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A lab-on-a-chip system integrating DNA purification and loop-mediated isothermal amplification for the quantification of the toxic diatom *Pseudo-nitzschia multistriata*

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Microfluidic technology can expedite nucleic acid testing by converting the functions of bulky laboratory instruments and protracted bench methodologies into easy-to-use and inexpensive miniaturised systems without compromising speed and reliability. We developed a lab-on-a-chip (LOC) platform that integrates a dimethyl adipimidate (DMA)-based functionalised silica DNA isolation and pre-concentration method with a rapid and real-time loop-mediated isothermal amplification (LAMP) for detecting domoic acid-producing phytoplankton, *Pseudo-nitzschia*. An optimised design of a lab on a chip extraction module achieved a maximum DNA capture capacity of 61.73 ± 0.98 ng μL^{-1}. The DMA-based method reduced reagent costs per sample by 97% compared to a commercial nucleic acid isolation kit. A subsequent on-chip LAMP process was capable of sensitively quantifying cytochrome P450 homologous to the *dabD* gene, coding for a component of the domoic acid toxin production pathway, with a limit-of-detection of 10 cells per mL. LAMP-based detection of the target gene was achieved using dry-preserved reagents with a shelf-life of five months without refrigeration. There was no significant difference in assay performance between the preserved LAMP and freshly prepared LAMP mixtures. The total analysis time at the LOD of 10 cells per mL, from sample to result, was achieved within one hour. Our results demonstrate the long-term stability of assay reagents, rapid turnaround, and cost-effectiveness, offering a simple and economical approach to environmental monitoring and environmental bio-hazard diagnostics.

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1. Introduction

Rapid and reliable nucleic acid amplification testing can provide effective advanced warning and surveillance of harmful algal blooms (HABs), which represent a risk to public health from the consumption of contaminated seafood.[1,2] Specifically, toxic *Pseudo-nitzschia* spp. blooms incur substantial economic losses resulting from disruption to fisheries, aquaculture and sports fishing industries.[3] They occur globally, and incidences are increasing in frequency and severity.[4] *Pseudo-nitzschia* cells produce a potent neurotoxin, domoic acid, which accumulates within the marine food network and can cause debilitating amnesic shellfish poisoning (ASP).[5] Recent research has identified the genetic basis for domoic acid biosynthesis, which is associated with the *dab* gene cluster, a cluster that is absent in certain species.[6] Therefore, the direct detection of the *dab* genes, as opposed to the inclusive detection of *Pseudo-nitzschia* (PN) cells, may be a better indicator of risk.

Traditionally, specialists use microscopy to detect and quantify PN cells by fixing and settling water samples before examining them for identification based on morphology. However, nucleic acid amplification methods provide enhanced sensitivity and specificity compared to cytological methods, particularly in differentiating toxic from non-toxic variants that may have overlapping morphological features.[7,8] Molecular methods, particularly polymerase chain reaction (PCR) combined with fluorometric DNA-binding probes, provide greater sensitivity and specificity and represent the gold standard method for detecting microbial gene sequences in environmental samples.[9,10] Alternative isothermal methods, including loop-mediated isothermal amplification (LAMP) are increasingly popular due to their simplified thermal incubation requirements.[11] LAMP is also compatible with real-time fluorometric and colorimetric DNA detection approaches, offering flexibility and ease of use.[12] This method enables rapid,

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accurate, and reliable quantification of microbial genes for various applications, including harmful algal bloom monitoring,[13,14] viral detection,[15–18] and bacterial surveillance.[19,20] However, both PCR and LAMP analysis rely on centralised laboratories, requiring sample preservation and transport. This process is costly, delays results by days, and increases the public health risk by limiting the opportunity for timely mitigation. A fieldable solution enabling near-sampling and near-real-time analysis would transform surveillance and early warning capabilities, creating efficiencies that are otherwise lost to the dependency on laboratories. This may be particularly impactful in remote or developing regions where laboratory infrastructure is limited.[21]

Microfluidic lab-on-a-chip (LoC) technology streamlines analytical workflows by miniaturising instrumentation and integrating sample processing, DNA amplification, and gene detection.[22] For *in situ* nucleic acid testing, an effective LoC should combine DNA pre-concentration and purification with amplification and detection, incorporating pre-formulated reagents that remain stable on-chip without refrigeration and activate upon hydration.[23–26] Analogous advanced systems such as the Cepheid GeneXpert and BioFire FilmArray enable genetic detection of pathogens directly at the point of care.[27,28] Consistent with the goal of achieving near-real-time hazard detection in marine environments *via* a fully automated miniaturised system, this study designs and demonstrates the components intended for integration into a deployable device capable of autonomous offshore operation. Here, we introduce a novel microfluidic system, "LAMPTRON" which combines an open, cost-effective DNA extraction method using dimethyl adipimidate (DMA) chemistry and silica beads and ambient-stable reagents for real-time LAMP amplification on a single LoC design. DMA-based DNA extraction provides a solvent-free alternative to traditional solid-phase extraction (SPE) using silica matrices, thereby avoiding the harsh organic solvents that can interfere with downstream reactions in LoC systems.[29,30] Cells are lysed directly in a DMA-containing buffer, and purified DNA is released by a simple pH shift, enabling fully integrated, solvent-free on-chip extraction. This combined extraction and amplification system has been evaluated for the detection and enumeration of toxigenic *P. multistriata* using the *dabD* gene biomarker over a broad concentration range (10–10^5 cells per mL). In tandem, a novel LAMP reagent preservation technique using vitrification enables stable storage of assay mixtures on-chip for up to five months without refrigeration.

2. Experimental

2.1. Culture conditions and the preparation of cell standards

Pseudo-nitzschia multistriata SZN-B954 was obtained from the culture collection at Stazione Zoologica Anton Dohrn Institute (SZN), Italy. The strain was cultured in a sterile filtered f/2 growth medium at 18 °C, under a 12-hour light : dark photoperiod.[31] Enumeration of cells was carried out using a light microscope (Carl Zeiss, Oberkochen, Germany) and a Beckman Coulter glass Sedgwick-Rafter cell counting slide (PYSER-SGI, Kent, UK). To obtain cell concentration standards, viable *P. multistriata* cells were serially diluted in sterile filtered f/2 medium to obtain 1.07×10^1, 1.34×10^2, 1.33×10^3, 2.26×10^4, 1.6×10^5 cells per mL in 10 mL of medium. Cells were then harvested by centrifugation (7100 rpm for 3 minutes at 4 °C) and resuspended in 200 μl of medium, then stored at −80 °C until further processing.

2.2. Working principle of the LAMPTRON microfluidic system

DNA detection from cells in seawater involves cell pre-concentration (optional but often required), cell lysis, DNA purification, DNA amplification, and detection, most often *via* optical detection of the amplified product. The LAMPTRON prototype employs a combination of chemical cell lysis, DMA-based DNA purification, and isothermal nucleic acid amplification (LAMP) to detect a target gene from *P. multistriata* cells (Fig. 1). The DNA extraction method implemented in LAMPTRON is based on a dual-mode adsorption mechanism involving amine-functionalised silica beads and the bifunctional crosslinker dimethyl adipimidate (DMA) (Alfa Aesar, UK). Previous studies have shown that amine-functionalised surfaces created by APTES silanisation of silica or polyester substrates provide a hydrophilic, positively charged interface that binds negatively charged DNA under controlled pH conditions.[32–36] Surface functionalisation can be achieved by hydrolysing the H-bonded silanol groups of the silica surface using ozone treatment, followed by exposure to APTES solution.[34,36] The DMA chemical structure contains bifunctional imidoester groups, which enable the capture of DNA by both electrostatic interaction and covalent bonding.[32,35] The amidine bonds between imidoester groups of the DMA and the amine group of DNA molecules are pH-dependent, allowing reversible DNA binding and elution by altering the pH. The optimum pH for binding DNA and RNA molecules occurs at a pH of approximately 8, while releasing molecules requires a high pH (>10).[32,36] To enable DNA binding, silica beads were first treated with oxygen plasma (Diener Electronic, Germany) to activate silanol groups as shown in the SI, section S3, followed by incubation with 10% (v/v) 3-aminopropyltriethoxysilane (APTES) (Sigma-Aldrich, UK) in 95% ethanol for 2 hours at room temperature to introduce positively charged amine groups. After washing and drying, the beads were packed into the microfluidic extraction chamber. Cells were lysed by mixing with a lysis buffer containing DMA (Alfa Aesar, UK) and introduced into the microfluidic extraction chamber pre-packed with amine-functionalised silica beads. The DMA protocol was implemented on a microfluidic chip. The DNA released from lysed cells was captured through electrostatic and covalent interactions with the DMA and the functionalised silica surface under controlled pH conditions (pH 8.0). After binding, the chamber was washed with phosphate-buffered

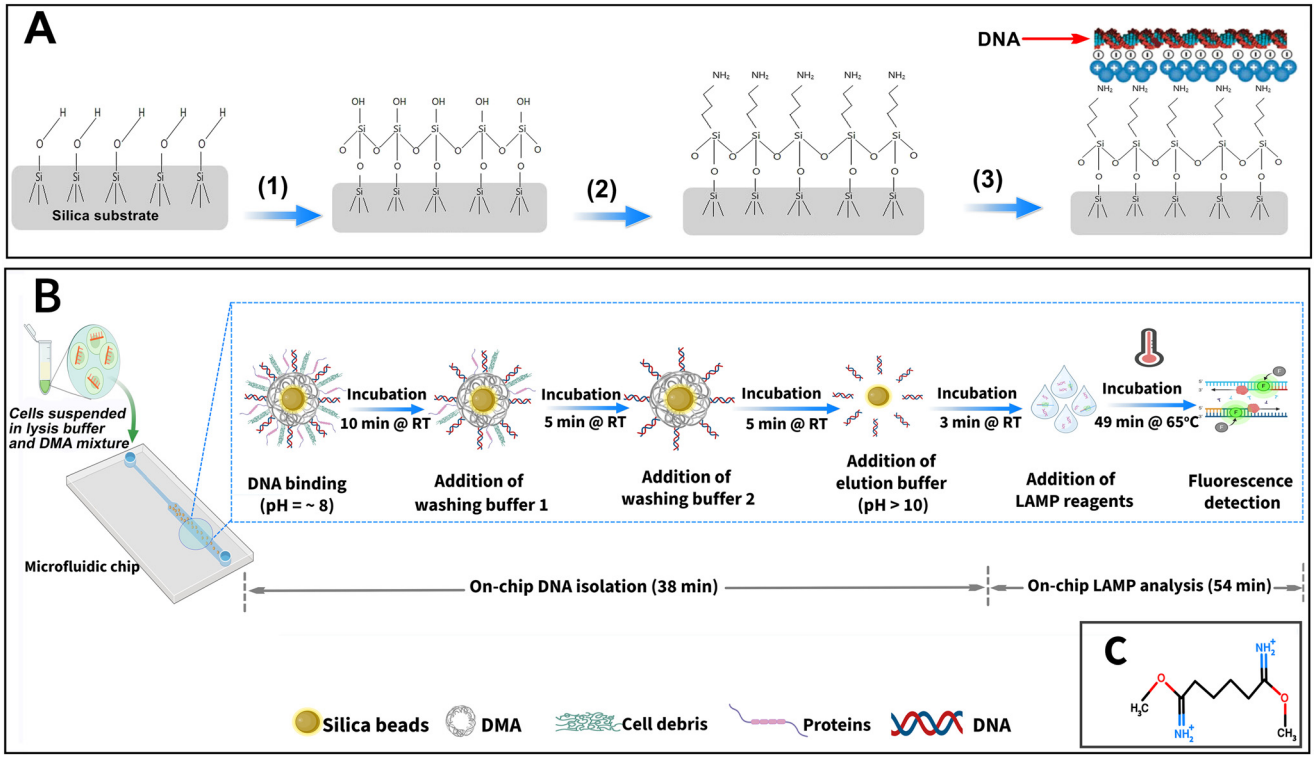

Fig. 1 Schematic representation of the lab-on-a-chip system for rapid and simple DNA isolation using the DMA-based DNA extraction method on silica beads, followed by real-time LAMP analysis. (A) Surface treatment of silica beads with APTES: (1) substrate hydroxylation by UV ozone treatment for 10 min, (2) APTES solution hydrolyses the exposed silanol groups on silica substrate by liquid deposition at 65 °C for 1 h, (3) adsorption of the negatively charged DNA molecule on the positively charged amine-modified silica surface. (B) Schematic process of DMA-based extraction of DNA sample. The chemical lysis of cells was carried out off-chip, and the resulting cell lysate was injected onto a chip with DMA as a binding agent, where the DNA binds to the amine-modified silica beads. Non-specific molecules are removed by washing and elution. Amplification of DNA occurs on the chip with the LAMP reagents, while no amplification occurs on the chip with the negative controls. (C) Chemical structure of DMA.

saline (PBS) (pH 8.0) (Sigma-Aldrich, UK) to remove cellular debris and unbound materials.[34,35] DNA was then eluted using 10 mM sodium bicarbonate (pH 10.6), enabling release through pH-dependent cleavage of amidine linkages. The extracted DNA was used directly for real-time LAMP amplification on the same platform (Fig. 1B).

2.3. Design and fabrication of the LAMPTRON system

The LAMPTRON system was manufactured to contain two functional modules for (1) DNA purification and (2) DNA analysis and detection.

2.3.1. DNA extraction module. The DNA extraction module was fabricated as a circular microfluidic chip (Ø = 119 mm) manufactured from two layers of dark polymethyl methacrylate (PMMA) with precision-milled microchannels (Datron Neo milling machine, DATRON Dynamics, Inc., Milford, England), as shown in Fig. 2B. Layers were solvent-bonded using chloroform vapour to induce partial reflow of micromilled PMMA surfaces. Following 2–3 min of vapour exposure, softened layers were aligned and pressed under moderate pressure, achieving irreversible bonding with minimal channel deformation. This treatment also reduces surface roughness from ~200 nm to <15 nm, enhancing optical clarity for downstream assays.[37] The microfluidic design was optimised using a combination of AutoCAD™ and Fusion 360 (Autodesk, Inc., San Rafael, CA) and included a network of microfluidic channels (approximately 950 μm wide and 950 μm deep) and two reservoirs (approximately 246 μL and 65 μL in volume respectively for a DNA purification and DNA amplification microchamber; both chambers were 950 um deep). Fluid control was achieved using five micro-inert solenoid valves (LFNA1250325H, The Lee Company, Connecticut, U.S.A.) mounted directly onto the chip. A custom-designed syringe pump unit was installed for pumping, comprising four glass barrels: one each for sample/lysis buffer mixture, first washing buffer, second washing buffer, and elution buffer (Fig. S8). The fluid flow to and from each barrel is controlled by two solenoid valves through an isolated fluidic circuit, enabling sequential control of multiple channels from a single actuation mechanism (Fig. S8). Perfluoro-elastomer moving seals (Polymax Ltd., UK) are fixed on the pump plungers on a stainless-steel shaft. All pump plungers are mechanically linked to operate simultaneously and are driven by a size 11 stepper motor (Haydon Kirk, U.S.A.). Magnetic field (Hall effect) sensors

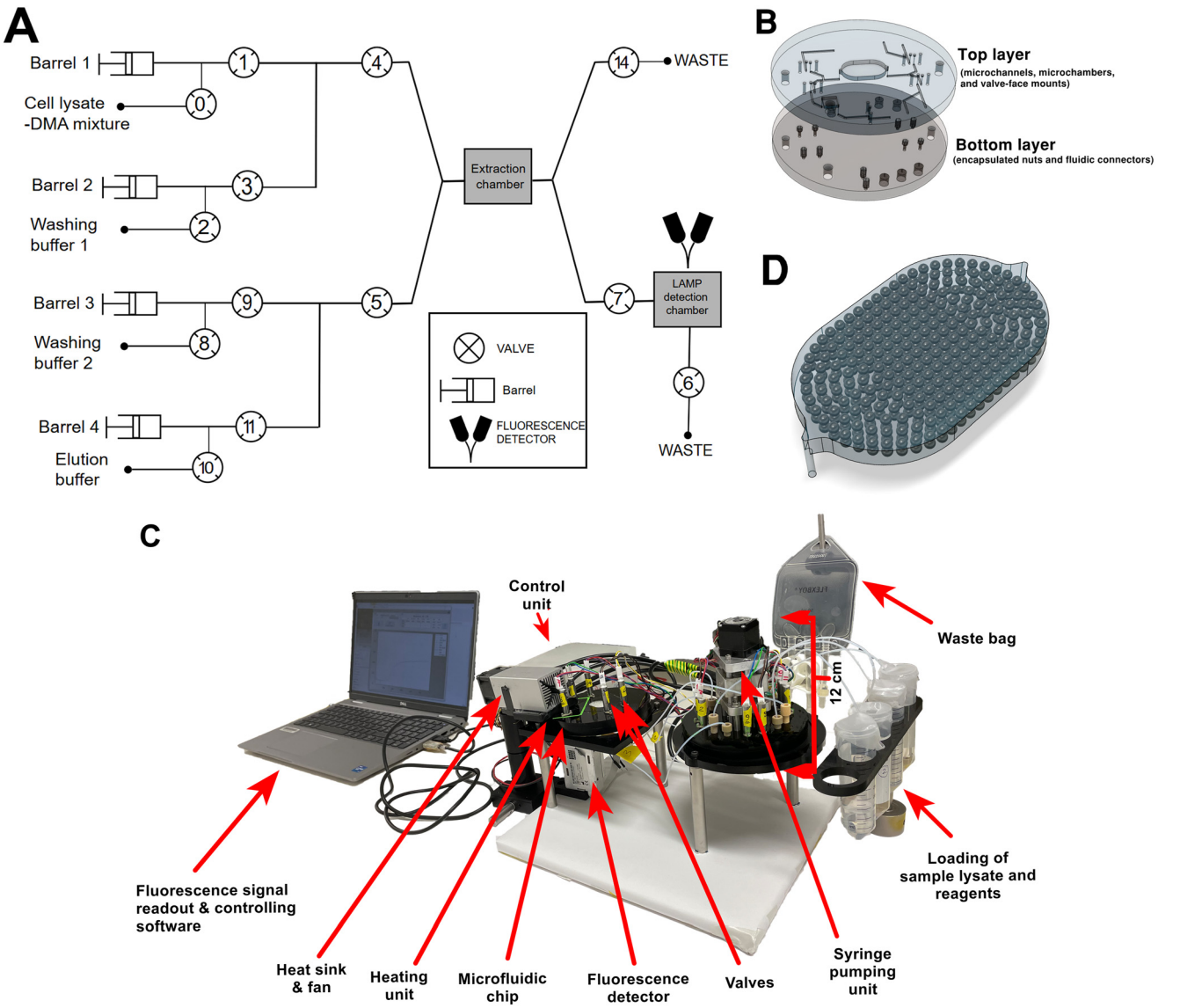

Fig. 2 LAMPTRON system: A lab-on-chip analyser combines sample processing and LAMP-enabled analysis of extracted DNA samples. A) Fluidic diagram shows the components that control the microfluidic working flow. B) Schematic of the microfluidic chip (119 mm in diameter) showing the PMMA layers of the system. C) Assembled LOC system that integrates microfluidic, mechanical, electronic, electronic, and optical components that support automated DNA isolation and result interpretation. D) Oblique view of the extraction microchamber (depth = 3 mm) packed with silica beads.

provide the pump control system with positional feedback. Sample and reagent solutions are pumped into the system from 50 mL conical tubes (Falcon™) *via* tubing (poly(tetrafluoroethylene) (PTFE), 1.6 mm OD × 0.5 mm ID) mounted on the outside layer of the chip *via* 1/4 in.-28 fluidic fitting (LT-115X, IDEX Health and Science LLC, Washington, U.S.A.). A fluid storage bag (Flexboy, Sartorius, UK) is hung behind the system to collect waste (Fig. 2C). Fluidic control is automated using a 32-bit microcontroller-based electronics package with 16-bit analogue-to-digital inputs, which can stream raw data (1 Hz) over USB and store it on an 8 GB flash memory card, as previously described in ref. 38.

2.3.2. Extraction module and preparation of amine-functionalised silica beads. 710 μm diameter Silica beads were purchased from Sigma-Aldrich, UK and were autoclaved prior to use. To activate silanol groups on the surface of beads, they were treated with oxygen plasma for 10 min under 70 sccm of O_2 flow rate using a plasma system (Diener electronic GmbH & Co. KG, Ebhausen, Germany). The beads were then immersed in a 2% H_2O solution of 3-aminopropyltriethoxysilane (APTES) (Sigma-Aldrich, UK) for 60 minutes at 65 °C, followed by washing with deionised water and drying under a nitrogen stream. The APTES-treated beads were stored in safe-lock tubes (Eppendorf, UK) at room temperature until use. The treated silica beads were packed into the extraction chamber from the exposed top side of the chip, which was designed with 150 μm-diameter access microchannels machined from both sides of the extraction

Table 1 Sequences of LAMP primers

Primer name	Sequence (5′---3′)
LAMP-F3	CGGAAAACACCATGCCCAA
LAMP-B3	TCTCGTTCGGGTACAGCA
LAMP-FIP	GGCCAGAACCTTTCGTCTCTGTCAAGGGT GATTCGGGGAATG
LAMP-BIP	AATCCCGACACTTTCGATCCCGTCGAAGC CCTTCCAGTCTG
LAMP-LB	GGTTCACCCGACCCTACAAGA
LAMP-LF	ATATTGTACAAGGGCAAAAAGATGT

chamber to trap the silica beads. The extraction chamber was sealed with PCR-compatible sealing tape for stable packing, and to enable the beads' convenient replacement, thus allowing sustainable use of the LAMPTRON.

2.3.3. LAMP primer design. The LAMP primers used in this study were designed using PrimerExplorer™ V5 software (Eiken Chemical Co., Ltd., Tokyo, Japan), with additional support from Geneious R11 Bioinformatics Software (Biomatters Ltd., Auckland, NZ). The LAMP assay targeted a 222 bp region of the cytochrome P450 gene homologous to the *dabD* gene, which has been implicated in the biosynthesis of the neurotoxin domoic acid in *Pseudo-nitzschia* species.[6,39,40] The development of the LAMP assay is described in the SI, section S5. The LAMP amplification primers were synthesised by Integrated DNA Technologies (IDT, Leuven, Belgium) and are listed in Table 1.

2.3.4. LAMP analytical module. The second component of the LAMPTRON system was designed to conduct the LAMP amplification reaction using the DNA-intercalating dye for real-time fluorescence readout. To maintain the on-chip LAMP reaction temperature, a Peltier thermoelectric heater (ET-127-08-15, Adaptive JUNIOR Temperature Controller, European Thermodynamics Ltd., Leicestershire, UK) was installed above the reaction microchamber. Temperature feedback control was achieved in real-time using a thermoelectric cooler controller (ADJ-48-450-UR, Adaptive JUNIOR Temperature Controller, European Thermodynamics Ltd., Leicestershire, UK). A FluoSens integrated fluorescence detector (QIAGEN GmbH, Hilden, Germany) was mounted beneath the reaction chamber to measure fluorescence emission during the LAMP reaction. To improve the platform's optical performance and prevent external or stray light from interfering with optical measurements, semi-transparent dark PMMA sheets were used for the microfluidic channel.[41] The distance between the detector light output and reaction microchamber was optimised to 7 mm for all LAMP reactions. An LED integrated into the FluorSens device enabled excitation of the sample at 465 nm and measurement of emission from the sample at 510 nm.

2.4. System testing

2.4.1. On-chip DNA isolation using DMA-based extraction on silica beads. The microfluidic chip-based DNA extraction process for the current study involves four main steps: sample mixing, DNA binding, washing, and elution, as illustrated in Fig. 1A. All solutions used for DNA extraction were prepared in ultrapure DEPC-treated water (Thermo Fisher Scientific, UK). Cells were pelleted by centrifugation at 7100 rpm for 3 min, and the culture medium was discarded and the resulting cell pellets were frozen at −80 °C until use. To initiate the on-chip DMA-based DNA extraction protocol, the cell pellets were thawed and mixed with an equivalent volume of DMA lysis buffer (1 : 1). The DMA lysis buffer contains 100 mM Tris-HCl (pH 8.0) (SERVA, Germany), 10 mM ethylenediaminetetraacetic acid (EDTA) (Invitrogen, UK), 1% sodium dodecyl sulfate (SDS) (Sigma-Aldrich, UK), and 10% Triton X-100 (Sigma-Aldrich, UK), 0.1 mg mL^{-1} proteinase K enzyme (Qiagen, Germany), and 100 mg mL^{-1} dimethyl adipimidate (DMA) agent (Alfa Aesar, UK). The cell lysate in DMA solution was loaded into the microfluidic chip at a flow rate of 350 μL min^{-1} and then incubated for 5 min at room temperature in the extraction microchamber to increase the binding of released DNA to the silica beads. Silica beads were washed twice with 0.01 M PBS (Sigma-Aldrich, UK) as washing buffer 1, and with 0.1 M trisodium citrate (Alfa Aesar, UK) in a 10% ethanol solution as washing buffer 2. The washing buffers were added to the extraction microchamber at a flow rate of 250 μL min^{-1} and incubated for 5 minutes. Finally, an elution buffer consisting of 10 mM sodium bicarbonate (pH 10.6) (Sigma-Aldrich, UK) was added at a flow rate of 150 μL min^{-1} through the silica beads, and the mixture was incubated for 3 min to recover the DNA sample. The DNA samples collected from the platform were evaluated for integrity by measuring their concentration and purity using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA) and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) using the dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA). A commercial kit (DNeasy Mini Kit, Cat. No. 69104, Qiagen, Germany) was used for an assessment of DNA recovery from the same sample, following the manufacturer's protocols. Relative DNA recovery efficiency was calculated by comparing the average DNA yield from LAMPTRON's extraction module to the yield obtained using the standard solid-phase extraction kit (DNeasy Mini Kit, Qiagen, Germany), which was defined as 100% recovery.[42]

To prevent any potential carryover of extraction between samples, a series of clean-up procedures was employed. These involved replacing the silica beads with freshly treated ones before each extraction, ensuring the removal of any bound debris, proteins, or other potential LAMP inhibitors. Subsequently, 80% ethanol (v/v) solution, DNA away solution (Thermo Fisher Scientific, UK), and 1 % w/v bovine serum albumin (BSA) (Sigma Aldrich, UK) in ultrapure nuclease-free water (Invitrogen, UK) were loaded into the chip using a 10 mL Luer slip syringe (BD Plastipak, UK). To completely dry the cleaned chip, it was dried in a convection oven (Heraeus, Thermo Fisher Scientific, UK) at 45 °C for 1 h.

We assessed several modification steps to the DMA-based DNA purification protocol using a microfluidic device and APTES-treated silica beads. The following modifications were

made: (i) optimisation of the volume of cells and DMA lysis buffer with input ratios of 1 : 1 and 1 : 2; (ii) testing of two washing buffers, 0.1 M trisodium citrate in 10% ethanol and 0.01 M PBS buffer (Sigma-Aldrich, UK); and (iii) evaluation of the impact of elution step retention times of 3 and 10 min on DNA yield and quality. The effects of these protocol modifications on DNA yield and purity are reported in section 3.2.

2.4.2. LAMP assay conditions. The fresh LAMP reaction was prepared using 1× Warm Start LAMP master mix (New England Biolabs, Hitchin, Hertfordshire, UK), 1× LAMP fluorescent intercalating dye (New England Biolabs, Hitchin, Hertfordshire, UK), 1.6 μM of FIB/BIP primers, 0.2 μM of F3/B3 primers, 0.8 μM of loop primers LP/LF, 6 μL DNA template, and nuclease-free water (Invitrogen, UK) to make a 50 μL reaction mixture. The vitrified LAMP mixture was prepared in a similar way to the fresh reaction, but the nuclease-free water was substituted with a solution containing 2% (w/v) trehalose (Acros Organics, Belgium), 0.5% (w/v) glycerol (Sigma-Aldrich, UK), 0.01% (w/v) polyoxyethylene glycol (PEG) (Sigma-Aldrich, UK), and 1.5% (w/v) bovine serum albumin (Thermo Fisher Scientific, UK).

To prepare vitrified LAMP reaction mixtures, an air pump (M361-C; Charles Austen Pumps Ltd., Surrey, England, UK) was used to air dry the reaction mixture with a continuous airflow of 0.45 m s^{-1} for 45 minutes in a sterile biosafety cabinet. Fresh and preserved LAMP reaction replicates were both prepared off-chip in clean 0.2 mL non-transparent tube strips (Roche Molecular Systems Inc, UK). The freshly prepared LAMP reactions were used on the same day, whereas the tubes containing vitrified LAMP reagents were stored at room temperature for up to five months in Aluminium film bags filled with MiniPax® Silica Gel Desiccant Sachet (Sigma-Aldrich, UK) before use. Vitrified preserved LAMP reagents were reconstituted by adding 5 mM dNTPs (New England Biolabs, Hitchin, Hertfordshire, UK) before being mix-pipetted with DNA sample extracted on-chip using the DMA-based method, resulting in a final reaction volume of approximately 50 μl. The reaction microchamber was then sealed with sealing tape (Applied Biosystems, UK) and the mixture was subsequently incubated for 49 min at 65 °C using the heating element fixed above the amplification chamber. Negative controls were triplicated throughout the on-chip DMA protocol and then analysed by both fresh and pre-stored LAMP reactions (negative controls were no template controls where the DNA template was replaced by nuclease-free water).

The compact FluoSense detector described above measured the real-time LAMP fluorescence signals for amplified DNA from triplicate samples of each cell standard concentration extracted using an on-chip DMA-based method. In total, 36 runs were performed on the LAMPTRON platform to evaluate the reproducibility of DNA analysis extracted from a tenfold serial dilution of *P. multistriata* cells, ranging from 10–10^5 cells per mL in tandem with the negative controls. DNA templates from triplicate samples of known cell concentrations were analysed by LAMP, and repeated in triplicate using fresh and preserved vitrified LAMP reagents. Raw fluorescence intensities were extracted from time points over 49 min of all LAMP reactions and normalised by the average baseline fluorescence of the first ten cycles for each amplification reaction.[43] The time to threshold (T_t) value of the LAMP reaction was determined as the time at which the fluorescence signal crosses the threshold fluorescence value.[44] The LAMPTRON chip was cleaned between runs as described in the above section 2.4.1 to eliminate any potential effect from sample-to-sample carryover. We extracted six DNA replicates from each culture concentration (10–10^5 cells per mL). For each concentration, three replicates were assayed using fresh LAMP reagents, while the remaining three used vitrified reagents. Each LAMP assay was conducted on separate days, resulting in independent calibration curves for both fresh and vitrified reagents (Fig. S5).

We evaluated the statistical significance of differences in DNA concentration and purity ratios between extraction methods using a paired *t*-test. We also conducted a two-way analysis of variance (ANOVA) to investigate the effects of reagent types and cell dilution on the time to threshold (T_t). Data were normally distributed, as confirmed by the Shapiro–Wilk test ($p > 0.05$). Statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS (version 31, IBM, UK).

3. Results and discussion

3.1. Working process of the LAMPTRON LOC system

The LAMPTRON system integrates on-chip DNA extraction with real-time LAMP amplification. By incorporating larger beads (710 μm in diameter), we effectively mitigated the aggregation, microchannel clogging, and bead wash-out challenges commonly associated with nano- and microbeads in microfluidic extraction systems.[45] This method recovered 34.13 ± 2.35% of the DNA compared to the solid-phase extraction kit (100% recovery), while the APTES-treated membrane recovered 21.42 ± 4.60%. After optimisation of buffer conditions and elution time, the recovery efficiency increased significantly, achieving up to 75% ± 3.86 with DMA-functionalised silica beads, compared to 47% ± 8.61 using APTES-treated membranes under the same optimised conditions. Sequential washing for 10 min successfully removed cellular debris, including proteins, lipids, and polysaccharides, and achieved DNA purity to a 260/280 ratio of 1.57 ± 0.06 (Fig. 3A), approaching the ideal value of ~1.8 for pure DNA. Furthermore, by increasing the pH to >10 for 3 min using a sodium bicarbonate buffer, the crosslinking bonds were rapidly reversed, resulting in DNA elution at a concentration of 35.8 ± 0.35 ng μL^{-1} and a purity ratio (260/280) of 1.65 ± 0.026 (Fig. 3A). Paired *t*-tests indicated that the DNA yield was significantly lower with on-chip extraction compared to the column-based method ($p = 0.0012$). Although both $A_{260/280}$ and $A_{230/280}$ purity ratios were lower with on-chip extraction, the differences were not statistically significant ($p = 0.068$ and $p = 0.072$, respectively).

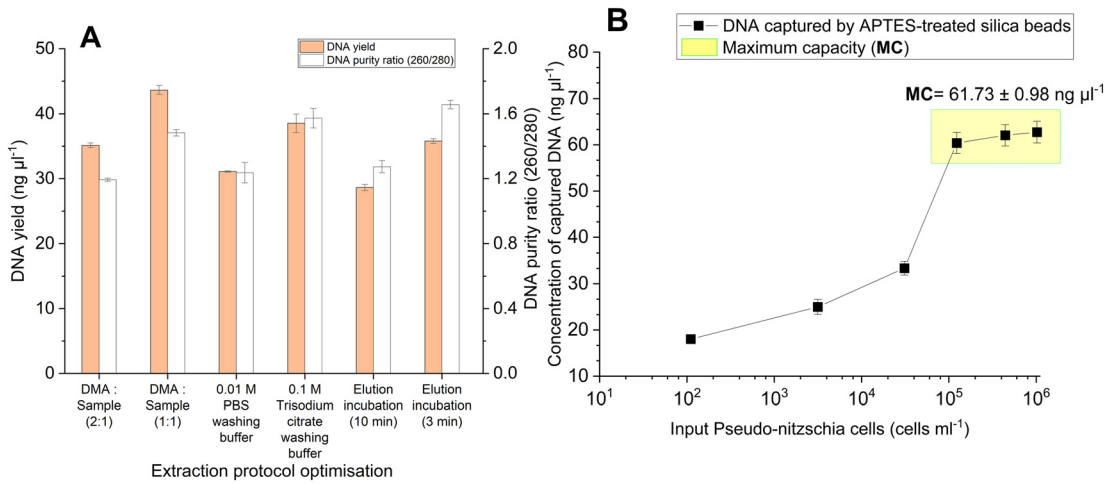

Fig. 3 Bar graph showing DNA yield and purity resulting from different experimental extraction protocols as determined by DNA concentration and absorbance ratios of 260/280 (A), and a log-linear line graph of captured DNA concentration at different sample concentrations, illustrating capacity for the DNA purification step, revealing the DNA-bead binding capacity of 61.73 ± 0.98 ng μL^{-1} (yellow). (B) Error bars denote the standard deviation of mean values between triplicate samples of the same *P. multistriata* culture.

3.2. Optimisation of the LAMPTRON DNA purification module

Five variations of the DMA-based extraction method were quantified using replicates harvested from the same *P. multistriata* cell number at 32 080 ± 240.54 cells per mL against a reference control labelled as DMA : sample (2 : 1) (Fig. 3A). The modified protocol using a 1 : 1 DMA : sample input ratio achieved the highest extraction efficiency, yielding a DNA concentration of 43.66 ± 0.66 ng μL^{-1} and an absorption ratio of 260/280 of 1.48 ± 0.02 (Fig. 3A). DNA samples are prone to co-elution with inhibitory substances such as humic acids, residual extraction reagents, and cellular debris, leading to reduced purity as indicated by a 230/280 (nm absorbance) ratio below 2.[46] To minimise inhibitor carryover, washing steps with 0.1 M trisodium citrate and 0.01 M PBS were implemented (Sigma-Aldrich, UK) resulting in higher DNA recovery, with a concentration of 38.53 ± 1.42 ng μL^{-1} and a 230/280 ratio of 1.57 ± 0.06, compared to 0.01 M PBS washing buffer (Sigma-Aldrich, UK), which yielded a DNA concentration of 31.1 ± 0.1 ng μL^{-1} and a 230/280 ratio of 1.23 ± 0.062 (Fig. 3A).

Elution duration significantly affects DNA yield.[30] Therefore, we examined the impact of elution times ranging from 3 to 10 minutes on DNA recovery. A 3-minute retention with 10 mM sodium bicarbonate (pH 10.6) elution buffer yielded 35.8 ± 0.35 ng μL^{-1} DNA with a purity ratio of 1.65 ± 0.026. In contrast, a 10-minute retention lowered the concentration to 28.63 ± 0.478 ng μL^{-1} and a purity ratio to 1.27 ± 0.037 (230/280). Based on these results, we selected a combination of three protocol modifications that improved DNA recovery for subsequent on-chip extraction experiments.

To assess the performance of an optimised protocol, the LAMPTRON extraction module processed *P. multistriata* samples with cell concentrations ranging from 1.02 × 10^6 to 1.11 × 10^2 cells per mL, following the extraction protocol in section 2.4.1. The DNA yield at the lowest cell concentration was 18.03 ± 0.84 ng μL^{-1}. DNA yield did not significantly increase between 4.42 × 10^5 and 1.02 × 10^6 cells per mL. Previous studies indicate that DNA extraction efficiency is dependent on bead capacity and cell loading to the extraction microfluidic system.[29] The maximum DNA binding capacity of LAMPTRON averaged 61.73 ± 0.98 ng μL^{-1} when extracting DNA from cells at concentrations ranging from 1.23 × 10^5 to 1.02 × 10^6 cells per mL. These results indicate that the available space on the surface of APTES-treated beads was saturated with DNA from cell numbers above 4.42 × 10^5 cells per mL, limiting DNA binding beyond the 61.73 ± 0.98 ng μL^{-1} threshold.

We compared the DNA capture efficiency between silica beads and glass fibre membrane, both received the same APTES treatment. Silica beads significantly improved DNA extraction efficiency, achieving a recovery rate of 75% ± 3.86 compared to 47% ± 8.61 with silica membranes, regardless of the sample source (SI, Fig. S1). DNA recovery in LAMPTRON was lower than with the commercial kit due to gentler, incomplete lysis compared to bead-beating techniques. On-chip DNA binding under laminar flow to silica beads is less efficient than in centrifugation-driven spin columns, where multiple washing steps effectively remove inhibitors. Incomplete elution from silica beads on the chip further reduces DNA yields. We propose optimising on-chip lysis with micromixers for complete cell disruption, testing alternative wash chemistries with varied buffer compositions, and enhancing elution efficiency using a heated elution step. To improve DNA recovery beyond the current capacity of the LAMPTRON system, we suggest increasing the size of the extraction microchamber to enhance the loading capacity of silica beads. Alternatively, enhancing DNA binding can be achieved by decreasing the size of silica beads, thereby increasing the surface area to volume ratio.[29]

However, incorporating nano- to micro-sized silica beads into microfluidic systems presents technical challenges, particularly the unintended migration of smaller beads beyond the extraction chamber, which can clog downstream microchannels or interfere with subsequent amplification steps, risking sample cross-contamination.[47]

3.3. Evaluation of quantitative LAMP detection on the LOC system

The performance of the LAMPTRON system in detecting and quantifying *P. multistriata* cells was assessed using on-chip DMA-based extractions followed by LAMP analysis. To evaluate the reusability of the extraction module, LAMPTRON was sequentially loaded with *P. multistriata* SZN-B954 cell suspensions in the following order: 1.6×10^5 cells per mL, 2.26×10^4 cells per mL, 1.33×10^3 cells per mL, 1.34×10^2 cells per mL, and 1.07×10^1 cells per mL. Between each run, clean-up procedures were rigorously performed to prevent any carryover (as described in section 2.4.1). Results demonstrated that reusing the extraction module of LAMPTRON yielded DNA of sufficient yield and quality across independent LAMPTRON runs, indicating minimal run-to-run variability, as shown in Fig. 4A (fresh reagents) and 5A (preserved and stored reagents). Coefficients of variation (CV%) for extraction ranged from 3.72% to 4.71% across input concentrations from 1.07×10^1 to 1.6×10^5 cells per mL. LAMP threshold-time (T_t) CVs ranged from 5.44% (1.07×10^1 cells per mL) to 7.91% (2.26×10^4 cells per mL) using fresh master mix, and from 4.26% to 5.79% with vitrified reagents. Combining extraction and amplification variability, total CV ranged from 6.62% to 7.20% (fresh reagents) and 6.35% to 6.86% (vitrified reagents) across tested concentrations. These findings demonstrate that LAMPTRON delivers reproducible DNA quantification across independent runs (SI, Fig. S6).

A stepwise increase in threshold time (T_t) was observed as the cell concentration decreased from 10^5 to 10 cells per mL, reflecting lower nucleic acid amounts from lower cells (Fig. 4A and 5A). DNA recovery improved with an increased input of cells into the DMA-based extraction module (Fig. 3B). Target-specific amplification was successfully demonstrated by the device, shown by gel electrophoresis results (Fig. 4B and 5B). Endpoint gel electrophoresis of LAMP products confirmed amplification but revealed minor non-specific bands, which are common in

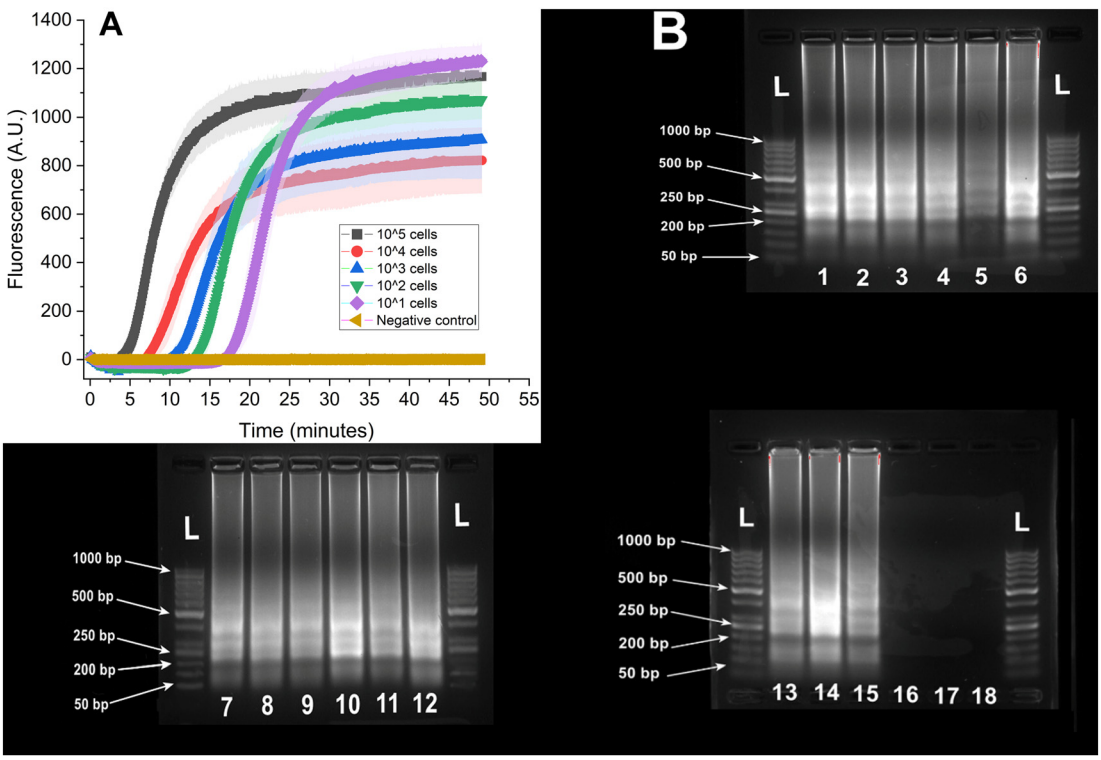

Fig. 4 Fluorescence real-time detection of *P. multistriata* cell standards using freshly prepared LAMP reagents (A). Samples were analysed on the LAMPTRON system in triplicates for each concentration as represented by coloured datapoints; (black; 1.6×10^5 cells per mL, red; 2.26×10^4 cells per mL, blue; 1.33×10^3 cells; green; 1.34×10^2, purple; 1.07×10^1 cells per mL; brown; non-template control (NTC). The LAMP reaction achieved a detection threshold of 1.07×10^1 cells per mL (n = 3). Error bars are represented as coloured filled area mean ± SD (n = 3 independent experiments). B) Gel electrophoresis data of the fluorescent LAMP products produced from the reactions in panel A, confirming the size of the LAMP amplicon at 222 bp. Lane L: DNA ladder (50 bp), lanes 1, 2, and 3 are replicates of 1.6×10^5 cells per mL amplicons, lanes 4, 5, and 6 are replicates of 2.26×10^4 cells per mL amplicons, lanes 7, 8, and 9 are replicates of 1.33×10^3 cells per mL amplicons, lanes 10, 11, and 12 are replicates of 1.34×10^2 cells per mL amplicons, lanes 13, 14, and 15 are replicates of 1.07×10^1 cells per mL amplicons, lanes 16, 17, and 18 are replicates of non-template control (NTC).

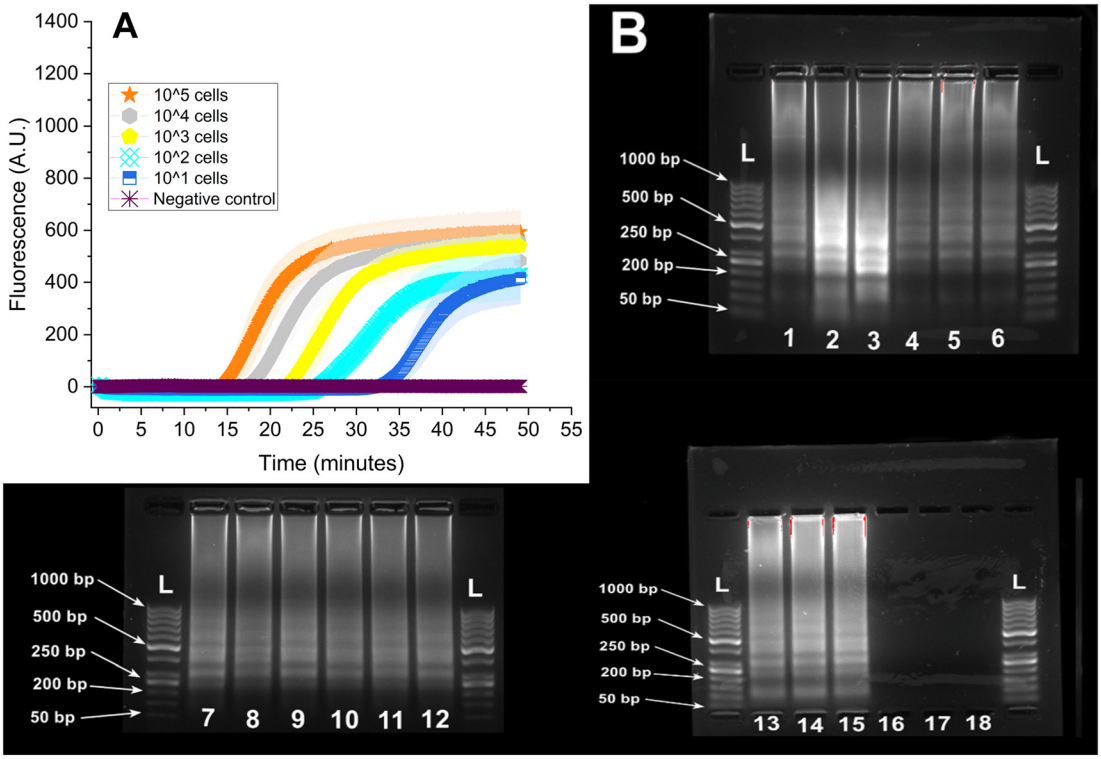

Fig. 5 Performance of pre-stored LAMP reaction of five months shelf-life. A) Amplification curves of amplified *P. multistriata* cell standards were represented by (orange; 1.6×10^5 cells per mL, grey; 2.26×10^4 cells per mL, yellow; 1.33×10^3 cells; cyan; 1.34×10^2, dark blue; 1.07×10^1 cells per mL; purple; non-template control (NTC). Each data point represents the average of independent triplicate reactions, and the coloured areas indicate standard deviation. LAMPTRON was cleaned between each extraction and amplification run to avoid carry-over contamination (section 2.4.1). A limit of detection was determined at approx. 1.07×10^1 cells per mL using vitrified LAMP reaction on LAMPTRON system (n = 3). B) Gel electrophoresis results of the vitrified LAMP products produced from the reactions in panel A. The lanes of electrophoretic agarose gel confirmed the specificity of LAMP amplicon at a size of 222 bp. Lane L represents DNA ladder (50 bp), lanes 1, 2, and 3 represent replicates of 1.6×10^5 cells per mL amplicons, lanes 4, 5, and 6 represent replicates of 2.26×10^4 cells per mL amplicons, lanes 7, 8, and 9 represent replicates of 1.33×10^3 cells per mL amplicons, lanes 10, 11, and 12 represent replicates of 1.34×10^2 cells per mL amplicons, lanes 13, 14, and 15 represent replicates of 1.07×10^1 cells per mL amplicons, lanes 16, 17, and 18 represent replicates of non-template control (NTC).

endpoint LAMP analysis (Fig. 4B and 5B and section S6). Amplification efficiencies, measured as isothermal doubling time (IDT), were 93.98 ± 7.41 for fresh reagents and 129.27 ± 16.55 for vitrified reagents. A strong log-linear relationship existed between T_t and known cell numbers, yielding R^2 = 0.98 for fresh reagents and R^2 = 0.97 for preserved reagents (Fig. S5). These findings confirm consistent amplification kinetics and reliable quantification of *P. multistriata* using the LAMPTRON across a broad range of cell concentrations. Detailed methods for standard curve generation and calculations of LAMP kinetics are described in section 2.4.2 and SI (section S6).

The long-term stability and robustness of LAMPTRON analysis were tested by preserving LAMP assays using an off-chip vitrification method for five months at room temperature prior to use on the LAMPTRON system. The existing vitrification methods for preserving LAMP reagents entailed adding enzymes and primers at the rehydration steps.[48–50] In contrast, we preserved all LAMP reaction components, including enzyme, primers, and reaction buffer at room temperature for up to five months. This protocol simplified the operation of LAMPTRON by eliminating additional pipetting or dilution steps, thereby reducing the risk of contamination and making the operating procedure more user-friendly for non-specialists.

To reconstitute the vitrified LAMP assays, we added DNA templates, as described in section 2.4.2. However, preserving LAMP reagents can lead to variability in diagnostic performance compared to freshly prepared assays, due to the decrease in enzyme reactivity or the reaction reactivity.[44,51] As demonstrated in the LAMP amplification curves (Fig. 4A and 5A), the fluorescence signals of fresh LAMP amplicons reached a saturation level at an earlier T_t value than vitrified LAMP amplicons. Specifically, the detection of DNA templates extracted from the same sample (10^5 cells per mL) was delayed by approximately 10 min when vitrified LAMP reagents were used. Similarly, fresh LAMP detected 1.07 × 10 cells per mL (LoD & LOQ) in less than 25 minutes, with a mean threshold time (T_t) of 20.16 ± 1.09 minutes (Fig. 4A). Using vitrified LAMP reagents, the same cell concentration was detected in under 40 minutes (T_t = 36.31 ± 1.55 minutes; Fig. 5A). To capture delayed positives, we therefore extended the reaction endpoint to 49 minutes. T_t variability between replicates at intermediate cell concentrations remained stable, and cell numbers were reliably quantified using preserved reagents. Compared to fresh LAMP

reagents (Fig. 4A), vitrified reactions generated reduced fluorescence intensity (Fig. 5A), reflecting partial degradation or diminished activity of the intercalating dye or enzyme mix over time. For deployment, we propose generating separate calibration curves for fresh and vitrified reagent formats to account for altered amplification kinetics and maintain quantitative accuracy. Despite reduced signal and amplification delay, LAMPTRON maintained consistent amplification performance with both fresh and vitrified reagents, even after extended ambient storage. A two-way ANOVA showed significant differences in T_t values between fresh and preserved reagents ($F = 2712.74$, $p < 0.001$) and across cell dilutions ($F = 80.91$, $p < 0.001$), where the F-value represents the ratio of variance explained by interaction to error variance, with a larger F-value indicating a stronger interaction effect. Similar discrepancies in T_t values have been previously reported for both fresh and preserved LAMP reagents.[44,50–52]

Using freshly prepared LAMP reagents, the coefficient of variation (CV) of LAMP T_t values for triplicate measurements of 1.07×10^1 and 1.6×10^5 cells input were 0.0544 and 0.0504, respectively. The CV of LAMP T_t values for triplicate measurements of 1.07×10^1 and 1.6×10^5 cells using preserved LAMP reagents with a shelf life of five months were 0.0426 and 0.0577, respectively. Detecting 1.07×10^1 cells per mL *P. multistriata* cells took less than 36.31 ± 1.55 minutes (*i.e.*, T_t value) following a sample processing time of approximately 25 minutes, resulting in a total analysis time of less than one hour. In comparison, conventional workflows involving DNA extraction and quantitative PCR (qPCR) typically require around two hours to complete, highlighting the time-saving advantage of the LAMPTRON system. The LAMP assay also sensitively detected *Pseudo-nitzschia* cells. Some qPCR measurements can be as sensitive as 1 cell per mL,[53] while other studies report quantification ranges of 10^3 cells per mL (ref. 54) and $1.2–8.9 \times 10^7$ cells per mL,[55] typically requiring thermal cycling and post-amplification processing steps that delay the time-to-result. In contrast, LAMP detects DNA amplification from 10 cells per mL of *P. multistriata* in a 20-minute assay (Fig. 4A).

The precision of LAMPTRON was evaluated by generating standard curves from LAMP T_t values of known cell numbers (Fig. S5). The limit of detection (LoD) was defined as the lowest concentration on the standard curve at which all replicates consistently yielded a detectable amplification signal,[56] while the limit of quantification (LoQ) was determined as the lowest concentration at which the amplification signal was both detectable and reproducible across repeated LAMP runs. The LoD and LoQ were established using real-time LAMP amplification curves (Fig. 4A and 5A) and the concentration providing reproducible quantitative data across multiple experiments, respectively (Fig. S6). LAMPTRON achieved LoD and LoQ of $\leq 1.07 \times 10$ cells per mL, the minimum concentration tested using both fresh and vitrified preserved reactions. The quality of the DNA template is critical for determining LAMP reaction kinetics and may adversely affect the LoD and delay detection time.[51] The LAMPTRON chip was cleaned through multiple steps to remove sample-to-sample inhibitors/cross-contamination by lipids, protein, DNA and reagents that may affect the LoD. Fresh LAMP demonstrated the highest overall performance and remains the preferred choice for applications requiring rapid field results. The run-to-run reproducibility of (T_t) suggests the system suits field deployment, particularly for presence/absence testing and threshold-based decisions for *P. multistriata*.

In Monterey Bay, an autonomous *in situ* platform, the Environmental Sample Processor (ESP) is routinely deployed for real-time detection and quantification of *Pseudo-nitzschia* blooms and their domoic acid toxin using sandwich hybridisation and ELISA, tracking bloom severity.[57–59] The ESP detected cell concentrations as low as 152 cells per mL,[58] while LAMPTRON achieved a lower detection limit of 10.7 cells per mL. The LoD of LAMPTRON demonstrates a 14.02-fold improvement in detection sensitivity compared to the statutory threshold of 150 cells per mL defined by UK monitoring guidelines for conducting emergency statutory testing of seawater and shellfish to avoid human health risks.[60]

To ensure performance in the field, we suggest testing the LAMPTRON system on both crude and filtered coastal seawater collected from multiple estuarine and brackish sites, including turbid bloom samples, to evaluate sensitivity, specificity, and robustness under real-world conditions. The sensor's pumping system has previously been implemented in autonomous lab-on-chip instruments deployed *in situ* for chemical analysis in a wide range of marine environments.[38] Incorporation of the pump unit onto a microfluidic chip reduces the need for external tubing and control modules. However, temperature control and fluorescence readout still rely on laboratory heaters and bench-top optical units. To develop a fully portable, battery-powered LAMPTRON system in future iterations, we suggest key engineering strategies: (i) a disposable cartridge with one-touch sample loading and automated internal valving to minimise operator steps; (ii) a single compact electronics module powered by a rechargeable battery; (iii) an integrated fluorescence system; and (iv) an integrated temperature control system capable of maintaining stable temperatures throughout the LAMP assay. These improvements will enable the transition from the current prototype to a portable, autonomous, field-deployable LAMPTRON system.

The LAMPTRON system presents a simplified and cost-effective method for DNA purification compared to commercially available kits. The on-chip DMA purification in LAMPTRON involves three steps from concentrated cells: lysis-binding, washing, and elution, taking 25 min. In contrast, commercially available kits require 25 manual + 7 centrifugation steps, requiring approximately 2 hours to extract DNA from the same *Pseudo-nitzschia* cells. Commercial kits however, can process up to 24 samples simultaneously on a standard 24-position centrifuge rotor, reducing hands-on time to roughly 5 minutes per sample, making them more efficient for high-throughput laboratory settings, while the LAMPTRON prototype processes samples sequentially, requiring about 25 minutes per sample. Reagent

costs per sample are estimated to decrease by 97.3% with the DMA-based extraction protocol, as summarised in Table S1, in the SI, providing a rapid and cost-effective alternative for DNA purification. Hence, the DMA-based technique is not only cost-effective but also demands minimal hands-on time for executing the streamlined analysis on LAMPTRON.

4. Conclusions

We designed and developed a microfluidic system that enables flexible customisation for lab-on-a-chip DNA extraction and real-time quantification. The DNA extraction process leverages the high surface-to-volume ratios of silica beads, using a dimethyl adipimidate (DMA) method to achieve high-efficiency DNA capture. By automating the repetitive liquid handling operations during the DNA extraction process, we were able to achieve high DNA yield using small reagent volumes, which reduces cost and minimises sample-to-sample cross-contamination. The LAMPTRON prototype also achieved reproducible on-chip-based LAMP analysis of the *Pseudo-nitzschia* toxin-associated target gene within 49 min. The LAMP quantification method offers faster analysis than traditional methods that require costly reagents, sophisticated equipment and a centralised laboratory. The streamlined on-chip operations for automated DNA extraction and LAMP testing for detecting as low as 10 cells per mL were completed in less than one hour. Such early-stage detection of *Pseudo-nitzschia* blooms can mitigate human health risks and economic costs.[61] Additionally, the preserved LAMP assay allows for flexible testing workflows in decentralised settings with minimal user intervention, avoiding potential sources of contamination or human-introduced error. The reusable chip, fluorescence detector, thermal profiling unit, and electronics minimise the cost, waste, and consequently the environmental footprint of LAMPTRON. The study demonstrated the simplicity of performing quantification using dried LAMP and the versatility of the DNA capture method from a range of microbial cells, which makes LAMPTRON potentially applicable in a variety of environmentally relevant settings. Future work will focus on engineering an autonomous system that performs a number of simultaneous DNA extractions coupled with nucleic acid amplification testing, amenable for field use, to bypass sample transportation and lab processing.

Author contributions

Ahmed I. Alrefaey: conceptualisation, investigation, methodology, validation, formal analysis, visualisation, writing – original draft, reviewing and editing, funding acquisition, project administration. Jonathan S. McQuillan: conceptualisation, methodology, validation, investigation, supervision, reviewing and editing. Allison Schaap: conceptualisation, methodology, validation, investigation, supervision, reviewing and editing. Fabrizio Siracusa: conceptualisation, methodology, validation, investigation, supervision, reviewing and editing. Christopher L. Cardwell: methodology, validation, investigation, reviewing and editing. John Walk: methodology, investigation, reviewing and editing. Daniel Rogers: methodology, validation, and reviewing. Reuben Forrester: methodology, validation, and reviewing. Matthew C. Mowlem: conceptualisation, methodology, validation, investigation, supervision, reviewing and editing, and funding acquisition. Julie C. Robidart: conceptualisation, methodology, validation, investigation, supervision, reviewing and editing, funding acquisition, project administration.

Conflicts of interest

Matt Mowlem and Chris Cardwell are shareholders of ClearWater Sensors, which manufactures lab-on-a-chip systems for non-genetic analytes (measuring seawater chemistry) and on-chip processes. The remaining authors declare no competing interests.

Data availability

All key data supporting the conclusions made in this paper have been included either in the main text or in the supplementary information (SI).

Supplementary information covering extraction bead preparation, extraction optimisation and development, and comparisons to traditional techniques, is available. See DOI: https://doi.org/10.1039/d5sd00135h.

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