1) / reaction) for F+IL+MB extraction samples of dilution series of model organisms E. coli (triangle, yellow), P. aeruginosa*
475 *(rhombus, grey), E. faecalis (square, blue) and the HF183 marker (circle, black); Values shown are mean values from two*
476 *biological replicates and three replicates of each in qPCR and LAMP analysis; standard deviation was always below 0.44*
477 *log_{10} (genome copies +1) / reaction for qPCR and below 3.48 minutes for LAMP;*

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4. Discussion

Although molecular diagnostic tools can provide rapid, sensitive, and highly specific detection, they are still not routinely applied in global water monitoring [9]. Their use in on-site applications is even rarer [25]. To address this gap, we developed a sample processing protocol compatible for the use in resource-limited and field settings and coupled it with isothermal amplification protocols to enable rapid and simple on-site detection of selected target organisms.

Previous studies have demonstrated the feasibility of field-ready water quality assessment and achieved reliable detection of waterborne pathogens, for example by employing portable devices or diagnostics workstations (e.g. [14,26]), coupling isothermal amplification with lateral-flow assays [27], microfluidic based platforms [28] or microchips [29]. Nevertheless, many still rely on multistep sample preparation, laboratory-dependent extraction protocols, or sophisticated and expensive amplification and readout equipment. Additionally, methods often exhibit low sensitivity, high limits of detection or lack validation with real water samples. Other approaches omit dedicated DNA extraction and instead introduce the filter directly into amplification reactions, which simplifies the workflow but increases susceptibility to amplification inhibition or reduces sensitivity due to low cell lysis efficiency. The potential of applying isothermal amplification for water quality assessment has been demonstrated in multiple studies on the detection of diverse indicators and pathogens, e.g. *E. coli* [30], enterococci [23,31], *P. aeruginosa* [32], as well as *Vibrio cholerae* [33]. Other isothermal amplification assays for waterborne pathogens include *Salmonella* [34], *Legionella* [35] and *Listeria monocytogenes* [36]. Additionally, assays for human-, ruminant-, pig- or poultry-associated source tracking markers have been published [24,37,38].

Unfortunately, very few studies address the issue of sample filtration and DNA extraction, which are essential for making isothermal amplification useful for water quality assessment in the field. Conventional DNA extraction techniques often depend on the usage of hazardous

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reagents or specialized equipment and tend to be labor-intensive, time-consuming and costly [13]. These limitations become particularly problematic in low-resource environments, where complex sample preparation remains a major barrier to implementing molecular diagnostics. ILs by contrast, have emerged as promising tools for simplifying and improving nucleic acid extraction workflows. They are a class of organic salts that remain liquid at temperatures below 100 °C and have been used to isolate nucleic acids from viruses [39], bacteria [13,15] and plants [40] as well as meat [41]. ILs can also be applied for more complex matrices such as whole blood [42] or urine samples [43]. For our DNA extraction method, the hydrophilic IL [C2mim][OAc] was combined with silica-coated magnetic beads (MBs) to enable rapid and straightforward DNA purification. In this approach, nucleic acids released during lysis bind to the bead surface in the presence of chaotropic agents, while unwanted cellular material is removed through successive washing steps. We demonstrated the compatibility of this DNA extraction method with prior water sample concentration by membrane filtration, establishing a novel approach to enable rapid and on-site water quality testing. When cells are collected on membrane filters before extraction, several challenges can arise. Cells often adhere strongly to the filter surface or become trapped within its pores, making detachment and complete lysis more difficult [44]. Furthermore, the filter material itself can interfere with extraction chemistry. These effects can reduce recovery, introduce contaminants, or hinder efficient access of lysis steps to the trapped cells [45]. In initial experiments, we adapted the DNA extraction method for the application on filters. We showed that adding an additional rinsing step of the filter improved extraction efficiency and was thereby comparable to direct extraction of the cell suspension. For the target bacteria *E. coli* and *P. aeruginosa*, the developed sample processing method achieved comparable or even higher extraction efficiencies than the commercial kits. The Gram-positive bacteria *E. faecalis* proved to be a more challenging target probably due to a thicker and multi-layered cell wall, making cells more resilient to lysis [15]. Future optimisation could therefore evaluate longer lysis durations or moderately increased lysis

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temperatures to improve DNA recovery from Gram-positive organisms. Interestingly, the sample processing method with prior filtration substantially enhanced recovery for *E. faecalis* and performed close to the commercial kit.

Beyond analytical performance, the three extraction methods also differed in cost, hands-on time and equipment requirements. Including the polycarbonate membrane filter, estimated reagent and material costs were approximately €4.20 per sample for F+IL+MB, €3.00 for phenol-chloroform extraction, and €12.20 for the PowerSoil kit. Estimated hands-on times were approximately 5-10 min for F+IL+MB, 30-35 min for phenol-chloroform extraction, and 15-20 min for the PowerSoil kit, while approximate total processing times were 30-35min, 75-85 min, and 40-45 min, respectively. Although phenol-chloroform extraction had the lowest reagent costs, it required bead beating, repeated high-speed centrifugation and the handling of hazardous organic solvents. In contrast, F+IL+MB required neither bead beating nor centrifugation and relied only on comparatively simple laboratory equipment. These practical differences support the suitability of F+IL+MB for decentralized or resource-limited settings, where access to specialised equipment and appropriate facilities for handling hazardous solvents may be limited. Importantly, this simplification did not substantially compromise analytical performance. Our sample processing approach exhibited a strong linear relationship between cell numbers and genome copies or marker equivalents detected by qPCR across the tested dilution series. When evaluated on environmental water samples, our method achieved results comparable to those obtained with the commercial PowerSoil kit and surpassed phenol-chloroform extraction for most water types and target organisms. One notable exception was *P. aeruginosa* in groundwater, where QPS yielded substantially lower genome copy numbers than F+IL+MB. As this reduction was not observed in the other water matrices, it may reflect method-specific variability and/or DNA loss during the more complex bead-beating and purification procedure rather than a consistent matrix effect. Both reference methods are well established for DNA extraction in environmental aquatic microbiology. Overall, our developed

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sample processing method combined shorter processing times, lower equipment requirements and avoidance of hazardous organic solvents with analytical performance that was comparable to established laboratory-based extraction methods.

Finally, the sample processing method was combined with LAMP. For the dilution series, our workflow showed consistent detection (3/3 replicates) of all targets across the entire concentration range. Reactions run on a heating block and when visualised by SYBR Green staining were in perfect accordance with those obtained from thermocycler-based amplification. The results indicated that 30 minutes of amplification time was sufficient to detect even low concentrations. These experiments demonstrated that the proposed on-site workflow performed highly competitive with laboratory-based workflows (i.e.: bead-beating based commercial kit or phenol-chloroform extraction combined with qPCR). The validation on spiked environmental water samples demonstrated the robustness of the approach for field application. All targets were reliably detected both with thermocycler-based amplification with real-time fluorescence monitoring and heating block amplification with endpoint-SYBR Green I staining and visual read-out. Across all tested water matrices and target organisms, the novel workflow consistently yielded the fastest detection times, very low variability between replicates and no significant effects of any background matrices. Additionally, there are indications that the IL-based sample processing was enhancing the LAMP assays as evident in shorter threshold times, which might prove beneficial for rapid on-site analysis.

Furthermore, we also found that, despite being regarded as a qualitative method, LAMP showed a clear inverse relationship between amplification time and template concentration. Under fluorescence-monitored conditions, this behaviour indicates that LAMP can offer at least semi-quantitative insights. This finding has also been explored by other publications, which have proposed quantitative isothermal amplification methods based on colour changes measured by visual inspection, real-time fluorescence measurements or specialized software for smartphone cameras [46–48]. The development of quantitative isothermal amplification methods would be

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583 an important step, as they enable reliable risk, process and method assessment beyond mere
584 detection of presence, and could thus advance their establishment in molecular water quality
585 analysis.
586 Several limitations and opportunities for further development should nevertheless be
587 considered. As the workflow is readily adaptable to additional microbial targets and
588 environmental water matrices, further studies should validate it using a broader range of water-
589 related pathogens and environmental water matrices. In addition, improving the detection limit
590 will be important for targets that may pose a health risk even when present at low
591 concentrations. For the same reason, our workflow could be particularly useful for the detection
592 of faecal indicators and microbial source tracking markers, as these targets often occur in high
593 concentrations. To further increase operational simplicity, reagents could be lyophilised and
594 supplied in ready-to-use reaction tubes. Integration of extraction and LAMP into a lab-on-a-
595 chip platform could ultimately enable compact and battery-powered diagnostic devices.
596 The proposed workflow still requires simple laboratory equipment and electric power. Its most
597 practical application may therefore lie in decentralized laboratory settings or mobile testing
598 units that lack advanced molecular biology infrastructure and specialized personnel. The
599 current workflow can thus be regarded as an intermediate implementation stage between
600 conventional centralised molecular diagnostics and fully integrated point-of-need systems.

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5. Conclusion

This study contributes to overcoming key barriers that have so far limited the widespread integration of molecular diagnostics into routine water quality monitoring. The successful combination of field-compatible DNA extraction with isothermal amplification enabled the rapid and reliable detection of selected targets in environmental water samples. The integrated workflow achieved performance comparable to established laboratory-based reference methods while reducing processing time, methodological complexity, and dependence on specialised instrumentation. By addressing critical time-based and logistical limitations inherent to conventional monitoring strategies, the workflow provides a practical basis for decentralised water quality testing and may support rapid screening and timely on-site risk assessment.

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Author contribution L.P. and G.H.R. designed the experiments. L.P. conducted the experiments and, together with G.H.R., prepared the manuscript. G.H.R. supervised the project. C.K. helped supervise the project. L.C., A.K.T.K., A.H.F. and C.K. provided critical feedback and helped shape the manuscript.

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References

1. Shayo GM, Elimbinzi E, Shao GN, Fabian C. Severity of waterborne diseases in developing countries and the effectiveness of ceramic filters for improving water quality. Bull Natl Res Cent. 2023; 47(1):113. doi:10.1186/s42269-023-01088-9.

2. Nnachi RC, Sui N, Ke B, Luo Z, Bhalla N, He D, et al. Biosensors for rapid detection of bacterial pathogens in water, food and environment. Environ Int. 2022; 166:107357. doi:10.1016/j.envint.2022.107357 PubMed PMID: 35777116.

3. Griffith JF, Cao Y, McGee CD, Weisberg SB. Evaluation of rapid methods and novel indicators for assessing microbiological beach water quality. Water Res. 2009; 43(19):4900-4907. doi:10.1016/j.watres.2009.09.017.

4. Li L, Mendis N, Trigui H, Oliver JD, Faucher SP. The importance of the viable but non-culturable state in human bacterial pathogens. Front Microbiol. 2014; 5:258 doi:10.3389/fmicb.2014.00258.

5. Girones R, Ferrús MA, Alonso JL, Rodriguez-Manzano J, Calgua B, de Abreu Corrêa A, et al. Molecular detection of pathogens in water - The pros and cons of molecular techniques. Water Res. 2010; 44:4325-4339. doi:10.1016/j.watres.2010.06.030.

6. Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): A rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother. 2009; 15:62-69. doi:10.1007/s10156-009-0669-9.

7. Green HC, Haugland RA, Varma M, Millen HT, Borchardt MA, Field KG, et al. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. Appl Environ Microbiol. 2014; 80(10):3086-3094. doi:10.1128/AEM.04137-13.

8. Reischer GH, Kasper DC, Steinborn R, Mach RL, Farnleitner AH. Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation

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of this method in alpine karstic regions. Appl Environ Microbiol. 2006; 72(8):5610-5614. doi:10.1128/AEM.00364-06.

9. Demeter K, Linke R, Ballesté E, Reischer G, Mayer RE, Vierheilig J, et al. Have genetic targets for faecal pollution diagnostics and source tracking revolutionised water quality analysis yet? FEMS Microbiol Rev. 2023; 47(4):fuad028. doi:10.1093/femsre/fuad028.

10. Farnleitner A, Zibuschka F. The intriguing new possibilities of the microbiological methodology and molecular diagnostics for water quality analysis. Österreichische Wasser- und Abfallwirtschaft. 2021; 73(11-12):446-449. doi:10.1007/s00506-021-00821-w.

11. Fernández-Baca CP, Spirito CM, Bae JS, Szegletes ZM, Barott N, Sausele DJ, et al. Rapid qPCR-Based Water Quality Monitoring in New York State Recreational Waters. Front Water. 2021; 3:711477. doi:10.3389/frwa.2021.711477.

12. Barbosa C, Nogueira S, Gadanho M, Chaves S. DNA extraction: Finding the most suitable method. In: Molecular Microbial Diagnostic Methods: Pathways to Implementation for the Food and Water Industries. San Diego: Academic Press; 2016. p. 135-54. doi:10.1016/B978-0-12-416999-9.00007-1.

13. Martzy R, Bica-Schröder K, Pálvölgyi ÁM, Kolm C, Jakwerth S, Kirschner AKT, et al. Simple lysis of bacterial cells for DNA-based diagnostics using hydrophilic ionic liquids. Sci Rep. 2019; 9(1):13994. doi:10.1038/s41598-019-50246-5.

14. Jiang YS, Riedel TE, Popoola JA, Morrow BR, Cai S, Ellington AD, et al. Portable platform for rapid in-field identification of human fecal pollution in water. Water Res. 2018; 131:186-195. doi:10.1016/j.watres.2017.12.023.

15. Kreuter J, Bica-Schröder K, Pálvölgyi ÁM, Krska R, Sommer R, Farnleitner AH, et al. A novel ionic liquid-based approach for DNA and RNA extraction simplifies sample preparation for bacterial diagnostics. Anal Bioanal Chem. 2024; 416(29):7109-7120. doi:10.1007/s00216-024-05615-z.

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674 16. Griffiths RI, Whiteley AS, O'donnell AG, Bailey MJ. Rapid Method for Coextraction of
675 DNA and RNA from Natural Environments for Analysis of Ribosomal DNA-and rRNA-
676 Based Microbial Community Composition. Appl Environ Microbiol. 2000; 66:5488-
677 5491. doi: 10.1128/AEM.66.12.5488-5491.2000.
678 17. Chern EC, Siefring S, Paar J, Doolittle M, Haugland RA. Comparison of quantitative
679 PCR assays for Escherichia coli targeting ribosomal RNA and single copy genes. Lett
680 Appl Microbiol. 2011; 52(3):298-306. doi:10.1111/j.1472-765X.2010.03001.x.
681 18. Lee CS, Wetzel K, Buckley T, Wozniak D, Lee J. Rapid and sensitive detection of
682 Pseudomonas aeruginosa in chlorinated water and aerosols targeting gyrB gene using
683 real-time PCR. J Appl Microbiol. 2011; 111(4):893-903. doi:10.1111/j.1365-
684 2672.2011.05107.x.
685 19. U.S. EPA Method 1611. Method 1611.1: Enterococci in Water by TaqMan ®
686 Quantitative Polymerase Chain Reaction (qPCR). Washington (DC): U.S.
687 Environmental Protection Agency, Office of Water; 2015. Report No.: EPA-820-R-15-
688 008.
689 20. U.S. EPA Method 1609. Method 1609: Enterococci in Water by TaqMan® Quantitative
690 Polymerase Chain Reaction (qPCR) with Internal Amplification Control (IAC) Assay.
691 Washington (DC): U.S. Environmental Protection Agency, Office of Water; 2013.
692 Report No.: EPA-820-R-13-005.
693 21. Hill J, Beriwal S, Chandra I, Paul VK, Kapil A, Singh T, et al. Loop-mediated isothermal
694 amplification assay for rapid detection of common strains of Escherichia coli. J Clin
695 Microbiol. 2008; 46(8):2800-2804. doi:10.1128/JCM.00152-08.
696 22. Dong K, Kang Z, Ji X, Zhang X, Cheng P, Sun B. A Loop-mediated Isothermal
697 Amplification With a Nanoparticle-Based Lateral Flow Biosensor Assay to Detect
698 Pseudomonas aeruginosa in Endophthalmitis. Transl Vis Sci Technol. 2021; 10(14):26.
699 doi:10.1167/TVST.10.14.26.

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700 23. Martzy R, Kolm C, Brunner K, Mach RL, Krska R, Šinkovec H, et al. A loop-mediated
701 isothermal amplification (LAMP) assay for the rapid detection of Enterococcus spp. in
702 water. Water Res. 2017; 122:62-69. doi:10.1016/j.watres.2017.05.023.
703 24. Khodaparast M, Sharley D, Best N, Marshall S, Beddoe T. In-field LAMP assay for rapid
704 detection of human faecal contamination in environmental water. Environ Sci Water Res
705 Technol. 2022; 8(11):2641-2651. doi:10.1039/d2ew00433j.
706 25. Martzy R, Kolm C, Krska R, Mach RL, Farnleitner AH, Reischer GH. Challenges and
707 perspectives in the application of isothermal DNA amplification methods for food and
708 water analysis. Anal Bioanal Chem. 2019; 411(9):1695-1702. doi:10.1007/s00216-018-
709 1553-1.
710 26. Alfahl Z, Biggins S, Higgins O, Chueiri A, Smith TJ, Morris D, et al. A rapid on-site
711 loop-mediated isothermal amplification technology as an early warning system for the
712 detection of Shiga toxin-producing Escherichia coli in water. Microbiology (Reading).
713 2024; 170(8):001485. doi:10.1099/mic.0.001485.
714 27. Batra AR, Cottam D, Lepesteur M, Dexter C, Zuccala K, Martino C, et al. Development
715 of A Rapid, Low-Cost Portable Detection Assay for Enterococci in Wastewater and
716 Environmental Waters. Microorganisms. 2023; 11(2):381.
717 doi:10.3390/microorganisms11020381.
718 28. Gowda HN, Kido H, Wu X, Shoval O, Lee A, Lorenzana A, et al. Development of a
719 proof-of-concept microfluidic portable pathogen analysis system for water quality
720 monitoring. Sci Total Environ. 2022; 813:152556. doi:10.1016/j.scitotenv.2021.152556.
721 29. Fu J, Chiang ELC, Medriano CAD, Li L, Bae S. Rapid quantification of fecal indicator
722 bacteria in water using the most probable number - loop-mediated isothermal
723 amplification (MPN-LAMP) approach on a polymethyl methacrylate (PMMA)
724 microchip. Water Res. 2021; 199:117172. doi:10.1016/j.watres.2021.117172.

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30. Manzanas C, Morrison E, Kim YS, Alipanah M, Adedokun G, Jin S, et al. Molecular testing devices for on-site detection of E. coli in water samples. Sci Rep. 2023; 13(1):4245. doi:10.1038/s41598-023-31208-4.

31. Kolm C, Martzy R, Brunner K, Mach RL, Krska R, Heinze G, et al. A Complementary Isothermal Amplification Method to the U.S. EPA Quantitative Polymerase Chain Reaction Approach for the Detection of Enterococci in Environmental Waters. Environ Sci Technol. 2017; 51(12):7028-7035. doi:10.1021/acs.est.7b01074.

32. Tran DH, Tran HT, Pham TNM, Phung HTT. Direct multiplex recombinase polymerase amplification for rapid detection of Staphylococcus aureus and Pseudomonas aeruginosa in food. Mol Biol Res Commun. 2022; 11(1):1–10. doi:10.22099/mbrc.2021.41503.1664.

33. Shen Z, Liu Y, Chen L. Qualitative and quantitative detection of potentially virulent vibrio parahaemolyticus in drinking water and commonly consumed aquatic products by loop-mediated isothermal amplification. Pathogens. 2022; 11(1):10. doi:10.3390/pathogens11010010.

34. Tourlousse DM, Ahmad F, Stedtfeld RD, Seyrig G, Tiedje JM, Hashsham SA. A polymer microfluidic chip for quantitative detection of multiple water-and foodborne pathogens using real-time fluorogenic loop-mediated isothermal amplification. Biomed Microdevices. 2012; 14(4):769-778. doi:10.1007/s10544-012-9658-3.

35. Furuhata K, Annaka T, Ikedo M, Fukuyama M, Yoshida SI. Comparison of Loop-Mediated Isothermal Amplification (LAMP) and Conventional Culture for the Detection of Legionella Species in Hot Spring Water Samples in Japan. Biocontrol Sci. 2005; 10(3):117-120. doi:10.4265/bio.10.117.

36. Nathaniel BR, Ghai M, Druce M, Maharaj I, Olaniran AO. Development of a loop-mediated isothermal amplification assay targeting lmo0753 gene for detection of Listeria

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monocytogenes in wastewater. Lett Appl Microbiol. 2019; 69(4):264-270. doi:10.1111/lam.13200.

37. do Nascimento MCA, Smith WJM, Gebrewold M, Liu Y, Simpson SL, Bivins A, et al. Development and evaluation of a colorimetric LAMP based-assay targeting the Bacteroides HF183 marker for tracking sewage pollution in environmental waters. Water Res. 2024; 264:122202. doi:10.1016/j.watres.2024.122202.

38. Kolm C, Martzy R, Führer M, Mach RL, Krska R, Baumgartner S, et al. Detection of a microbial source tracking marker by isothermal helicase-dependent amplification and a nucleic acid lateral-flow strip test. Sci Rep. 2019; 9(1):393. doi:10.1038/s41598-018-36749-7.

39. Fister S, Fuchs S, Mester P, Kilpeläinen I, Wagner M, Rossmanith P. The use of ionic liquids for cracking viruses for isolation of nucleic acids. Sep Purif Technol. 2015; 155:38-44. doi:10.1016/j.seppur.2015.03.035.

40. Gonzalez García E, Ressmann AK, Gaertner P, Zirbs R, Mach RL, Krska R, et al. Direct extraction of genomic DNA from maize with aqueous ionic liquid buffer systems for applications in genetically modified organisms analysis. Anal Bioanal Chem. 2014; 406(30):7773-7784. doi:10.1007/s00216-014-8204-y.

41. Ressmann AK, García EG, Khlan D, Gaertner P, Mach RL, Krska R, et al. Fast and efficient extraction of DNA from meat and meat derived products using aqueous ionic liquid buffer systems. New J Chemistry. 2015; 39(6):4994-5002. doi:10.1039/c5nj00178a.

42. Emaus MN, Cagliero C, Gostel MR, Johnson G, Anderson JL. Simple and efficient isolation of plant genomic DNA using magnetic ionic liquids. Plant Methods. 2022; 18(1):37. doi:10.1186/s13007-022-00860-8.

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774 43. Kreuter J, Piglmann L, Priselac K, Martzy R, Ante M, Walter D, et al. A rapid ionic
775 liquid-based DNA extraction method for molecular diagnostics of urinary tract
776 infections. Microbiol Spectr. 2026; 14(4):e0319125. doi: 10.1128/spectrum.03191-25.
777 44. Shi XJ, Liu G, Zhang M, Zhao J, Li H, Yang Z, et al. Membrane-sensitive bacterial DNA
778 extractions and absolute quantitation of recovery efficiencies. Sci Total Environ. 2020;
779 708:135125. doi:10.1016/j.scitotenv.2019.135125.
780 45. Putri RE, Vrouwenvelder JS, Farhat N. Enhancing the DNA yield intended for microbial
781 sequencing from a low-biomass chlorinated drinking water. Front Microbiol. 2024;
782 15:1339844. doi:10.3389/fmicb.2024.1339844.
783 46. González-González E, Lara-Mayorga IM, Rodríguez-Sánchez IP, Zhang YS, Martínez-
784 Chapa SO, Santiago GT De, et al. Colorimetric loop-mediated isothermal amplification
785 (LAMP) for cost-effective and quantitative detection of SARS-CoV-2: The change in
786 color in LAMP-based assays quantitatively correlates with viral copy number. Anal
787 Methods. 2021; 13(2):169-178. doi:10.1039/d0ay01658f.
788 47. Sullivan BP, Chou YS, Bender AT, Martin CD, Kaputa ZG, March H, et al. Quantitative
789 isothermal amplification on paper membranes using amplification nucleation site
790 analysis. Lab Chip. 2022; 22(12):2352-2363. doi:10.1039/d2lc00007e.
791 48. Zhang Y, Li X, Zhang S, Ma T, Mao C, Zhang C. Quantitative Loop-Mediated
792 Isothermal Amplification Detection of Ustilaginoidea virens Causing Rice False Smut.
793 Int J Mol Sci. 2023; 24(12):10388. doi:10.3390/ijms241210388.
794

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Supporting information

S1 Table. Oligonucleotides used for qPCR and LAMP assays in this study. Summary of primers and probes used for qPCR and LAMP detection of the investigated microbial targets.

S2 Table. Reaction compositions and amplification conditions of the qPCR and LAMP assays used in this study. Summary of reaction compositions and amplification conditions for the target-specific qPCR and LAMP assays for *E. coli*, *P. aeruginosa*, *E. faecalis*, and HF183.

S1 Fig. Establishment of the filter-based IL+MB DNA extraction procedure using *E. coli*. The previously established IL+MB extraction method was adapted for DNA extraction from membrane filters. Direct extraction of the corresponding cell suspension using the QIAamp DNA Mini Kit (Qiagen) and the original IL+MB protocol served as reference methods. For optimization of the filter-based procedure, different volumes of ionic liquid (IL; 500 µL, 250 µL, and 90 µL) and different approaches to ensure contact between the filter and IL (shaking or vortexing) were evaluated. Following lysis, DNA was purified using magnetic beads (MB). Boxes represent the 25th–75th percentiles, the horizontal line indicates the median, and whiskers indicate minimum and maximum values. Data represent two independent biological replicates, each analysed in technical triplicate.

S2 Fig. Effect of an additional filter rinsing step on DNA recovery using the F+IL+MB procedure.

The effect of additional filter rinsing was evaluated using *E. coli*. Direct extraction of the corresponding cell suspension using the QIAamp DNA Mini Kit (Qiagen) and the original IL+MB protocol served as reference methods. Following IL-mediated lysis, filters were rinsed either once with 765 µL, twice with 383 µL, or three times with 255 µL of 10 mM Tris (pH 8) before magnetic bead-based DNA purification. Boxes represent the 25th–75th percentiles, the horizontal line indicates the median, and whiskers indicate minimum and maximum values.

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Data were obtained from three independent biological replicates, each analysed in technical triplicate.

S3 Table. Absolute genome copies obtained during validation of the sample processing method with bacterial reference strains.

Measured genome copies per µL obtained after DNA extraction using the filter-based F+IL+MB procedure, the QIAamp DNA Mini Kit, and the original IL+MB protocol without prior filtration. Values are presented as means from three independent biological replicates, each analysed in technical triplicate, with the corresponding standard deviations.

S4 Table. Cell counts of bacterial overnight cultures determined by epifluorescence microscopy.

Cell concentrations of the bacterial cultures used in this study as determined by epifluorescence microscopy. Values from three independent biological replicates, their means, and corresponding standard deviations are shown.

S3 Fig. Epifluorescence microscopy of bacterial reference cultures used for DNA extraction experiments.

Representative images of freshly grown bacterial cell suspensions used for DNA extraction experiments. Cells were fixed with paraformaldehyde, filtered through 0.2-µm Anodisc 25 filters, and visualized by epifluorescence microscopy. (A) *E. coli*, (B) *P. aeruginosa*, and (C) *E. faecalis*. Scale bar, 10 µm.

S5 Table. LAMP threshold times obtained from dilution series of the selected microbial targets.

Results from dilution series analysed using the respective target-specific LAMP assays following DNA extraction by phenol-chloroform extraction (P/C), the Qiagen PowerSoil Kit (QPS), or the developed filter-based IL+MB procedure (F+IL+MB). Each dilution step was analysed in two independent biological replicates (A and B). Values represent mean threshold times (tt, min) obtained from three amplification replicates.

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S4 Fig. Visual endpoint detection of LAMP products from an *E. coli* dilution series following F+IL+MB extraction.
DNA extracted using F+IL+MB was amplified on a heating block for 60 min at 66°C and subsequently stained with SYBR Green I. (A) Visual evaluation of amplified products by the naked eye. Samples from left to right: negative control followed by dilution steps 7 to 0. (B) Visualization of the same amplification products under UV illumination. Samples are arranged from the highest dilution (dilution step 7) to the undiluted sample (dilution step 0).

S6 Table. LAMP threshold times obtained from spiked environmental water samples.
Wastewater, surface water, and groundwater samples were individually spiked with *E. coli*, *P. aeruginosa*, *E. faecalis*, or HF183 and processed using phenol-chloroform extraction (P/C), the Qiagen PowerSoil Kit (QPS), or the developed filter-based IL+MB procedure (F+IL+MB). Threshold times are reported in minutes. Colour shading indicates the threshold time relative to the minimum and maximum values obtained for the respective target. Values represent means from three amplification replicates.

S5 Fig. Visual endpoint detection of LAMP products from spiked environmental water samples following F+IL+MB extraction.
DNA extracted from spiked wastewater, surface water, and groundwater samples using F+IL+MB was amplified on a heating block for 60 min at the respective target-specific LAMP temperature and subsequently stained with SYBR Green I. Samples from left to right: negative control, wastewater samples (1–2), surface water samples (3–4), and groundwater samples (5–6). Each water matrix was analysed in two independent biological replicates. (A) *E. coli*, (B) *P. aeruginosa*, (C) *E. faecalis*, and (D) HF183.

S6 Fig. Relationship between LAMP threshold time and qPCR-derived target concentration for the three extraction methods.
Comparison of LAMP threshold times (min) with target concentrations determined by qPCR for dilution series of the investigated targets. qPCR results are expressed as log10(genome

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874 copies + 1) per reaction for bacterial targets or log10(marker equivalents + 1) per reaction for
875 HF183. Results are shown for phenol-chloroform extraction (P/C; circles), the Qiagen
876 PowerSoil Kit (QPS; triangles), and the developed filter-based IL+MB procedure (F+IL+MB;
877 diamonds). Panels show (A) *E. coli*, (B) *P. aeruginosa*, (C) *E. faecalis*, and (D) HF183. Values
878 shown are mean values from two biological replicates and three replicates of each in qPCR and
879 LAMP analysis.
880

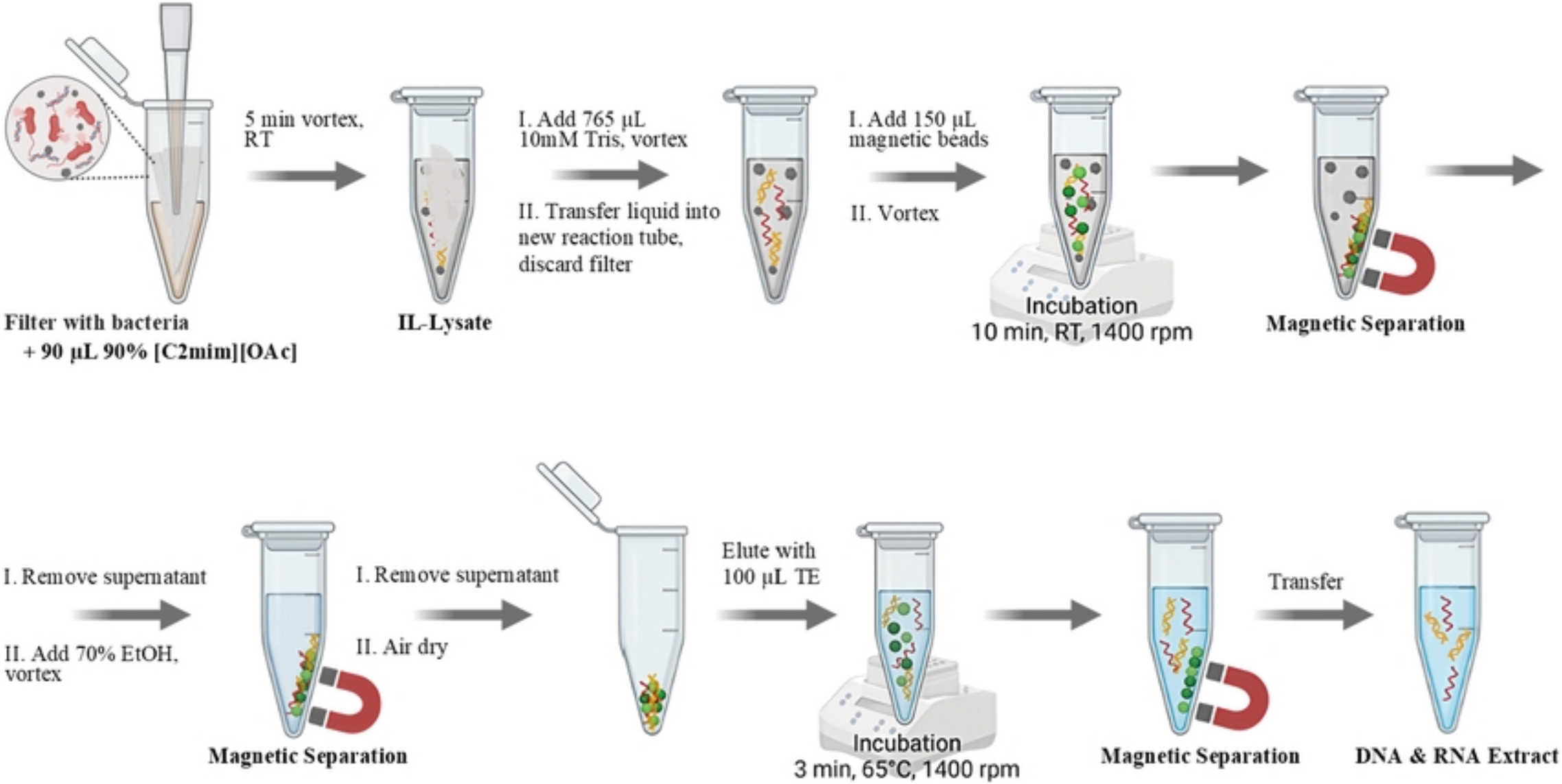

5 min vortex, RT
I. Add 765 µL 10mM Tris, vortex
II. Transfer liquid into new reaction tube, discard filter
I. Add 150 µL magnetic beads
II. Vortex
Filter with bacteria
+ 90 µL 90% [C2mim][OAc]
IL-Lysate
Incubation
10 min, RT, 1400 rpm
Magnetic Separation
I. Remove supernatant
II. Add 70% EtOH, vortex
Magnetic Separation
I. Remove supernatant
II. Air dry
Elute with 100 µL TE
Incubation
3 min, 65°C, 1400 rpm
Magnetic Separation
Transfer
DNA & RNA Extract

Figure_1

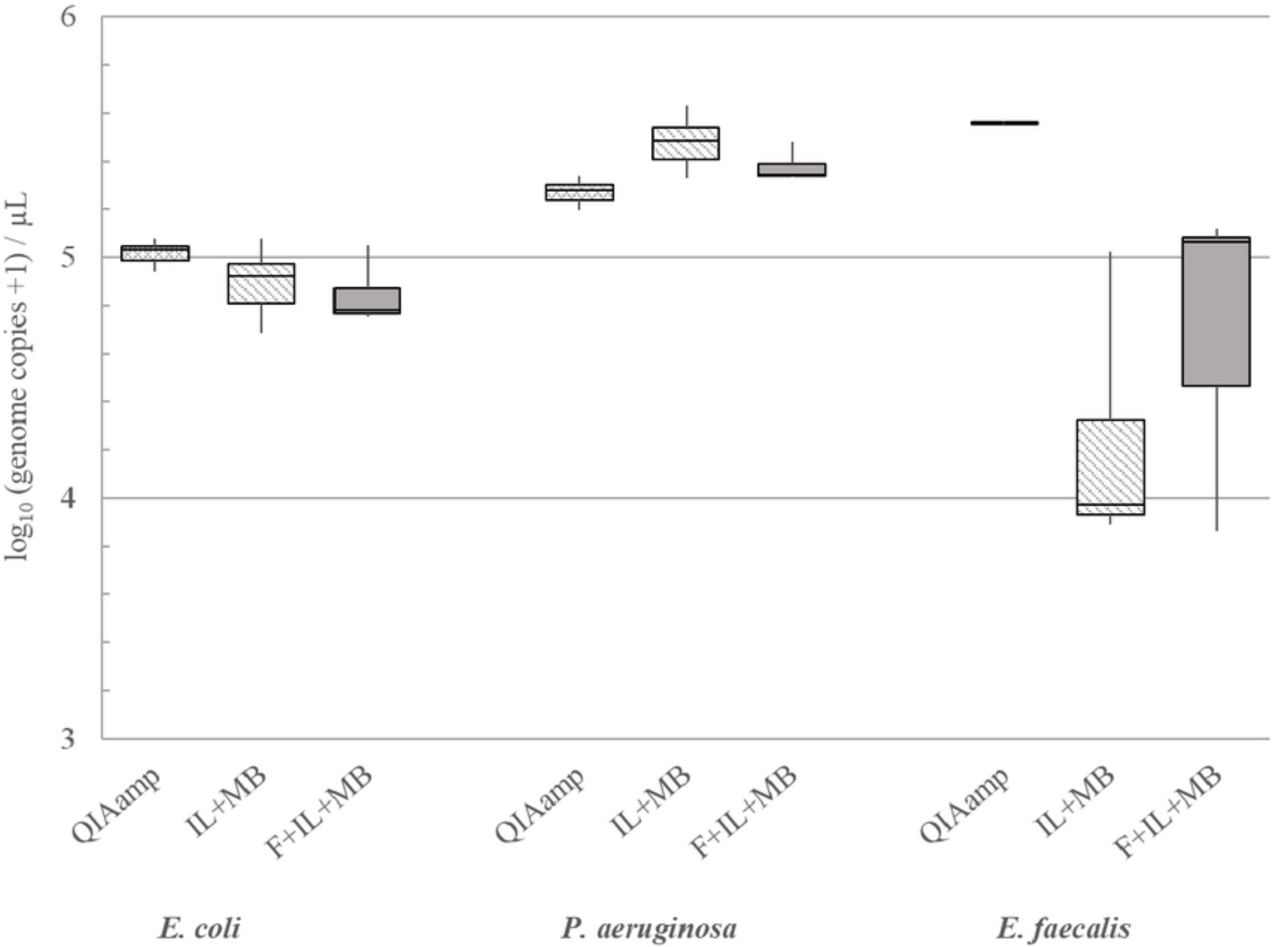

6
5
4
3
log10 (genome copies +1) / µL
QIAamp
IL+MB
F+IL+MB
QIAamp
IL+MB
F+IL+MB
QIAamp
IL+MB
F+IL+MB
E. coli
P. aeruginosa
E. faecalis

Figure_2

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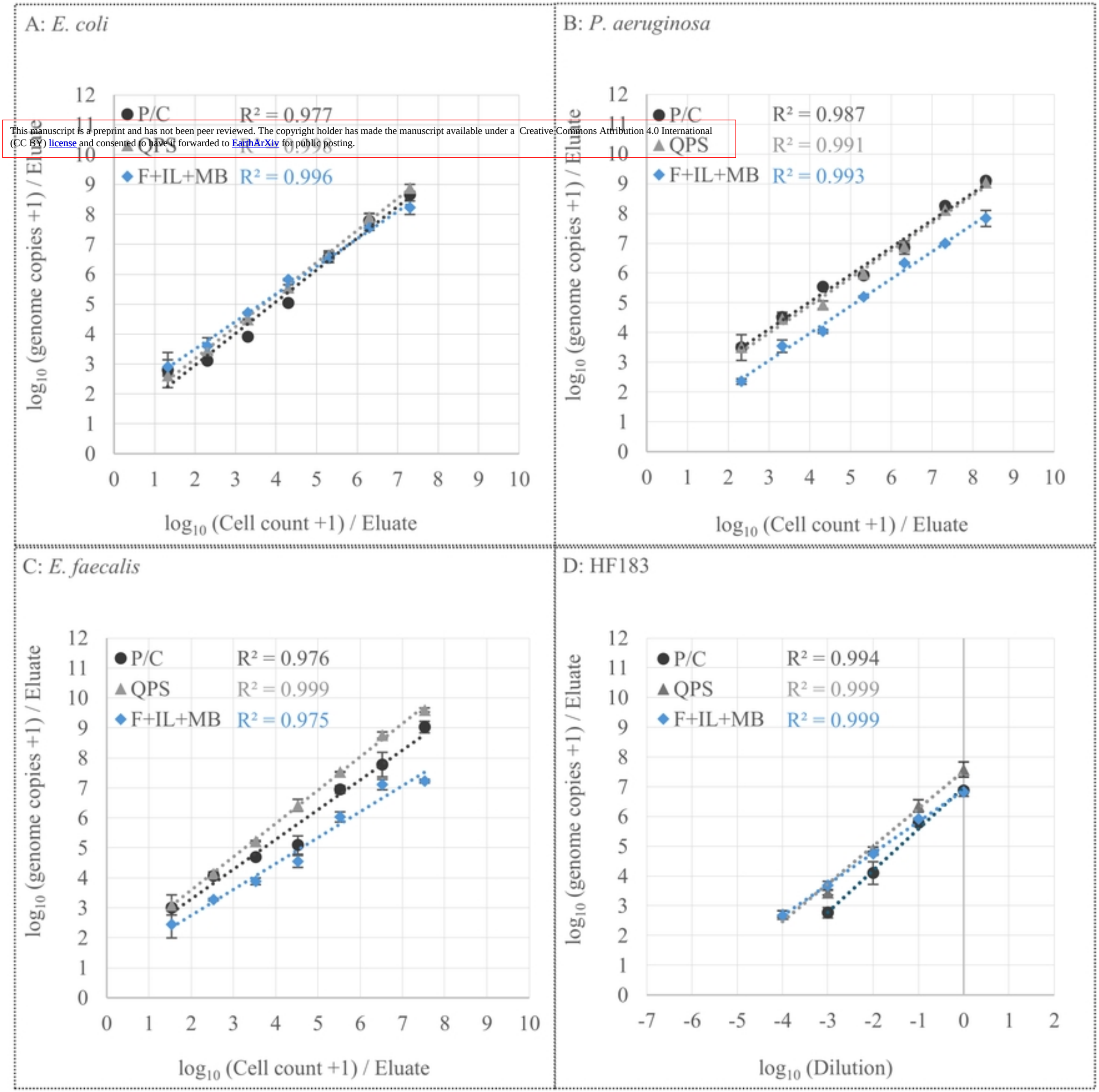


Figure_3

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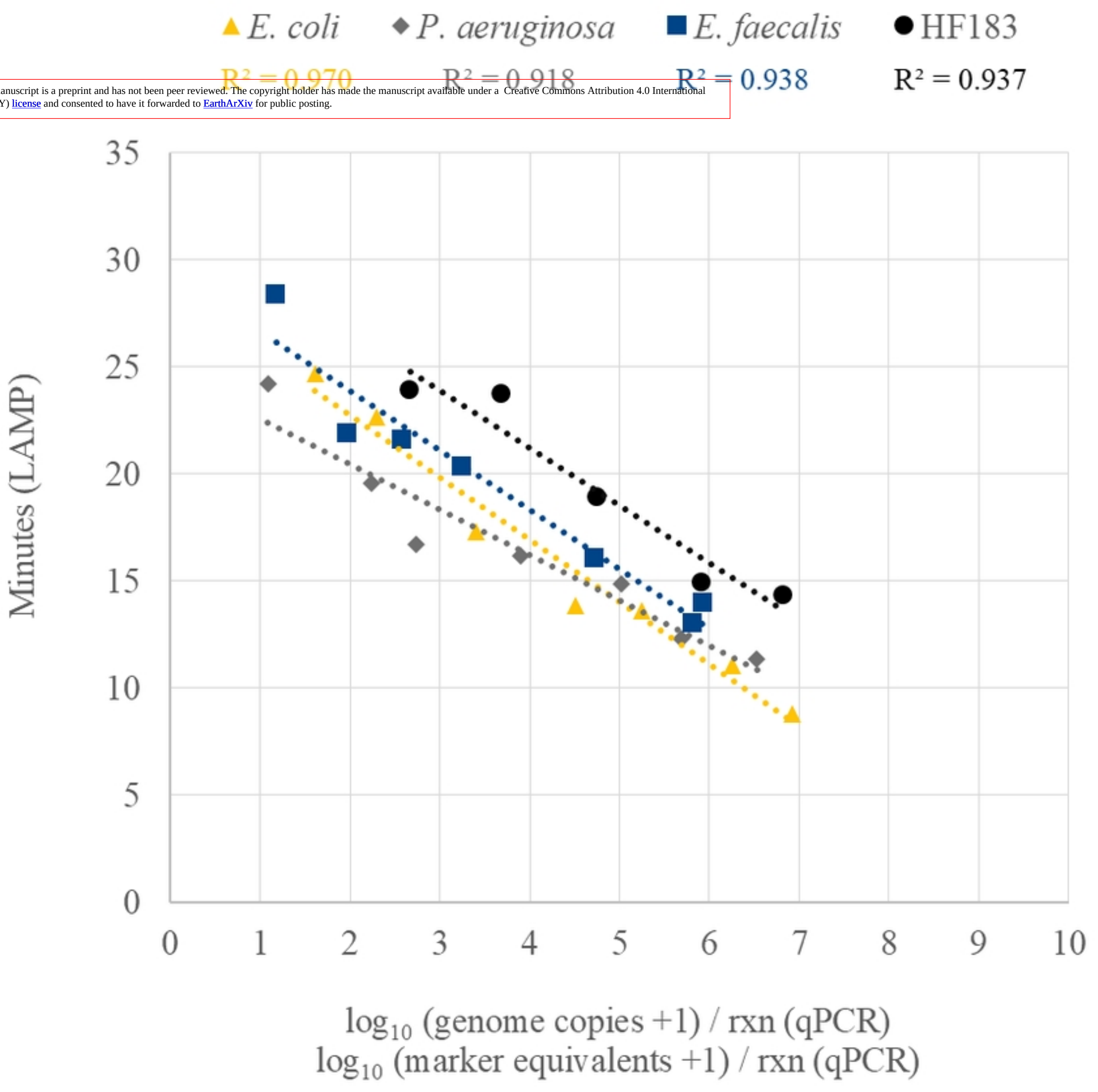


Figure_4