



A new design of pressure-driven microbiological filter unit suited for ‘on-the-filter’ cell lysis in DNA-testing applications

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ABSTRACT

DNA analysis techniques such as Polymerase Chain reaction are invaluable for the detection of microorganisms in a range of sample types, many of which are bulk liquids such as natural and potable waters, homogenised foodstuffs, blood and other clinical fluids. In each case, the first stage is the collection and pre-concentration of cells and particles using filtration, followed by the preparation of a lysate for DNA extraction and testing. For this purpose, single-use pressure-driven filter units and on-filter lysis protocols are often preferable for speed and practicality, as well as to reduce contamination. This study presents a new design of pressure filter unit and a battery-powered device to achieve this sample preparation in a manner that may be advantageous over current, commercially available units and typical laboratory apparatus and practices. The filter design is thin, flexible and features an inflatable filter chamber that accommodates lysis reagents. The device efficiently heats and agitates the filter membrane in a direct manner resulting in the generation of a lysate, which can be easily removed for further processing. Testing against a market leader, Sterivex™, the new design was shown to support the generation of DNA with equivalent or greater yield, dependent on starting sample, and equivalent quality. The development and adoption of the new design may, for some applications, offer a more convenient and cost-effective approach.

1. Introduction

DNA extraction followed by Polymerase Chain Reaction offers a sensitive, specific and rapid alternative to cytological methods such as microscopy and differential-selective culture for the detection of hazardous microorganisms in clinical, food and environmental samples (Bouchez et al., 2016; Butz and Patócs, 2019; Dutta et al., 2022). It is particularly valuable for the surveillance of water quality indicators including faecal indicator bacteria, water-borne enteric pathogens and toxigenic algae because, unlike traditional methods, PCR can detect target cells in a background of overlapping morphological or biochemical characteristics and is not limited to the analysis of only the viable and culturable portion of diverse microbial communities (Silva and Domingues, 2015; Walker et al., 2017; Oon et al., 2023). It is also well-suited for automation and point of sample testing because it requires low (microlitre-scale) reagent volumes and produces a ‘simple’ optical (fluorometric/colourimetric) signal derived from mixtures that

can be stabilised for storage and transport, then activated on need (McQuillan and Robidart, 2017; Scholin et al., 2017). However, a successful PCR test is dependent on a robust sample processing workflow to pre-concentrate, extract and purify DNA, and this can be challenging to implement without proper facilities.

Usually, the first stage in the preparation of DNA from a water sample is the collection and concentration of microbial cells and eDNA on a filter membrane using a pressure-driven filter device. Re-usable assemblies for vacuum filtration such as those based on the Buckner funnel design are commonly employed in a laboratory setting, however if working on multiple samples the need to decontaminate the assembly to avoid cross-contamination can be time-consuming and laborious. In contrast, single-use, disposable designs such as Sterivex™, a small and self-contained plastic filter cartridge which connects to a syringe or fluid line, offer speed and convenience, and are a preferred choice for water analysis applications, particularly when collecting field samples (Majaneva et al., 2018; Andreou et al., 2023).

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After filtration, the next step is to expose the retentate to a chemical solution which promotes the detachment and lysis of captured cells, the release of nucleic acids and the denaturation of nucleases in preparation for DNA purification and PCR (Shehadul Islam et al., 2017). Heat and mechanical agitation are usually important to maximise the yield and quality of extracted DNA, particularly when working with robust cell types such as phytoplankton and Gram-positive bacteria. This stage of the workflow is critical because the efficiency of cell capture and lysis is proportional to the final yield and integrity of DNA, and therefore the overall sensitivity of the approach. For practical reasons it is often preferable to carry out this step directly within the filter chamber. However, Sterivex™ and similar existing designs are poorly suited for this purpose. They are constructed with a cylindrical, thermally-insulative and rigid plastic shell, which is difficult to interface with external heaters and mechanisms in a way which promotes efficient thermal or kinetic energy transfer to the interior. Additionally, their rigid housings incur a pressure imbalance as fluid and air displace one another when filling or evacuating the chamber unless both inlet and outlet are open (i.e., the unit is vented), complicating overall operation and increasing the likelihood of leakage, contamination or sample loss. Additionally, they are expensive and can create large quantities of

plastic waste and therefore a design that achieves a reduction in cost and materials would be advantageous.

In this study, we present and demonstrate a novel design and prototype of a single-use, pressure-driven filter unit that addresses these issues. We also describe a custom-built, portable and battery-powered device engineered to work specifically with the new design, to efficiently heat and agitate the filter membrane enabling cell lysis directly within the filter chamber. The system was evaluated in comparison with Sterivex™ in a workflow designed to pre-concentrate, extract and amplify bacterial DNA from natural water samples with a view towards applications using targets nucleic acid amplification assays.

2. Materials and methods

2.1. Filter unit design and fabrication

The design was generated using Fusion360 Software (AutoDesk, USA). The design was based on laminating plastic film and adhesive layers directly with a filter membrane material, with the outer layers of the laminate being embossed to create a pliable and inflatable filter chamber, but the native structure remaining flat. The overall

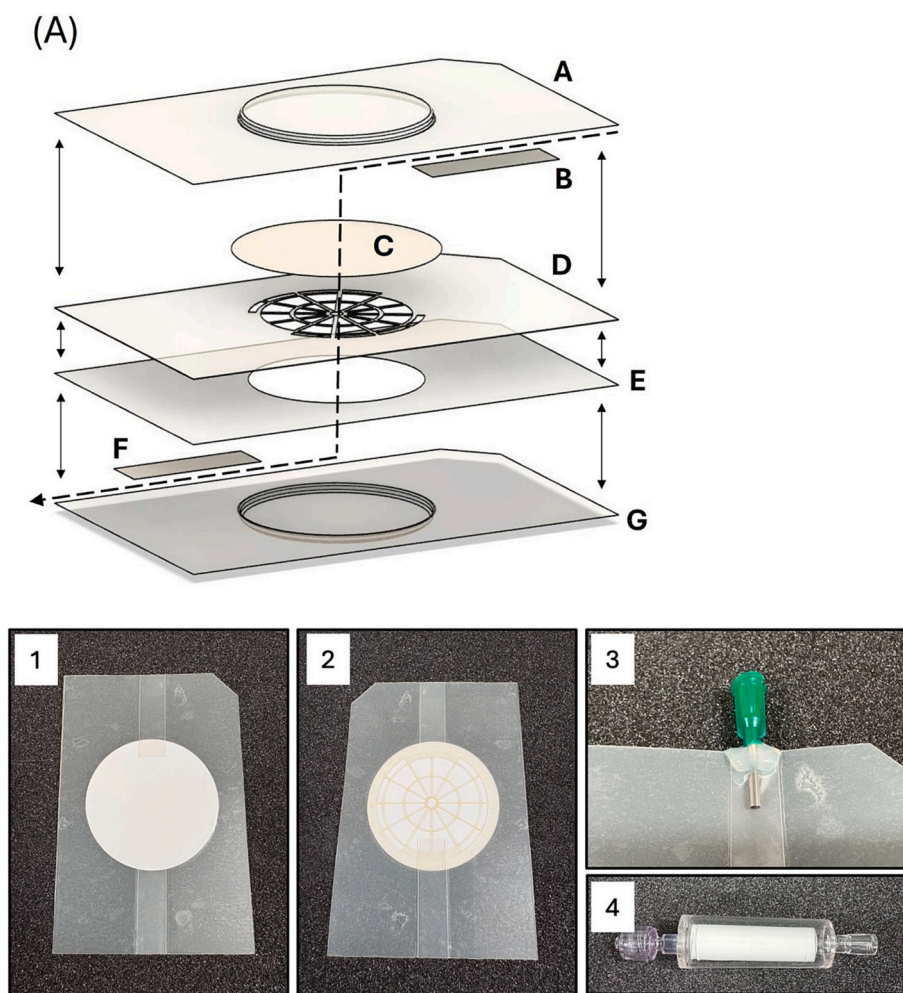


Fig. 1. A laminate filter design and prototype, constructed by laminating layers of polyester film, a microbiological-grade filter membrane material and sheet adhesive. Panel (A) shows an exploded view of the design, illustrating the order of layering: A and G are embossed 23-μm polyester films; B and F are 23-μm polyester films that form inlet and outlet channels with A and G respectively; C is a 47 mm diameter filter membrane circle; D is an acrylic adhesive overlaid onto a 100-μm thick polyester film, which acts as a filter membrane support; E is an acrylic adhesive that bonds D to G. The top surface of component D is adhesive, therefore directly bonds and seals the perimeter of the filter membrane such that the sample must pass through, rather than around the membrane. The dashed line indicates the path of fluid flow. Below are photographs of the filters: images 1 and 2 show an assembled laminate filter unit viewed from the top and bottom respectively. Image 3 shows a Luer-taper fitting secured with thermo-polymer glue to produce an interface for a fluid line or syringe. Image 4 shows, for comparison, a Sterivex™ unit, an archetypical and commercially available, rigid-bodied pressure-driven filter unit.

construction is shown in the results section, Fig. 1. Materials were selected based on their physical and chemical robustness. The main structure was constructed from biaxially oriented polyethylene terephthalate (BoPET/‘Mylar®’) (RS Components, UK). The layers were bonded using a water- and temperature-resistant acrylic-based sheet adhesive, 468MP (3 M, UK). Film and adhesive parts were cut to size and shape using a CNC LASER cutter with an 80 W CO₂ LASER (Speedy 400, Trotec Laser GmbH, Austria). The filter membrane material was a 47 mm diameter Mixed Cellulose Esters (MCE) 0.45µm-pore-size membrane (Merck, UK). The outer layers were embossed prior to assembly using a custom 3D-printed cold-embossing apparatus (Bambu X1-E with PLA-CF filament). The embossing apparatus was used to create a feature approximately the same diameter as the filter disc and the extent of the emboss was proportional to the ‘hold-up’ volume of the filter unit, which for the initial demonstration of the design was configured to be 1.9 mL, approximately matching that of the Sterivex™ units (Merck, UK) that were used for comparative testing. An inlet and outlet were created using BoPET strips to mask adhesive layers and create channels leading to and from the filter chamber on opposite sides of the membrane. External fluidic Interfaces were created at the channel ends by inserting blunt-end steel needles with luer-taper type connectors (RS Components, UK), secured with low-melt thermoplastic polymer (EVA) glue. During assembly, proper alignment of the layers was achieved using a series of 3D-printed plastic ‘jigs’.

2.2. Device design and fabrication

The device, Fig. 2, was designed using Fusion360 software (Autodesk, USA) and constructed from 3D-printed (Bambu X1-E with PLA-CF filament) and LASER-cut (Speedy 400, Trotec Laser GmbH, Austria) parts. A heated interface was created by mounting a circular metal-ceramic heater element (Thorlabs Ltd., UK) on a 3D-printed, heat-resistant Polyamide pedestal capped with a ‘Nomex’ thermal insulating sheet (RS Components, UK). The heater element was overlaid with a thermal gap-pad material, 467MP (3 M, UK) and stainless-steel disc, approximately the same size as the filter membrane material. Temperature measurements were made using a K-type thermocouple mounted against the underside of the heated surface and enclosed with thermal paste. Temperature control was achieved using an Arduino MKR WiFi 1010 microcontroller and an Arduino MKR Thermocouple shield (Arduino, Italy), using the Pulse Width Modulation (PWM) function of a digital output pin to assign a duty cycle based on a Proportional Integral Derivative (PID) algorithm to maintain a set temperature within ± 0.5 °C. The heater was powered from a 12 V DC rechargeable Li-ion battery with logic level switching using an N-channel MOSFET (IRLZ44N, RS Components, UK).

Mechanical agitation to the filter unit was provided from an Eccentric Rotating Mass (ERM) motor (Farnell, UK), mounted on the top side of a plastic pad, that was positioned adjacent to the filter membrane when a filter unit was installed with the device. A small potentiometer was used to set the speed of the motor and the intensity of the mechanical agitation. The motor was powered from the 12 V DC battery

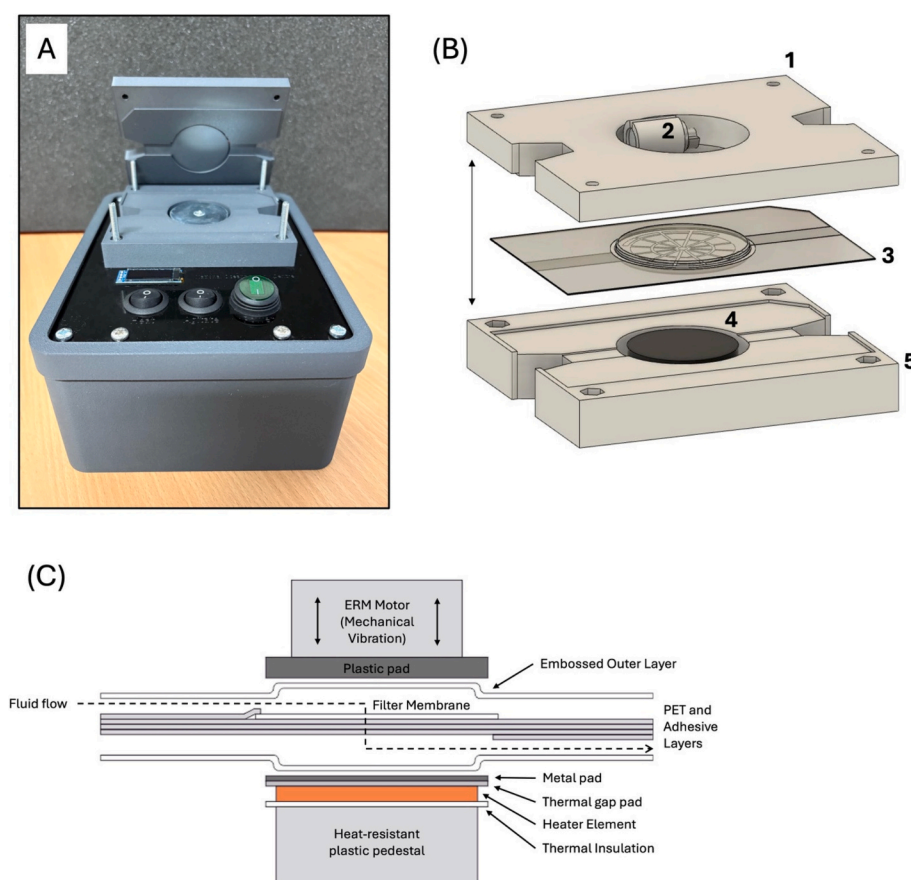


Fig. 2. A custom-built prototype device to provide heat and mechanical agitation to the laminate filter unit for the purpose of producing a cell lysate within the filter chamber. (A) shows a photograph of the device. The device surface presents a filter unit enclosure, rocker switches to activate device power, heating and agitation functions, and a small OLED screen to indicate heater temperature. (B) shows a simplified 3-dimensional illustration of the device-filter interface in an exploded view; A, enclosure lid presenting a thin plastic pad, supporting an ERM motor; B; C, a flexible filter unit; D, a pedestal presenting a heater element and thermocouple; E, enclosure bottom. (C) shows a simplified 2-dimensional illustration of the device-filter interface in cross-section. Further details of the device are given in the Methods, Section 2.2.

supply via a 12 V–5 V DC voltage convertor.

2.3. Filter unit operation

Two types of pressure driven filter unit were used in this work. Sterivex™ units were operated by connecting a syringe directly to the inlet aperture and forcing a liquid sample through the unit manually. Afterwards, the filter chamber was emptied by ‘chasing’ the liquid through with air. Alternatively, flexible filter units were operated with the assistance of the custom-built device. The units were secured between the heater and mechanical agitator interfaces (see Fig. 2C) before a liquid sample was pushed through the filter membrane using a syringe and fluid line (silicone tubing with an inner diameter of 2 mm), connected to the filter unit using luer fittings. Introduction of the fluid caused the filter chamber to inflate against the device's interfaces. After the sample was passed, excess liquid in the filter chamber (the hold-up volume) was removed by retracting the syringe connected to the inlet; the hold-up volume was withdrawn and the filter returned to a deflated state.

2.4. Bacterial cell culture

Two species of faecal indicator bacteria were used in this study. All culture media were purchased from Oxoid UK, prepared with deionised water and sterilised by autoclaving at 121 °C for 15 min. *Escherichia coli* type strain (NCTC 9001) was maintained on Nutrient agar at 37 °C, and prior to experiments was cultured in Nutrient broth medium at 37 °C with aeration (200 rpm). *Enterococcus faecalis* type strain (NCTC 775) was maintained on Columbia blood agar, and prior to experiments was cultured in Nutrient broth at 37 °C with aeration (200 rpm). Experiments were carried out using overnight (18 h) cultures, which had reached a stationary phase.

2.5. On-filter cell lysis

All tests were completed using samples prepared from a base of seawater water collected from the Empress Dock, Southampton, UK (grid reference: 50.891174, –1.3939665) with or without added bacterial suspensions from overnight cultures. For each type of filter, 200 mL of water sample was passed, and the tests were repeated using 5 filters of each design. In each case, on-filter lysis was achieved using reagents available in the DNeasy PowerWater Sterivex Kit (Qiagen, UK) and following the manufacturers' recommended protocol. First, the filter chambers were emptied of residual sample, followed by the addition of 0.9 mL of solution ST1B. Then, the filters were subjected to ‘gentle’ agitation for 10 min. Sterivex™ were agitated using a mains-powered rotary vortex platform (Vortex-Genie 2, Scientific Industries, UK) at ~500 rpm, whereas laminate filters were agitated using the battery-powered device shown in Fig. 2, with the ERM motor speed set to ~500 rpm. Subsequently, 0.9 mL of solution MBL was added and the filters were heated for 5 min. Sterivex™ were heated by placing them in a mains-powered laboratory incubator set to 95 °C. Laminate filters were heated using the device, with the PID-controller set to 95 °C. Finally, the Sterivex™ and laminate filters were subjected to ‘vigorous’ agitation using the rotary vortex or device respectively, each set to a speed of 3000 rpm. For clarity, an illustration of the overall workflow used to compare and evaluate the filter types is given in Fig. 3.

2.6. DNA extraction

DNA was extracted from the lysate prepared using either the Sterivex™ or laminate filter types as described above. Extraction was carried out using the reagents and materials provided by the DNeasy PowerWater Sterivex Kit (Qiagen, UK) and following the manufacturers' recommended protocol exactly. Purified DNA was analysed spectrophotometrically, using a NanoDrop 1000 spectrophotometer

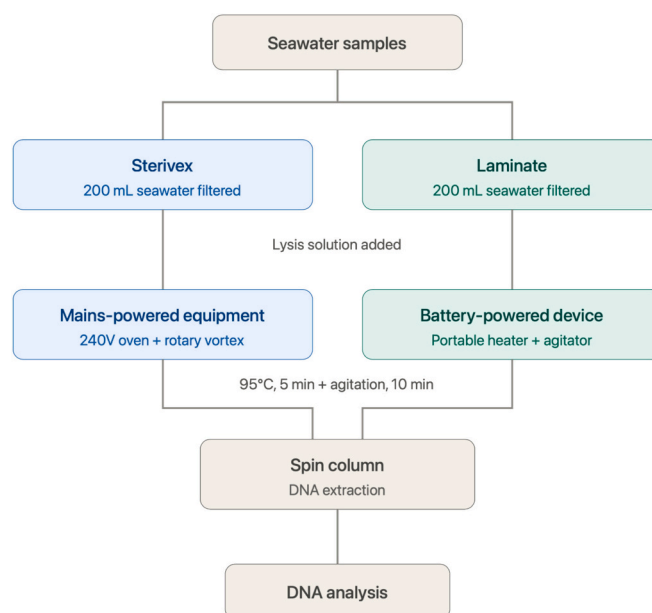


Fig. 3. A schematic of the experimental workflow adopted during the comparative evaluation of the two filter designs. The workflow diverged at step 2, in which the same samples are filtered using either Sterivex™ or the laminate design, followed by the use of either lab equipment or a custom-built device respectively to apply heat and mechanical forces to the filter membranes during the lysis step. After lysis, the same DNA purification and PCR protocols, and the same reagents and formulations were used for each type.

(ThermoFisher Scientific, UK) and fluorometrically using a Qbit fluorometer with the Qbit HS DNA assay kit (ThermoFisher Scientific, UK).

2.7. Real-time polymerase chain reaction

Real time PCR was used to compare the amplification rate and inhibitor content of DNA prepared using either laminate filters and the custom-built device or the commercial Sterivex™ filters and laboratory equipment. This was done using DNA isolated from seawater spiked with *E. coli* and the *E. coli*-specific primer and probe sequences given in Table 1, using an optimised assay (McQuillan and Wilson, 2019). All qPCR reactions were prepared using the GoTaq® G2 PCR System (Promega, UK). Each reaction contained GoTaq® Colourless PCR Buffer at the manufacturer's recommended concentration: 1 mmol l⁻¹ of MgCl₂, 0.5 mmol l⁻¹ each of dATP, dTTP, dCTP and dGTP, 400 nmol l⁻¹ of primers, 200 nmol l⁻¹ of hydrolysis probe, 1 U of GoTaq® G2 polymerase and 1 µL of template DNA; the final volume was 25 µL. The reactions were prepared in 0.2 mL nuclease-free polycarbonate tubes with optically clear lids (ThermoFisher Scientific, UK). The reactions were completed using an MXpro3005 real-time PCR instrument (Agilent Technologies, USA). Thermal cycling conditions were 95 °C for 2 min followed by 35 cycles of 95 °C for 15 s and 60 °C for 45 s. DNA dilutions

Table 1
Oligonucleotides used in this study.

Oligo Name	Type	Sequence (5' - 3')
ybbWPCRf	qPCR forward primer	TGATTGGCAAAATCTGGCCG
ybbWPCRr	qPCR reverse primer	GAAATCGCCCAATCGCCAT
ybbWHP	qPCR Hydrolysis probe	[FITC]-CCGCCG[ZEN] AAAACGATATAGATGCACGG-[IABkFQ]

FITC = Fluorescein Isothiocyanate; ZEN = ZEN internal fluorescence quencher; IABkFQ = Iowa Black Fluorescence Quencher.

were made in PCR-grade water (Qiagen, UK).

2.8. Statistical testing

The results of the spectrophotometric and fluorometric DNA quantification of DNA extracts prepared using the Sterivex™ and laminate filters (as shown in Table 2) were subjected to a student's *t*-test using Excel (Microsoft Corporation, USA) to look for any statistically significant differences in DNA quantity achieved using each filter type.

3. Results and discussion

The aim of this study was to demonstrate a new design of disposable, pressure-driven filter unit and an associated portable device intended for on-filter cell lysis prior to DNA extraction and PCR. We chose seawater as an appropriate medium to conduct the initial testing of the design given that there are numerous applications in which cell capture and DNA extraction from seawater are relevant including, for example, the detection of hazardous microorganisms such as faecal indicator bacteria or toxigenic phytoplankton using simple nucleic acid amplification tests (NATs). However, with additional development and testing the design may also be suitable for other sample types including freshwater, clinical fluids and homogenates, and additional applications such as microbial community analyses; although further trial of the system will be necessary to confirm this. The design was achieved by laminating a microbiological-grade filter membrane material within thin and pliable layers of a thermoplastic polyester film and was unique from commercially available designs that offer a cylindrical and rigid construction. The result, shown in Fig. 1, was an essentially 2-dimensional filter unit, 656- μm thick (0.65 mm), with embosses in the outer layers that allowed the structure to respectively inflate or deflate with the introduction or removal of fluid or air. The external layers were 23- μm thick to enable close-coupling of a heater and mechanical agitator to the embedded membrane. This was intended to support efficient thermal and kinetic energy transfer, to improve cell lysis efficiency and to minimise power consumption. The inflatable filter chamber was included to improve the general operation of the unit in comparison to rigid-bodied designs by enabling the user to wholly fill and evacuate the unit via the inlet without the structure being vented via the outlet. With rigid designs, there must be some path for air to exit or enter the structure as a lysis solution is introduced and the lysate recovered, which increases the complexity of operation and leads to pressurisation and potential leakage of sample and chemicals. With the new design, the fluid within the filter chamber can be evacuated by collapsing the chamber as fluid is drawn out, ensuring no fluid remains in the chamber. This ensures complete removal of excess fluid prior to the introduction of lysis reagents, and complete recovery of lysate to maximise DNA yield.

3.1. Functional testing and characterisation

Testing and evaluation were done with reference to the Sterivex™ type throughout. This is, at the time of writing, the market leader and is widely adopted for applications requiring the collection of microorganisms and eDNA from water samples. Alternative, commercial designs

are of a similar, rigid and (typically) cylindrical construction. The new design incorporated a 47 mm diameter Mixed Cellulose Esters (MCE) membrane with an effective filtration area of 7.95 cm², however this could be easily changed to suit different applications. Sterivex™ filter units contain a Polyether Sulfone (PES) membrane with an effective filtration area of 10 cm². Each filter type had a 0.45- μm pore size, and a hold-up capacity of approximately 1.9 mL. This pore size is widely used for the retention of bacterial cells such as *E. coli* (as used for evaluation of the design) and to reduce clogging versus 0.22- μm filters. The integrity of the new, laminate design was tested by capping-off the outlet and pushing water into the unit using a syringe connected to a digital pressure gauge. The laminates could withstand an internal pressure of between 8.7 and 11.6 PSI (0.6 and 0.8 bar) before failure; however, the point of failure was always the thermo-polymer glue affixing the inlet fixture (see Fig. 1D) and it was not possible to determine the pressure limit of the laminate itself. As the glue is a temporary solution during the early development of this design, it is anticipated that significantly greater pressure tolerance can be achieved with the adoption of advanced manufacturing techniques in which the components would be welded. Nonetheless, when operated at lower pressures the laminate design offered a flow rate of approximately 20 mL min⁻¹, which was sufficient to complete additional testing. In comparison, the mass manufactured Sterivex™ design is, according to the manufacturer, rated to 45 PSI (3.1 bar), and offers typical flow rates of around 150 mL min⁻¹.

3.2. On-filter cell lysis using a custom-built device

After initial characterisation, the new filter design was directly compared with the Sterivex™ type for the collection and pre-concentration of suspended bacterial cells from seawater followed by on-filter cell lysis in preparation for DNA extraction. The comparison was done using the cell lysis buffers available in the DNeasy PowerWater Sterivex Kit from Qiagen. Like most filter-based lysis protocols, heating and agitation of the filter membrane is necessary to efficiently lyse the trapped cells and increase DNA yield. This was achieved differently for each type. Sterivex™ were processed using conventional laboratory equipment including a mains-powered rotary vortex and laboratory oven, and the laminate design was processed using a custom-built, portable and battery powered device, shown in Fig. 2 A. The device was designed specifically for use with the laminate filters, to clamp the unit during operation and prevent any structural distortion, reinforce against delamination and improve the flow rate, and to interface the top and bottom of the filter unit with a heater and agitator respectively, shown in Fig. 2B/C. During testing, the laminates were secured between the device interfaces, and a water sample (200 mL) was pushed through the unit after which the small amount of leftover sample in the chamber was removed by retracting the syringe. Then, the outlet of the filter unit was capped with a luer-plug, and lysis reagents were introduced via the inlet using a fresh syringe, as described in the materials and methods. This remained attached to the filter during operation and served to cap-off the inlet. The introduction of lysis reagents inflated the chamber so that the thin outer layers pressed against the device. Heat and agitation were applied by the user activating independent rocker switches mounted on the device housing. After cell lysis, the lysate was removed

Table 2
DNA extraction results.

Sample Type	Filter Type	Mean DNA Yield ng μL^{-1} (Standard Error; n = 5)		Mean Absorbance Ratio (Standard Error; n = 5)	
		Spectro-photometry	Fluorimetry	A _{260/280}	A _{260/230}
Natural Seawater	Sterivex™	3.47 (0.34)	3.32 (0.32)	1.85 (0.09)	0.79 (0.10)
Natural Seawater	Laminate	3.27 (0.30)	3.16 (0.30)	1.81 (0.04)	0.85 (0.07)
Natural Seawater + <i>E. coli</i>	Sterivex™	48.52 (20.11)	43.74 (16.99)	1.85 (0.02)	2.09 (0.04)
Natural Seawater + <i>E. coli</i>	Laminate	62.20 (5.10)	54.56 (3.86)	1.84 (0.02)	2.07 (0.02)
Natural Seawater + <i>E. faecalis</i>	Sterivex™	19.85 (2.60)	17.32 (2.34)	2.02 (0.06)	0.45 (0.06)
Natural Seawater + <i>E. faecalis</i>	Laminate	47.08 (3.84)	41.96 (3.49)	1.87 (0.02)	0.58 (0.06)

by retracting the connected syringe until the filter chamber was fully deflated. For clarity, we have included an illustration of the overall workflow for the comparative testing in Fig. 3.

Multiple sample types were tested: (i) seawater spiked with a stationary-phase culture of Gram-negative *Escherichia coli*, (ii) seawater spiked with a stationary-phase culture of Gram-positive *Enterococcus faecalis*, and (iii) unaltered seawater. Stationary phase cells were used because they are generally more resistant (difficult to lyse) than actively replicating cells, and more representative of the cells recovered from the environment (Kolter et al., 1993; Nyström, 2004). Each test was repeated 5 times (i.e., using 5 individual filter units of each type, for each sample).

After on-filter lysis, DNA extraction was carried out using the DNeasy PowerWater Sterivex Kit exactly according to the manufacturer's recommendations. Table 2 shows the quantity and purity of the extracted DNA. Yield was confirmed using two DNA quantification methods, spectrophotometry and fluorimetry. For native seawater, the yield was approximately equivalent regardless of the way in which the samples were filtered and lysed. Analysis of the spectrophotometry results using

a simple student's *t*-test reported no significant difference between DNA yields (equal variances assumed, $t = 0.423$, $p = 0.683$). Extractions from seawater spiked with *E. coli* were also not significantly different, although sample number (5 repeats) and therefore statistical power was low in this case (equal variances assumed, $t = -1.323$, $p = 0.222$). Conversely, extractions from seawater spiked with *E. faecalis* showed a strong statistical significance (equal variances assumed, $t = -5.87$, $p = 0.00038$). Taken together we conclude that the samples prepared using the laminate design and device contained the equivalent or more DNA than those generated using Sterivex™ and mains-powered equipment, depending on the sample type.

DNA purity estimations based on $A_{260/280}$ (protein contamination), and $A_{260/230}$ (salts and organic compound contamination) were approximately equivalent using each filter type. UV-visible spectra collected from DNA samples produced using seawater and seawater spiked with *E. faecalis* produced $A_{260/230}$ readings of <2.0 , indicating the presence of contaminants; it was unclear if this was the result of the sample matrix or the preparation method, but it was consistent for each filter type and was not observed when processing the *E. coli* samples.

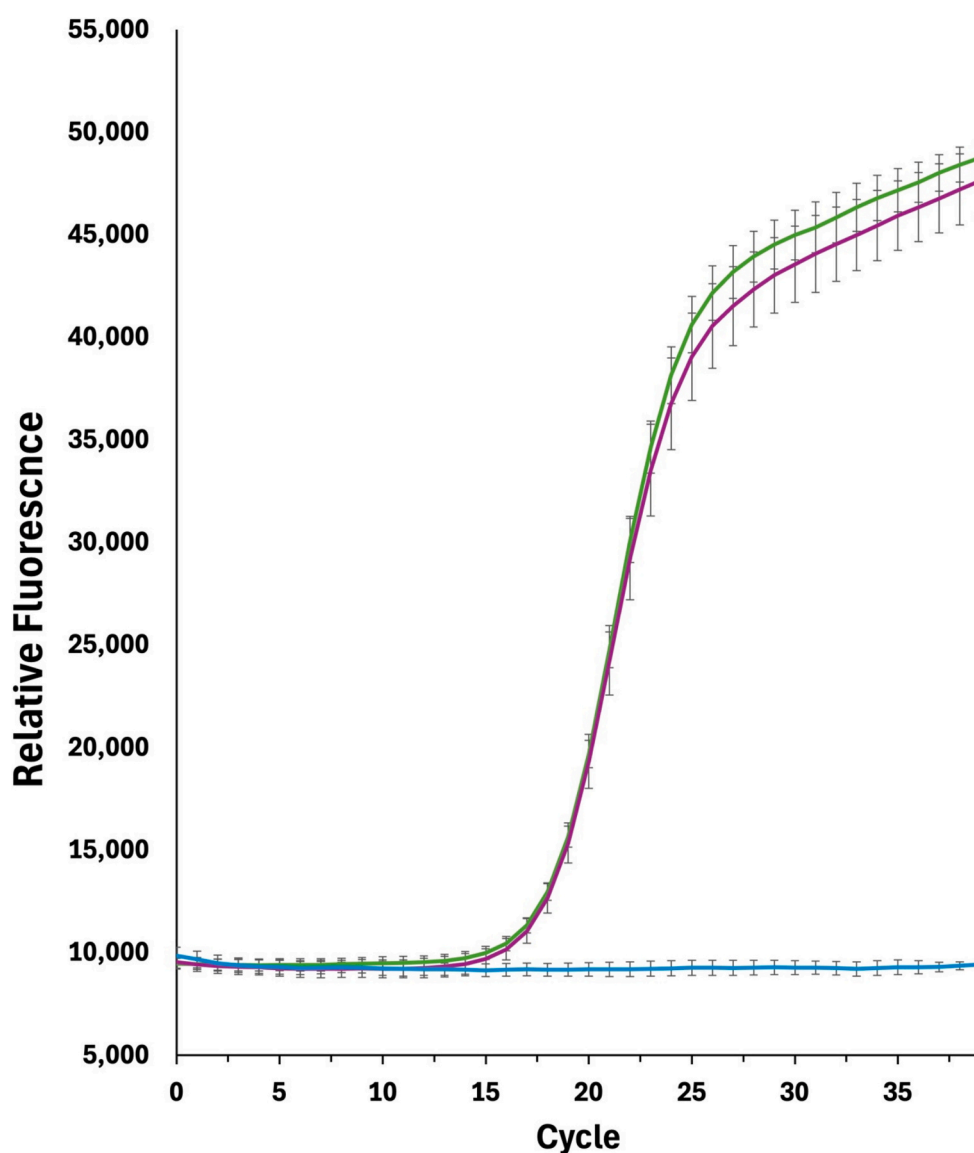


Fig. 4. Real-time fluorometric PCR amplification of DNA extracted on a laminar Filter unit (dark grey line), a rigid Sterivex™ filter unit (black line), or null amplification from a 'no template' control (light grey line). Each PCR reaction was carried out in triplicate; the error bars represent the Standard Error of the mean. Each amplification reaction contained 1 ng of template DNA. Threshold cycle (C_t) and overall DNA (fluorescence) yield were approximately equivalent for DNA extracted using either filter design. The experiment was repeated 3 times with similar results.

To fully validate the laminate design, it was important to confirm that DNA could be amplified in a consistent manner to that generated from a commercial Sterivex™ filter. This was necessary because, although the DNA had been subjected to a rigorous purification procedure, carryover of PCR inhibitors and DNA degradation is frequently an issue when working from environmental samples such as seawater. To do this, a separate experiment was conducted in which DNA was prepared using each filter type, from seawater spiked with *E. coli* exactly as described above. First, a sample of DNA from each filter type was prepared to an equivalent concentration and used as a template for the amplification of an *E. coli* gene sequence to directly compare amplification rate (C_t Value). Fig. 4 shows amplification plots generated from PCR reactions containing 1 ng of *E. coli* DNA prepared using either the Sterivex™ or laminate filter design; in each case the C_t values obtained were equivalent. A second experiment was carried out to look specifically for evidence of differences in PCR inhibitor carry-over. To do this, the extracted DNA was diluted 10-fold in series, and each dilution was used as template in additional PCR reactions. Analysis of the threshold

cycle (C_t) values obtained using each dilution indicated a strong linear relationship over each dilution series; PCR efficiency calculated from the slope of a linear fit to the data was 95.1% or 94.7% for DNA collected using the Sterivex™ or laminate filters respectively (based on the mean of 3 repeats). The results are shown in Fig. 5. Based on these findings, there was no indication the use of the new design and device impacted PCR amplification efficiency or DNA sample inhibitor content compared to the Sterivex™.

3.3. Improved DNA yield using the laminate design and device

Using seawater spiked with bacterial cultures, the cell lysates prepared on the laminate filters with the device contained slightly more DNA, with equivalent DNA quality, than those prepared using Sterivex™. This was unexpected because the laminates incorporated a membrane with a reduced total filtration area compared to the Sterivex™ (by approximately 80%). Although the filter membranes were different (laminate filter membranes were cellulose-based, Sterivex™

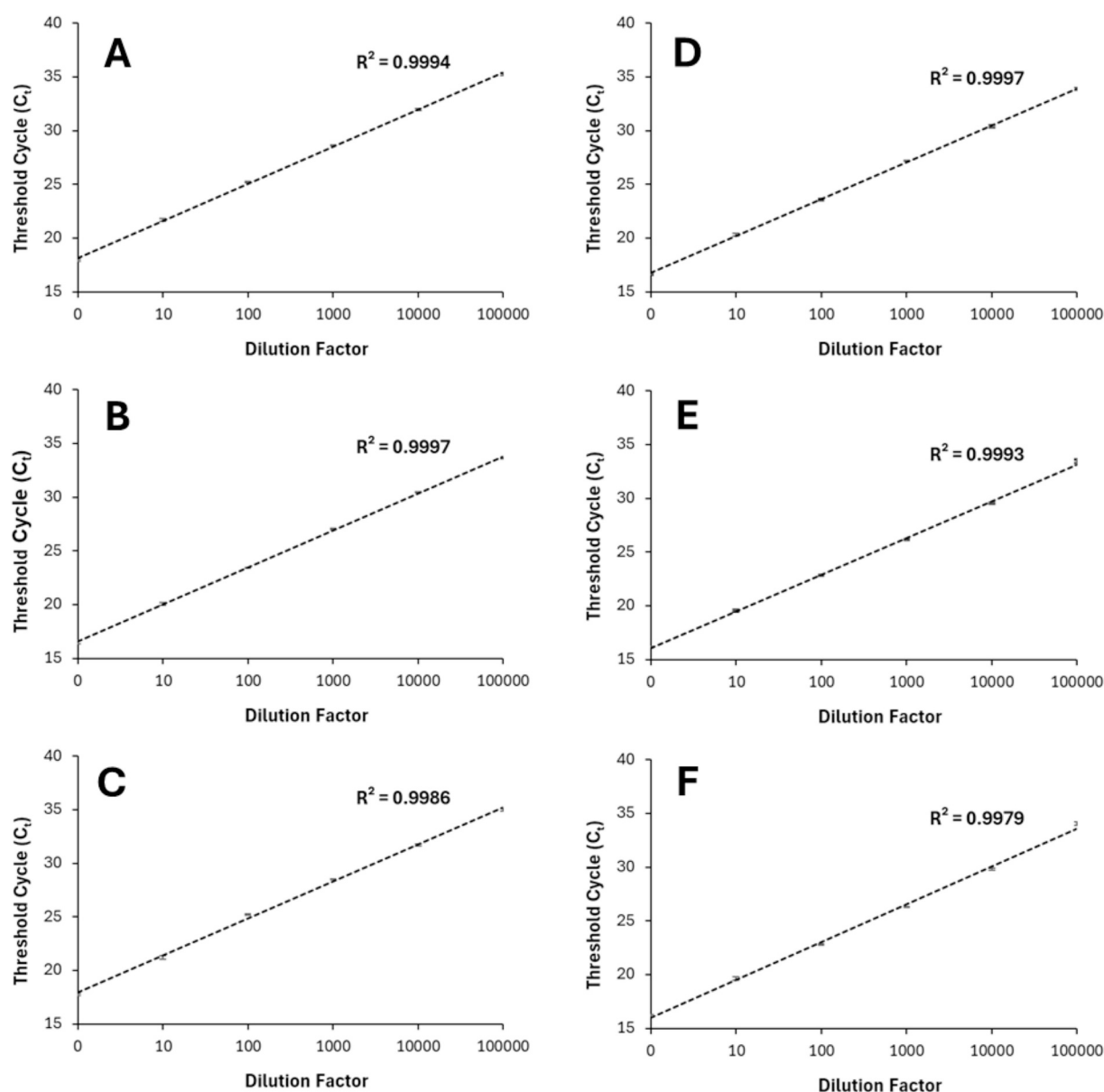


Fig. 5. Threshold cycle values obtained from PCR amplification of DNA extracted using triplicate laminate filter units (plots A, B, C) and triplicate Sterivex™ filter units (plots D, E, F) across a 10-fold DNA sample dilution series. The dashed lines represent a linear regression, used to determine goodness of fit (R^2) and therefore the linearity of each dataset. Data obtained from each filter displayed an almost linear trend, indicating the absence of PCR inhibition.

filter membranes were PES), both membranes had an equivalent pore size (0.45- μm), are constructed from hydrophobic materials, and are generally considered appropriate for the intended application. Therefore, improved DNA recovery may reflect differences in the effectiveness of the cell lysis process and, in agreement with this theory, the increase in DNA yield was greater when the starting sample contained the Gram-positive *E. faecalis*, which are more resistant to lysis than the Gram-negative *E. coli*.

The retentate on each filter was lysed using the same chemical reagents, with heat and agitation applied for the same duration, however the mode of heating and agitation differed. The laminate filters were used in tandem with the device to bridge heat directly to the filter chamber through a very thin-walled (23- μm) layer, whereas Sterivex™, which have much thicker enclosures (1.5 mm), were heated within a convective laboratory oven. Sterivex™ enclosures are manufactured from Easstar™ copolyester and have a Thermal Resistance (R) of 0.00714 $\text{m}^2 \text{K}^{-1} \text{W}^{-1}$ (based on the material thermal conductivity of 0.21 $\text{W m}^{-1} \text{K}^{-1}$). The laminate outer layers were BoPET film with a Thermal Resistance (R) of 0.00018 $\text{m}^2 \text{K}^{-1} \text{W}^{-1}$ (based on the material thermal conductivity of 0.13 $\text{W m}^{-1} \text{K}^{-1}$). In each case the filters were heated to a 'set' (estimated) temperature of 95 °C according to the recommended protocol of the DNA extraction kit used to conduct the comparison. However, in practice the interior of each filter never reached this temperature during the 5-min heating step. If a thermocouple temperature probe was inserted into each filter type the interior of a Sterivex™ filter never exceeded 75–80 °C. In contrast, the laminate design heated on the device reached temperatures more than 90 °C indicating that this constituted a far more efficient heating system. Improved transfer of thermal energy to the filter membrane was a key consideration when creating the new design for the purpose of potentially improving lysis efficiency, however it is unclear whether this is the only factor that influenced the DNA yield. Filter membrane properties can also influence DNA yield (Hinlo et al., 2017; Majaneva et al., 2018). In this study, two different materials (MCE in the flexible design and PES in the Sterivex design) were used in this comparison. We selected MCE membranes for the laminate design because these are standard choice in applications such as faecal indicator testing by the membrane filtration method; this was one of the intended applications during the development of the laminate. Sterivex are not presently available with the same material; only PES or PVDF variants are available, of which PES is most like MCE in terms of overall material properties (e.g., both hydrophilic, similar protein retention, similar physical properties) and therefore was selected to make the comparison.

Another difference in the two lysis procedures was that the Sterivex™ were agitated lying horizontally on a benchtop rotary vortex instrument that produced primarily lateral acceleration forces acting in the same plane as the orientation of the filter membrane. In contrast, the custom-built device featured an oscillating pad that aligned in parallel to the filter surface and generated forces acting in a perpendicular plane. In this case, however, the purpose of this agitation was to dissociate cells from the membrane, rather than induce a mechanical lysis (which would require the inclusion of an abrasive particle type in the lysis formulation such as glass microbeads), and therefore it is unclear if this could have influenced the results.

3.4. Ease of operation

Removal of a lysate from a Sterivex™ is usually done by first capping-off the outlet and then pushing air into the unit with a syringe to create a positive internal pressure, which allows lysate to be drawn out under gravity. Typically, users will see a small amount of lysate remain in the chamber. In contrast, the laminate design has a pliable filter chamber that can inflate and deflate as a user adds or removes fluid, enabling the recovery of lysate without a positive internal pressure and irrespective of gravity. The user retracts the plunger on a syringe connected to the inlet, causing the chamber to collapse as fluid is recovered

in full. This eases the operation of the filters with respect to both removing residual fluid from the chamber before introducing a lysis solution (i.e., to purge sample that may be rich in inhibitors from the pre-filter chamber), and for recovering as much lysate as possible to maximise DNA yield.

3.5. Environmental sustainability

The laminate design was achieved using approximately 3.3 g of plastic, which is less than half the total plastics composition of a standard Sterivex™ unit (~7.5 g). The PET films used in its construction are widely recyclable (Blanzeanu et al., 2026; Borda et al., 2026); however this early prototype incorporated a non-recyclable acrylic sheet adhesive, which with development for scaled manufacture could be eliminated by direct bonding of layers using ultrasonic or thermal processes. Additionally, with shaping of the design around the filter membrane, a large portion of the plastic could be eliminated without compromising its function. These factors are important because DNA analysis and other molecular biological procedures generate large quantities of plastic waste. Furthermore, even though the filter units could be made recyclable, they are commonly used in environmental or clinical diagnosis applications for which spent units would be classified as Offensive waste and subject to disposal routes such as autoclaving or incineration that incur a high environmental and economic footprint (Singh et al., 2022). The potential to generate equivalent amounts of DNA from filter units that reduce overall plastic consumption could be advantageous, and the sheet-type construction would be well-suited for high volume and low-cost rotary die cutting manufacturing processes. Scaled production of filter units for DNA sample preparation with potentially lower cost would be supportive of wider applications for DNA testing.

3.6. Field applications

The laminate filter and device were designed to maximise energy transfer and lysis efficiency, but also with consideration of applications where the device and filters might be used in low-resource settings without mains power. The device described in Fig. 2 is battery powered, and easily portable (approximate dimensions are 15 cm × 18 cm × 11 cm, w × d × h; and < 1 kg) and could easily be operated the field if required. The system presented here is only relevant to the first stages of a DNA analysis workflow, however it could be combined with additional technologies that offer in field DNA extraction and amplification to form part of an integrated lab-independent solution. This would have numerous benefits for microbiological testing because of the high cost and long delay associated with centralised laboratories. It would be particularly suited for the detection of sudden, stochastic or short-lived microbiological hazards that are often missed through the delays from laboratory testing. In applications requiring filters to be returned to a laboratory, the use of a thin, flat configuration may aid in sample transit and, where the use of a laminate filter and device may promote a more efficient heating of the filter membrane through close-coupling of a heat source, conversely it could be modified to enable rapid and efficient cooling to preserve the integrity and composition of the sample immediately after collection.

CRedit authorship contribution statement

Jonathan S. McQuillan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Daniel Rogers:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Wahida T. Bhuiyan:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Andrew Morris:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Katherine Hartle Mougou:** Writing – review & editing,

Conceptualization. **Electra Gizeli:** Supervision, Funding acquisition. **Martha Valiadi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

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