**Supporting information**

# Limited Resource Preparable Chitosan Magnetic Particles for Extracting Amplification-Ready Zeptomole-Range Nucleic Acid from Complex Biofluid

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**Table S1: Comparison of previously reported methods for chitosan-dependent microdevices and magnetic particles for nucleic acid extraction**

| **Types of Device/material** | **Preparation of Device**<br>**Instrument required**<br>**Preparation time** | **Type of extracted nucleic acid using the microdevice/particle.**<br>**Capture efficiency** | **Device extracts nucleic acid from complex biofluid/lysate?** | **Post-extraction application, PCR or real time PCR**<br>**Limit of detection (LoD)** | **Post-extraction application, isothermal amplification**<br>**Limit of detection (LoD)** | **In situ (on bead, on-device, or on particle) amplification?** |
|---|---|---|---|---|---|---|
| γ-$Fe_2O_3$@Chitosan@Polyaniline hybrid for nucleic acid extraction[1] | Polymerization of aniline on the surface of the $Fe_3O_4$@Chi magnetic particles<br>Prep Time: 38 h for $Fe_3O_4$@Chi MNPs and 48 h for γ-$Fe_2O_3$@Chitosan@Polyaniline hybrid<br>Instruments needed: Magnetic stirrer, vacuum oven. | 15% adsorption efficiency for 10 min of incubation, increased to 70% for 145 min incubation time for 5 mg of nanoparticle with 100 ng/μL input DNA solution | Genomic DNA from blood extracted | PCR<br>Not for detection purpose, PCR has been done to check the extracted DNA using magnetic device is PCR compatible or not. | No | No |
| Paper based origami microdevice for nucleic acid amplification on nucleic acid from live cells[2] | Molds engraved using CNC milling machine, then immersed in PDMS solution overnight. Separately, paper treated with $O_2$ plasma, then | Viable and non-viable cells from *E. coli* and *Salmonella sp*. culture was added to directly to | No | No | End point LAMP with visual readout<br>LoD is $10^3$ | Yes |

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| | immersed into 1% w/v chitosan was anchored for nucleic acid capture.<br>Prep Time: 18 – 24 h<br>Instruments needed: CNC milling machine, $O_2$ plasma machine. | microdevice<br>Efficiency not ascertained, but device can capture nucleic acids up to $10^9$ cells | | | CFU/mL | |
| Pipette-actuated capillary array comb with integrated DNA extraction[3] | Pipette-actuated capillary array comb with (poly(methyl methacrylate)) base embedded with glass filter paper(chitosan) discs and glass capillaries<br>Prep time: more than 12-15 h.<br>Instruments needed: Driller, diamond wire cutting machine, $O_2$ plasma reactor. | Capture efficiency over 97% for 10 – 50 ng of pre-extracted bacteriophage genomic DNA | Cell lysate and mock clinical (urine) sample | Real time PCR on pre-extracted bacteriophage virus DNA.<br>LoD not ascertained. | End point LAMP performed on<br>a. genomic DNA of *E.coli, S. aureus, K. pneumonia*<br>b. lysed *E. coli* cells<br>c. spiked *E. coli* cells on urine sample (mock clinical sample)<br>LoD: 200 cfu $mL^{-1}$ for | Yes |

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| | | | | | *E.coli* cells | |
| Chitosan coated nanoceria[4] | $CeO_2$/chitosan modified electrode device was synthesized by drop-casting method.<br>DNA probe was then immobilized.<br>Prep time: more than 24 h<br>Instruments needed: magnetic stirrer, hydrothermal, muffle furnace. | Optimal sensor functionality obtained at 0.1 μM concentration of extracted *C. perfringens* genomic DNA.<br>Electrochemistry mediated readout detected for pM concentration *C. perfringens* genomic DNA in water and spiked milk. | *C. perfringens* genomic DNA spiked milk | N/A | N/A | N/A |
| PDMS coated with chitosan-magnetic particle by template-assisted soft lithography technique[5] | Chitosan-coated lanthanum strontium manganese oxide magnetic nanoparticles in deionized water was cross-linked with sodium tripolyphosphate cross-linker.<br>Prep time: more than 18 h.<br>Instruments needed for preparation: Syringe pump.<br>Nucleic acid extraction | Capture efficiency measured on performed on *E. coli* pure genomic DNA is 90 – 97% | The device performs in situ mechanical lysis on cell culture solutions of *Escherichia coli, Salmonella typhimurium, Shigella boydii, Klebsiella pneumoniae, Pseudomonas aeruginosa* and | PCR<br>LoD is $10^3$ CFU/mL for PCR conducted on 16S rRNA gene (using a non-integrated thermal cycler) from the extracted genomic DNA from microdevice-enabled bacterial | No | No |

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| | requires syringe pump | | *Acinetobacter baumannii,* then captures the DNA | lysis | | |
| Chitosan-coated microparticles[6] | Chitosan solution reacted with epoxide coated silica microparticles and vortexed.<br>Prep time: more than 12 h.<br>Instruments needed: Magnetic stirrer, vortex | Upper limit of capture is 500 copies of DNA/μg of particles for pBR322 plasmid DNA and human genomic DNA | Human genomic DNA detection from 1 μL blood (following lysis)<br>Spiked pBR322 plasmid in blood | Quantitative real time PCR<br>Detected $10^4$ copies for plasmid spiked in blood and DNA detection from 1 μL blood | No | Yes |
| Cellulose-chitosan porous membrane[7] | Chitosan powder of 0.5, 1.0, 2.0, and 3.0 wt% was incubated in a mixture of a strong acid.<br>Prep Time: more than 72 h.<br>Instruments needed: Magnetic stirrer, radio frequency plasma reactor | Capture efficiency 72 – 78% for 3.5 kbp plasmid DNA | No | PCR<br>LoD not ascertained | No | No |
| Chitosan magnetic silica microparticles[8] | Chitosan microparticles were fabricated by reacting chitosan solution with epoxy silica magnetic beads<br>Prep time: more than 12 h.<br>Instruments needed: Homogenizer, magnetic | Capture efficiency over 95 – 100% for plasmid DNA and *E. coli* genomic DNA | No | Quantitative real time PCR<br>Detected $10^4$ copies for plasmid DNA | No | Yes |

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| | stirrer, vortex, centrifuge | | | | | |
| $Fe_3O_4$@silica@chitosan nanoparticles[9] | $Fe_3O_4$@silica@chitosan nanoparticles were synthesized by alkaline precipitation method.<br>Preparation time-8 h<br>Instruments needed: Magnetic stirrer, ultrasonicator | Capture efficiency 98% for calf thymus DNA | Extraction from saliva | No | No | No |
| Chitosan gold nanoparticles[10] | Nanoparticles have been made by sol gel method.<br>Preparation time-1 h<br>Instruments needed: Heat bath | Capture efficiency not calculated | Genomic DNA extraction from sputum | PCR on extracted DNA<br>Limit of detection not ascertained | No | No |
| Chitosan functionalized magnetic particle with silica coating[11] | Chitosan magnetic nanoparticles were prepared by coprecipitation method followed by silica coating.<br>Preparation time- more than 12 h<br>Instruments needed: Magnetic stirrer, nitrogen purging system. | Calf thymus DNA capture efficiency 86% and elution efficiency 96% | Extracted from soybean lysate. | PCR on extracted DNA<br>Limit of detection not ascertained | No | No |
| Chitosan-coated silica bead and chitosan-modified multichannel extraction | Chitosan coating of clean silica beads was accomplished through | Pre-extracted human genomic DNA capture | DNA was extracted from | PCR on extracted DNA | No | No |

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| chip[12] | incubation with cross linker.<br>Instruments: multichannel has been made using lithography. PDMS layer on the top of PMMA plate, followed by binding of chitosan using cross-linker<br>Preparation time: over 12 h | efficiency 88% for beads, 77% for microchannel | blood sample | Estimated 7.0 ng of human genomic DNA from blood was extracted and amplified | | |
| Chitosan coated silica microdevice and silica particles[13] | Chitosan oligosaccharide coated silica beads were packed on microdevices using standard photolithographic techniques.<br>Preparation time: over 20 h<br>Instruments needed: diamond tip drill bit, rotor, vortex, sonicator.<br>Syringe pump required for nucleic acid extraction | Capacity for λ-phage DNA 118 ng on microdevice and for rRNA 67 ng<br>rRNA has been extracted.<br>Extraction efficiency 71-81% | RNA from buccal cells and alveolar rhabdomyosarcoma cancer (~~ARMS~~) cell line has been extracted. | Reverse transcriptase PCR on RNA extracted from ~~ARMS~~ alveolar rhabdomyosarcoma cell line.<br>LoD not ascertained | No | No |
| Chitosan-coated nylon membrane[14] | Method A: Chitosan oligosaccharide cross-linked using N,N-carbonyldiimidazole with the nylon membrane<br>Method B: To prepare | Over 90% capture efficiency measured for salmon sperm DNA | No lysate has been used. | Quantitative real time PCR<br>LoD: 2 copies.<br>Also, 25 molecules of DNA in 50 mL | In situ LAMP<br>LoD: 20 copies | Yes |

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| | hydrogel coated membranes, a chitosan solution added to the LoProdyne membrane along with glutaraldehyde cross-linker.<br><br>Preparation time: 2 – 4 h for both methods.<br><br>Instruments needed: Lite spin coater, Vacuum evaporator. Flow controlled syringe/luer lock required for nucleic acid capture. | | | was detected | | |

**Materials and Methods**

$FeCl_3 \cdot 6H_2O$ (#GRM165), $FeSO_4$ ,$7H_2O$ (#TCE119), 2X real-time SYBR mastermix (#MBT074) were purchased from HiMedia. Chitosan (medium molecular weight, #18824) and the rest of the chemicals were purchased from SRL Chemicals unless mentioned otherwise. Bst 2.0 enzyme, dNTP mix were procured from New England Biolab, USA. Fetal bovine serum was procured from Sigma Aldrich and was heat-inactivated prior to usage. MCF-7 genomic DNA was a gift from Prof. Subhabrata Sen's lab at Department of Chemistry, Shiv Nadar University, India. DNA concentration estimations using $UV_{260}$ were carried out at Thermo MultiSkan Go plate reader. Real-time LAMP and PCR experiments were carried out in BioRad CFX Maestro or Connect instrument. Gel and colorimetric LAMP experiments were carried out at the Eppendorf master cycler.

**Synthesis of coprecipitation-cured chitosan-coated magnetic nanoparticles (CCCMP).** The synthesis was carried out as described elsewhere with slight modification[22]. The process was carried out in a 50 mL conical flask. 5 mL of 2 M $FeCl_3 \cdot 6H_2O$ (HiMedia) (2.7 gm in 5 mL) and 5 mL of 1.5 M $FeSO_4$ ,$7H_2O$ (HiMedia) (2.1 gm in 5 mL). The procedure started with mixing of 293 μL of $FeSO_4$,$7H_2O$ (final concentration 0.04 M) and 440 μL $FeCl_3 \cdot 6H_2O$ (final concentration 0.08 M) with preheated (50°C) 9.3 mL 1% medium molecular weight chitosan in 1% acetic acid (total reaction mixture volume-10 mL). The dosing of 4 mL of the aqueous ammonia at 200 μL/min was started with constant stirring. After that reaction mixture was kept at 50°C for the next 20 minutes. The resulting magnetic particles were then subjected to magnetic decantation-mediated washing with deionized water with the help of a permanent magnet until pH increased to 7, resuspension to 10 mL water, followed by continuous stirring for two hours at 90°C ("curing"). The particles were then washed 5 times with 10 mL 0.05 M MES buffer each (in each step incubated with MES buffer for 10 minutes) and finally, 5 times washed with 10 mL autoclaved water each using magnetic decantation. The magnetic particles (MPs) were stored in water at 4°C after the concentration (mg/mL) was calculated.

**Preparation of bare iron oxide magnetic particles.** The bare iron oxide particles were prepared using alkaline coprecipitation methods from $FeCl_3$ and $FeSO_4$ exactly as described above except the use of chitosan solution and without the follow-up curing step (heating at 90°C for 2 h). The magnetic particles (MPs) were stored in water at 4°C after the concentration (mg/mL) was calculated.

**Synthesis of electrostatically cross-linked chitosan magnetic particles (ECCMP).** The electrostatically cross-linked chitosan coated iron oxide particles were adopted as previously reported with the following modification[23]. 9 mL of 1% chitosan in 1% acetic acid was mixed with 1 mL 0.5 mg/ml bare iron oxide magnetic particles in a vial in a preheated water bath at 60°C for 10 mins. The vial was placed on the magnetic stirrer (700 r.p.m) and added with 1.5 mL of sodium tripolyphosphate (STPP) solution (stock concentration 0.5% in water) with continuous stirring. The reaction was carried out for 10 mins. Then the reaction mixture was washed with 10 mL of autoclaved water each 10 times. The magnetic particles (MPs) were stored in water at 4°C after the concentration (mg/mL) was calculated.

**Characterization and sample preparation of magnetic particles for FE-SEM, EDX, FT-IR, DLS, Zeta Sizer and XRD.** Morphology and size of the CCCMP, ECCMP, and bare iron oxide were determined by field emission scanning electron microscopy (FE-SEM, Sigma-Carl Zeiss). The presence of elements in all types of magnetic particles was identified using energy-dispersive X-ray spectroscopy (EDX) attached with FE-SEM. X-ray diffraction spectroscopic (XRD) analysis was carried out for the determination of the crystalline structure of prepared magnetic particles. Fourier transform infrared spectra (FT-IR) of the samples were recorded on an FTIR spectrometer (Perkin Elmer) from 500 to 4000 $cm^{-1}$.

Dynamic light scattering (DLS) was employed to determine the hydrodynamic diameter and zeta potential of chitosan coated iron oxide nanoparticles dissolved in MiliQ water at 25 ± 0.1 °C. For hydrodynamic diameter determinations, a backscattering detection angle of 173° was employed. The ζ-potential was estimated using the Smoluchowski equation from the laser Doppler electrophoretic mobility measurement at 25 ± 0.1 °C.

**Genomic DNA isolation from *E.coli*.** *E.coli* DH5α strain was cultured on tryptic soya broth (TSB) at 37°C for 12 – 15 h. 10 mL of culture were pelleted down at 4000 r.p.m for 5 minutes and the pellet is resuspended in 1 mL of lysis buffer (10 mM Tris-HCl, 0.1 M NaCl, 5 mM EDTA,

0.5% [w/v] sodium dodecyl sulfate, Proteinase K (100 ng/mL), pH 7.8). The cell suspension was incubated at 37°C, 1 mL of 10M ammonium acetate is added, and centrifuged at 12000 r.p.m at 4°C. The supernatant fluid was transferred into a new sterile tube. Subsequently, cold isopropanol was added to the supernatant to the final 50% concentration and kept at −20 °C for 20 min. After this stage, the solution was centrifuged at 12000 r.p.m and the supernatant was discarded. The pellet is dissolved with 1 mL of cold 70% ethanol, centrifuged at 12000 r.p.m, and the supernatant was discarded. DNA template was air-dried and dissolved in 50 μL sterile distilled water and stored at −20 °C until PCR amplification.

**Cell lysate preparation from bacterial culture.** *E.coli* DH5α strain was cultured on tryptic soy broth (TSB) at 37 °C for 12 h. 25 μL of culture containing $10^9$ cells was mixed with 25 μL of 2X lysis buffer (20 mM Tris-HCl, 2 mM EDTA, 2% [v/v] Triton X100, 1.0% Tween-20, pH-8). For the limit of detection assays, the cell suspension was serially diluted to $10^1$ – $10^5$ cells/50 μL using 1X lysis buffer. The cell suspension was incubated at 95°C for 15 minutes and neutralized with 50 μL of 0.05 M MES buffer pH-5.2 before magnetocapture experiments.

**$UV_{260}$ quantification of DNA binding capacity of CCCMP, ECCMP, and bare iron oxide with pure genomic DNA.** 1.0, 2.5, or 5.0 mg of wet CCCMP, ECCMP, or bare iron oxide was taken from storage and the supernatant was removed by magnetic decantation. 100 μL 0.05 M MES buffer (pH - 5.2) was added and incubated for 10 mins (charging Step) on the benchtop with occasional finger tapping. The supernatant was removed by magnetic decantation. The magnetic particles were then incubated with 50 μL 500 ng/μL *E. coli* genomic DNA solution in 0.05 M MES buffer (pH - 5.2) on the benchtop with occasional finger tapping or vortex-enabled shaking. The supernatant was separated from magnetic particles by magnetic decantation. The particles were washed by resuspension once by addition of 50 μL 0.05 M MES buffer (pH - 5.2) and the supernatant was separated from magnetic particles by magnetic decantation. The particles were incubated with 10, 25, or 50 μL of elution buffer 10 mM Tris HCl-pH-8.5 (for 1.0, 2.5, or 5.0 mg magnetic particles, respectively) on a benchtop with occasional finger tapping or vortex-enabled shaking. The eluent (supernatant) was separated from magnetic particles using magnetic decantation and quantified with $UV_{260}$ in a Thermo MultiSkan Go plate reader nanodrop. Assuming a linear correlation of DNA adsorption for 1.0, 2.5 or 5.0 mg magnetic particles, eluted DNA (in ng) was plotted against weight (in mg) of magnetic particles and a linear fitting was applied. The slope of the linear fit was calculated as the amount of DNA captured and eluted per mg of wet magnetic particles.

**DNA binding assay with CCCMP, ECCMP with pure genomic DNA, and crude cell lysate**. 2.5 mg of wet CCCMP or ECCMP was taken from storage and the supernatant was removed by magnetic decantation. 100 μL 0.05 M MES buffer (pH - 5.2) was added and incubated for 10 mins (Charging Step) on the benchtop with occasional finger tapping. The supernatant was removed by the magnet. Next, 25 μL MES 0.05 M pH 5.2 solution containing $10^1$ – $10^5$ copies of *E. coli* gDNA (in case of genomic DNA) or 100 μL of neutralized (above) heat lysate from $10^1$ – $10^5$ cells (in case of crude lysate) was added to the particles and incubated for 10 mins on the benchtop with occasional finger tapping. The supernatant was separated from magnetic particles by magnetic decantation. The particles were washed by resuspension twice by addition of 25 μL 0.05 M MES buffer (pH 5.2) each time and the supernatant was separated from magnetic particles by magnetic decantation. 25 μL elution buffer (10 mM Tris HCl, pH 8.5) was added and incubated for 10 mins on the benchtop with occasional finger tapping. The supernatant was collected as elution and subjected to NAAT procedure as described below. The magnetic particles left out are called beads and resuspended in 25 μL autoclaved water and stored in 4°C.

**LAMP with elution and beads obtained from DNA binding assay.** The LAMP reaction was conducted with the elution and beads obtained from DNA magnetocapture assay with $10^9$ copies of gDNA or heat lysate from $10^9$ cells. The final LAMP reaction (total 25 μL) contained the primer pairs in the following final concentrations: 0.2 μM outer primers, and 1.6 μM forward and backward inner primers. The loop primers, when utilized were used at final concentrations at 0.8 μL. The reaction mix also contained 2.5 μL of 10× Bst 2.0 DNA polymerase reaction buffer [1× containing 20 mM Tris-HCl, 50 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Tween-20, pH 8.8], 1.4 mM dNTPs, 1 μL of an 8 U/μL concentration of Bst 2.0 DNA polymerase, 6 mM $MgSO_4$ and 5 μL of elution as template. In the case of beads, 5 μL of beads resuspended in autoclaved water is used as the template. In case of no template control, 5 μL of autoclaved water is used instead of beads or elution obtained from DNA binding assay.

**Real-time LAMP to determine LoD for detection of bacterial genomic DNA from the aqueous and crude lysate.** Elutions from magnetocapture experiments performed on $10^1$ – $10^5$ copies of aqueous *E. coli* gDNA solutions or heat lysate from $10^1$ – $10^5$ *E. coli* cells were subjected to real-time LAMP experiments. The final LAMP reaction (total 25 μL) contained 0.2 μM outer primers, 1.6 μM forward inner primers, 2.5 μL of 10× Bst 2.0 DNA polymerase reaction buffer [1× containing 20 mM Tris-HCl, 50 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Tween 20, pH-8.8], 2.5 μL SYBR I (final concentration 1X diluted from 10,000X), 1.4 mM dNTPs, 1 μL of an 8 U/μL concentration of Bst DNA polymerase (New England Biolabs), 6 mM $MgSO_4$ (2 μL) and 5 μL of elution as template. Real-time LAMP was set at the following settings for each cycle; 69°C for 30 s, 68°C for 30 s, 67°C for 30 s, 66°C for 60 s with fluorescence monitoring at the last step. The cycles were repeated 30 times in a CFX Maestro or CFX connect real-time PCR machine (BioRad). This was immediately followed by the default standard melt curve analysis protocol present in the instrument, where the temperature was gradually increased from 65°C to 95°C every 5 s by 0.5°C. Alongside, the fluorescence was recorded at each temperature increment step.

**DNA extraction with CCCMP, ECCMP with mammalian genomic DNA from aqueous solution and complex biofluid.** The magnetocapture extraction-amplification assay was tested for detecting human genomic DNA sample (obtained from MCF-7 cells) spiked in aqueous solution or 50% (final) heat-inactivated fetal bovine serum (FBS) samples. In each case, the assay was performed on $10^4$ copies, $10^3$ copies, or $10^2$ copies present in 25 μL solution. The aqueous solution or the serum was added with 25 μL 0.1 M MES pH 5.2 buffer. The DNA was captured using 2.5 mg of either CCCMP or ECCMP by 10 min benchtop incubation with occasional finger tapping. Following two successive washing with 25 μL 0.05 M MES pH 5.2, the bound DNA was eluted in 25 μL 10 mM Tris-HCl buffer pH 8.5. 5μL of the elution was subjected to real-time PCR.

**Real-time PCR to determine LoD for detection of human genomic DNA from the aqueous sample and complex biofluid.** In each case, the assay was performed in 25 μL solution, where the template was the 5 μL elution from the magnetocapture of $10^4$, $10^3$, $10^2$ copies of MCF-7 genomic DNA. The elution was added with 2X proprietary real-time PCR mix (12.5 μL), forward and reverse primer (final concentration 0.4 μM, *actin B* gene), and molecular grade water. PCR was set at the following settings: 95°C for 180 s, then 39 cycles of 95°C for 10 s, 55°C for 10 s, and 72°C for 30 s, where the last step consisted of fluorescence monitoring. This was followed by the default program of melt curve analysis where the temperature was gradually increased from 65°C to 95°C every 5 s by 0.5°C. Alongside, the fluorescence was recorded at each temperature increment step.

**Table S2.** Oligonucleotide primer sequences (5′ to 3′) used in this study for loop-mediated isothermal amplification (LAMP) and real-time PCR

| **Primer Name** | **Primer Sequence (5′ to 3′)** |
|---|---|
| For LAMP (*malB* gene in *E. coli*)[15] | |
| F3 | GCCATCTCCTGATGACGC |
| B3 | ATTTACCGCAGCCAGACG |
| BIP | CTGGGGCGAGGTCGTGGTATTCCGACAAACAC CACGAATT |
| FIP | CATTTTGCAGCTGTACGCTCGCAGCCCATCATG AATGTTGCT |
| Loop forward | CTTTGTAACAACCTGTCATCGACA |
| Loop backward | ATCAATCTCGATATCCATGAAGGTG |
| For real-time PCR (*actin B* gene in *H. sapiens*)[16] | |
| Forward primer | TGG CAC CAC ACC TTC TAC AAT |
| Reverse primer | GGT CTC AAA CAT GAT CTG GGT CA |

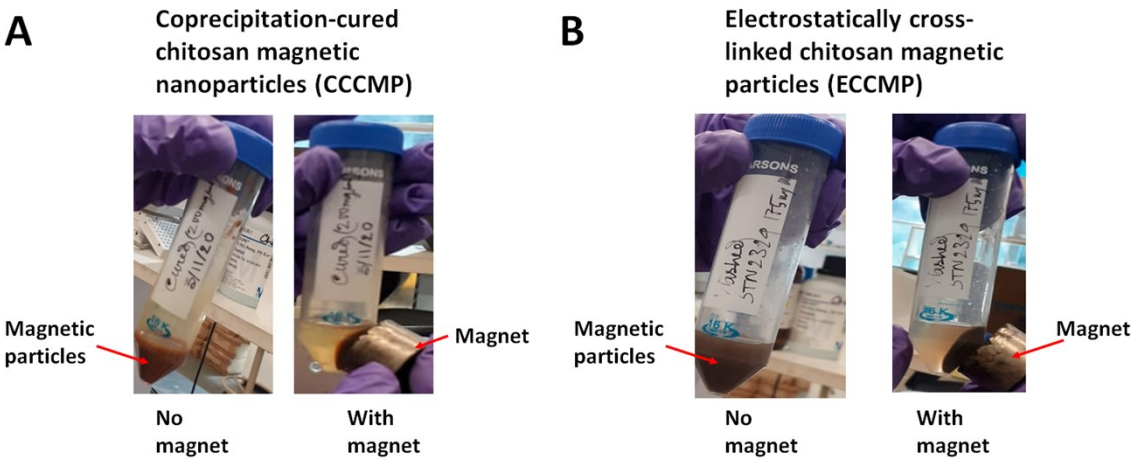


**Fig. S1.** Images of CCCMP (A) and ECCMP (B) particles in the presence or absence of the magnet.

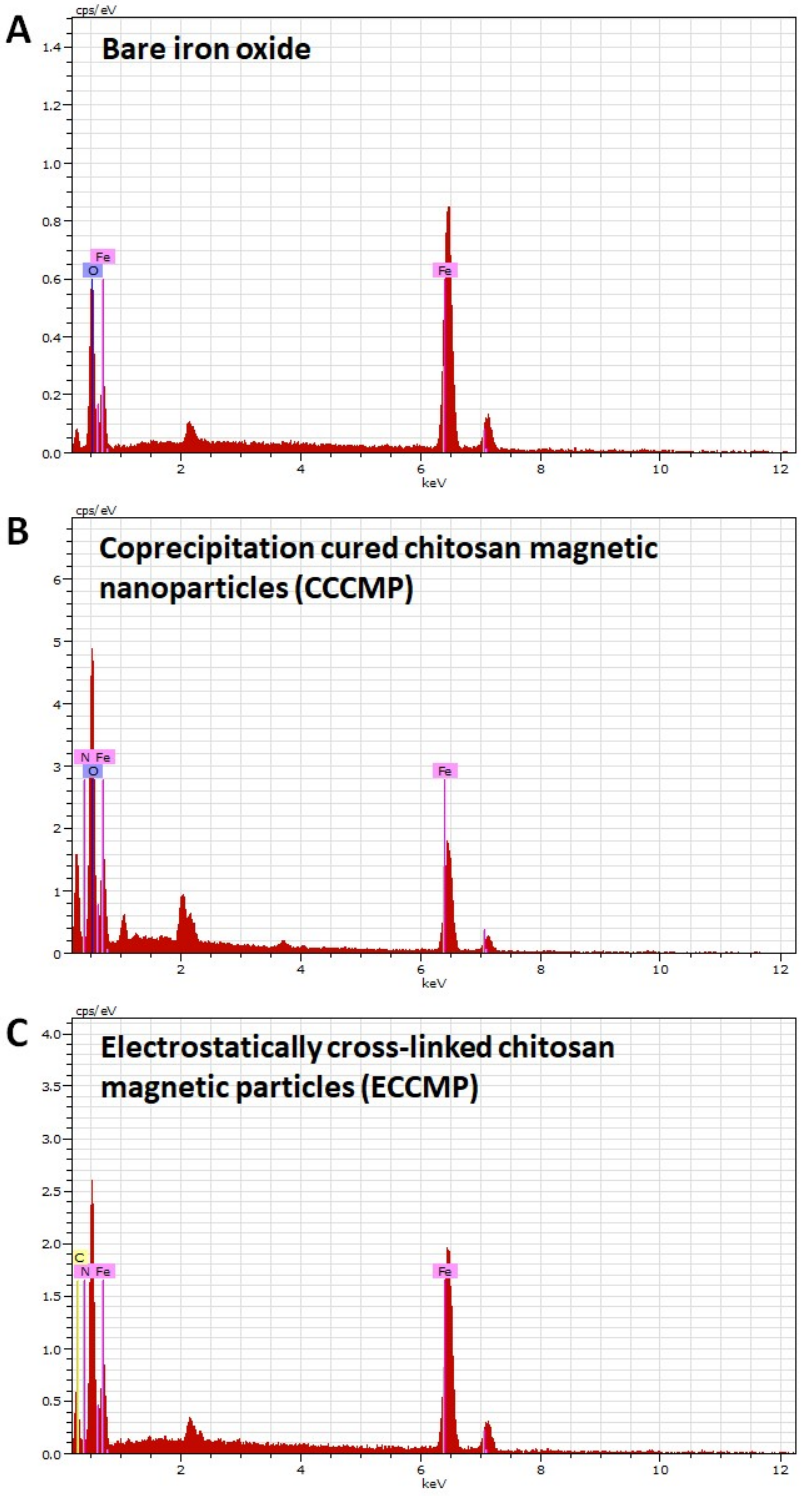


**Fig. S2.** EDX characterization of bare iron oxide (A), CCCMP (B), and ECCMP (C) magnetic particles.

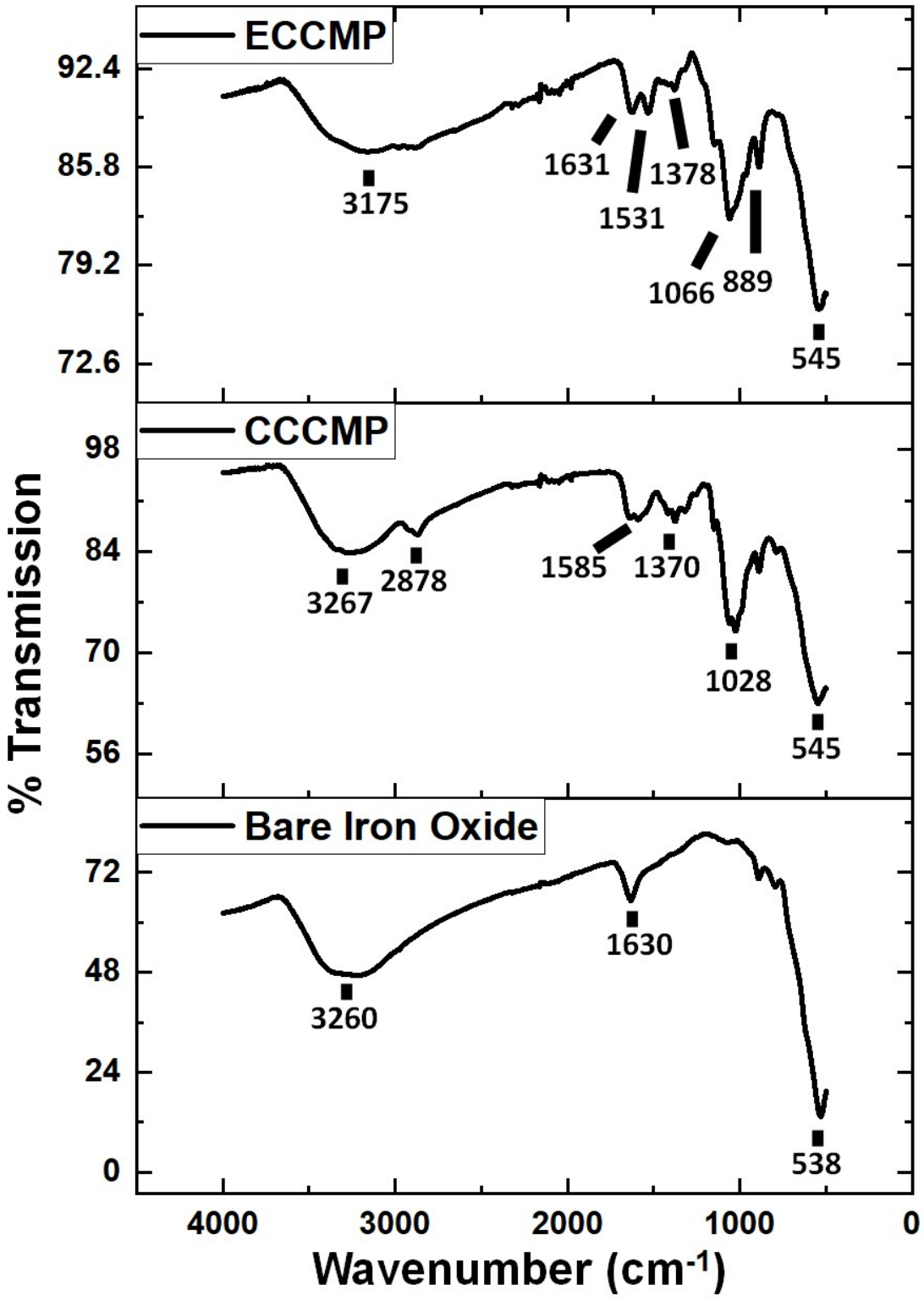


**Fig. S3.** FT-IR characterization of CCCMP, ECCMP, and bare iron oxide magnetic particles.

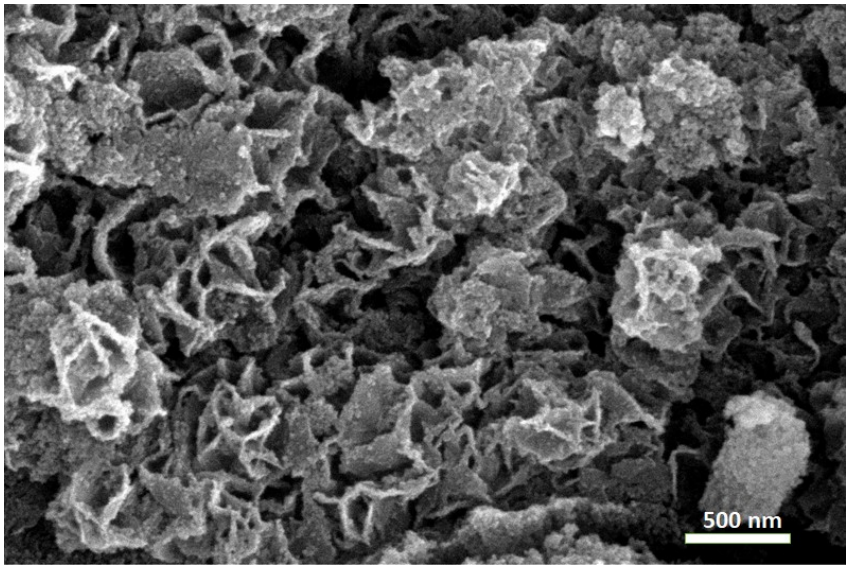


**Fig. S4.** Visualization of filament-like structure in CCCMP in FE-SEM analysis.

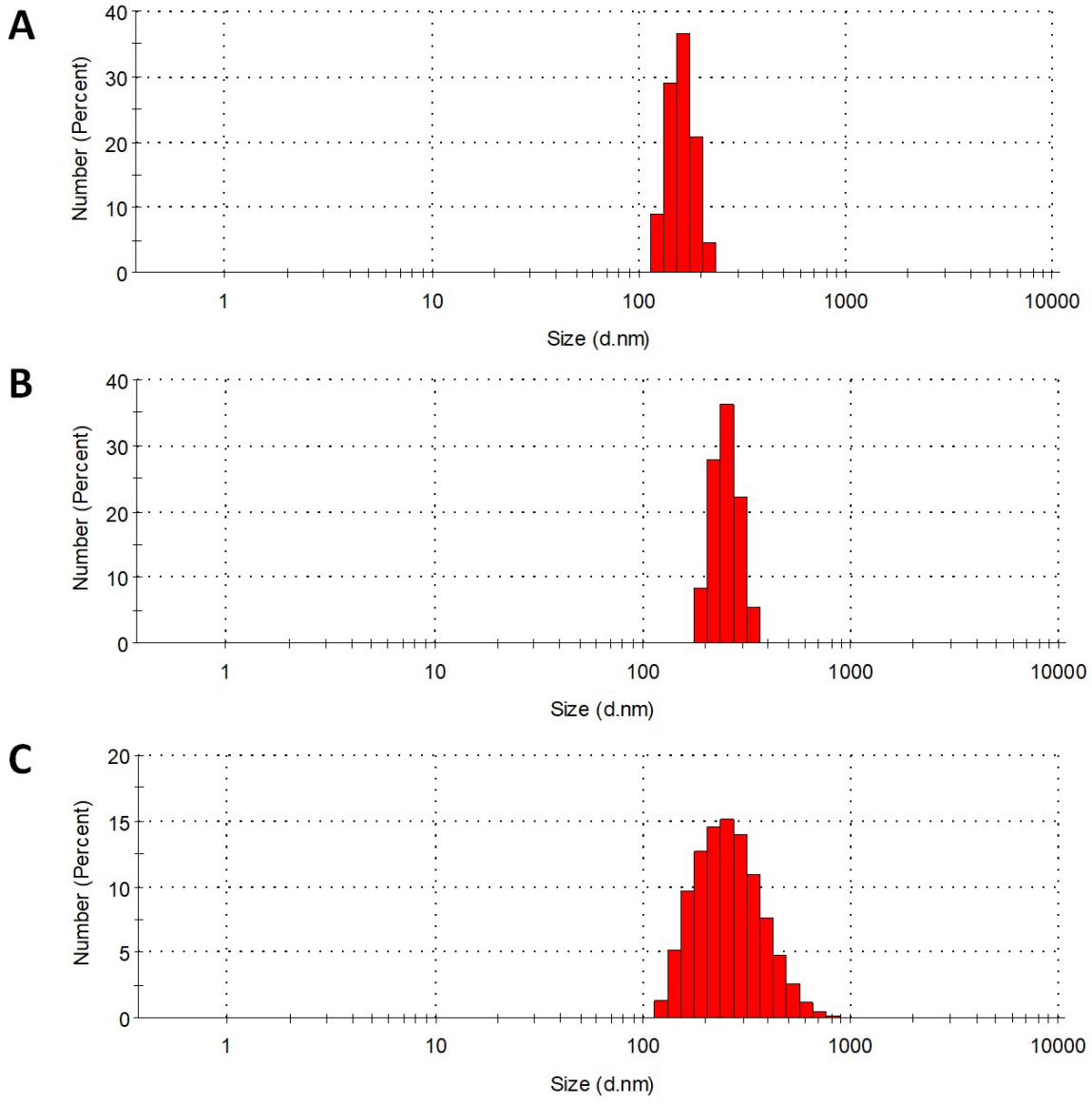


**Figure S5.** Dynamic light scattering (DLS) studies of bare iron oxide nanoparticles (panel A), CCCMP (panel B), and ECCMP (panel C)

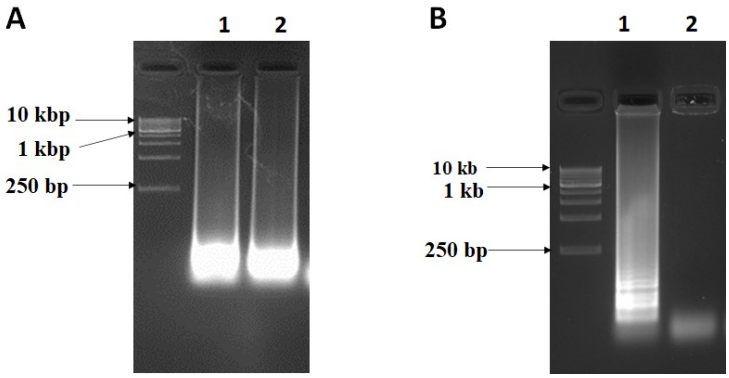


**Fig. S6.** Loop-mediated isothermal amplification for detecting *malB* gene in *E. coli*. A, non-specific amplification in the presence of loop primers analyzed in 1.5% agarose gel. Lane 1, in presence of *E. coli* genomic DNA. Lane 2, in the absence of *E. coli* genomic DNA. B, amplification in the absence of loop primers analyzed in 1.5% agarose gel. Lane 1, in presence of *E. coli* genomic DNA. Lane 2, in the absence of *E. coli* genomic DNA. The leftmost lanes in both gels represent a 10 kb ladder.

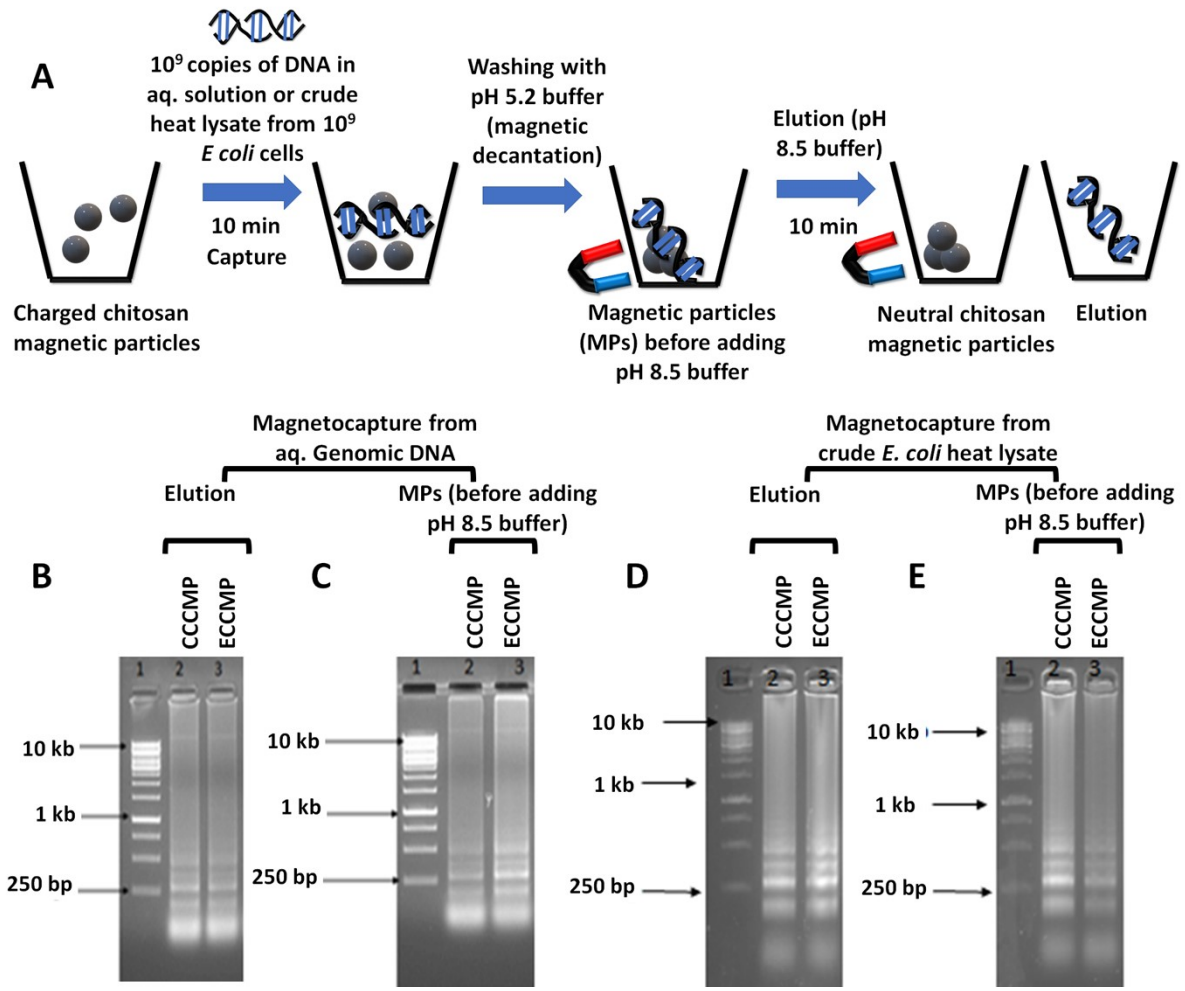


**Fig. S7.** Magnetocapture, elution, and loop-mediated isothermal amplification (LAMP) on $10^9$ copies of *E. coli* genomic DNA (gDNA) from aqueous solution or crude lysate. A, Scheme of magnetocapture assay. B, LAMP assay on pH 8.5 buffer elution from magnetocapture on gDNA in aqueous solution. C, LAMP assay on magnetic particles (MPs) itself after pH 5.2 buffer washing but before pH 8.5 buffer elution from magnetocapture on gDNA in aqueous solution. D, LAMP assay on pH 8.5 buffer elution from magnetocapture on crude lysate. E, LAMP assay on magnetic particles (MPs) itself after pH 5.2 buffer washing but before pH 8.5 buffer elution from magnetocapture on crude lysate. For crude lysate, $10^9$ cells were heat treated (95°C for 15 min) in lysis buffer (10 mM Tris-HCl, 1 mM EDTA, 1% Triton X-100, 0.5% Tween 20 pH 8) before magnetocapture. All experiments were analysed in 2% agarose gel electrophoresis where the leftmost lanes represent 10 kb ladder.

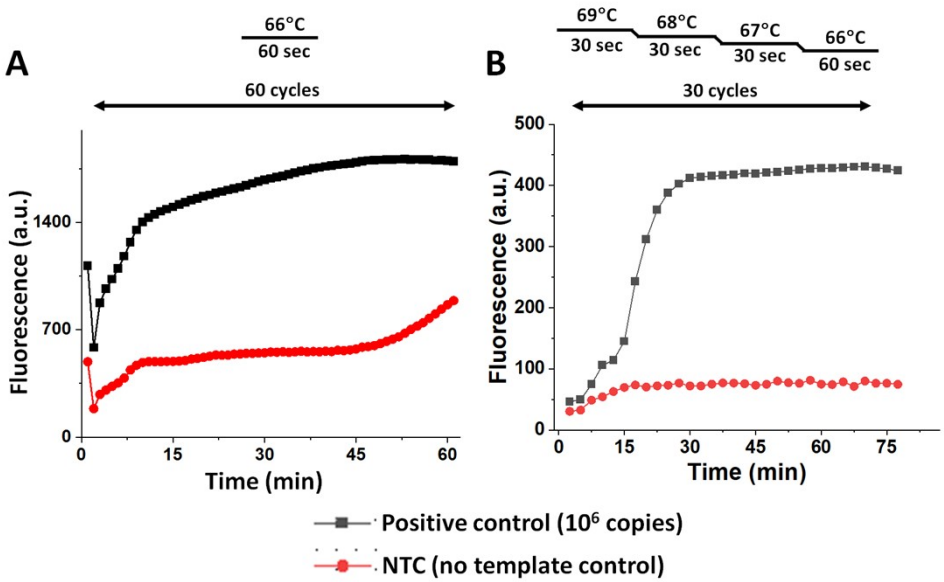


**Fig. S8.** Comparison between real-time loop-mediated isothermal amplification (LAMP) (panel A) and touchdown real-time LAMP (panel B) along with respective temperature cycling information. In both cases, the fluorescence was monitored at the 66°C step of the cycles. The experiments were conducted on $10^6$ copies of *E. coli* genomic DNA and no template control (NTC).

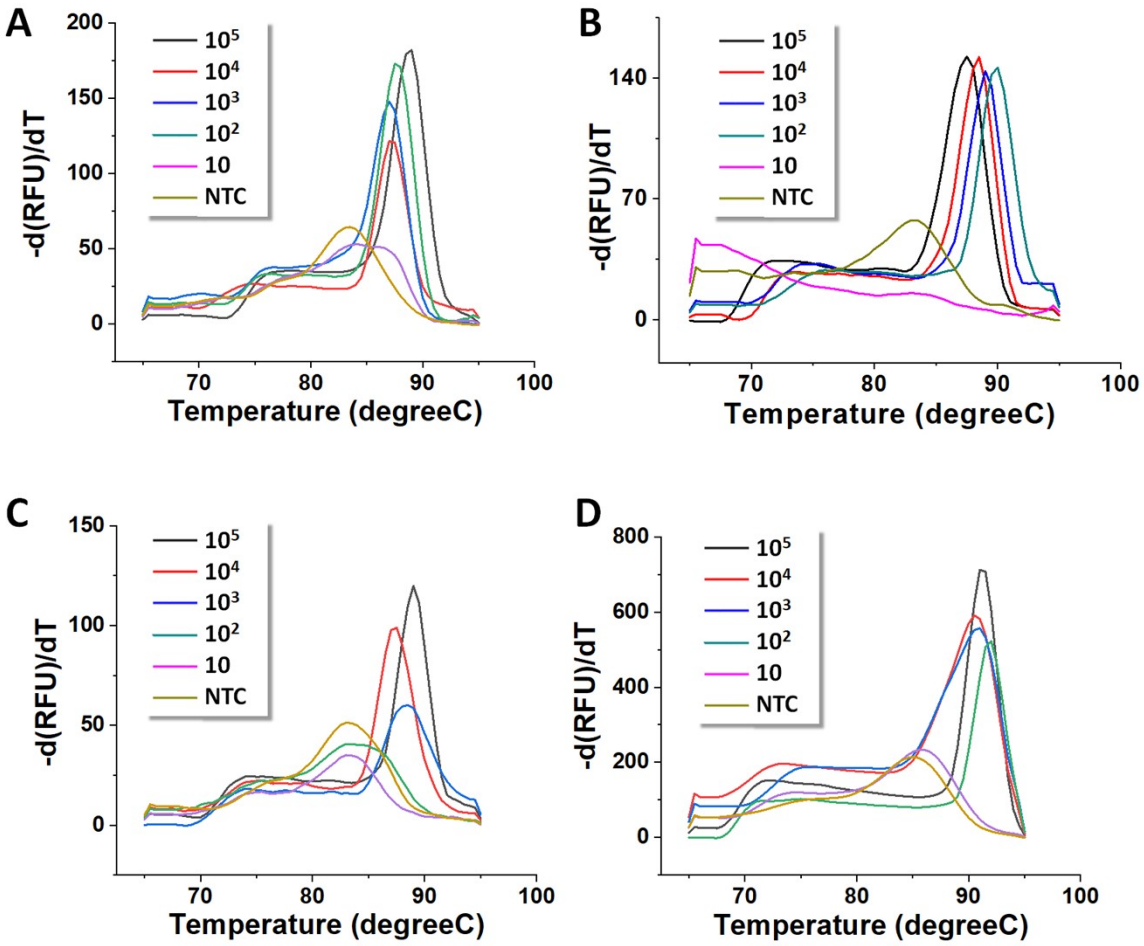


**Fig. S9.** Derivative melt curve analysis for real-time LAMP experiments on the elution from magnetocapture performed on $10^1$ – $10^5$ copies of *E. coli* genomic DNA in aqueous solution or crude lysate. A, real-time LAMP on elution from CCCMP magnetocapture on aqueous gDNA. B, real-time LAMP on elution from CCCMP magnetocapture on crude cell lysate. C, real-time LAMP on elution from ECCMP magnetocapture on aqueous gDNA. D, real-time LAMP on elution from ECCMP magnetocapture on crude cell lysate. For crude lysate, the cells were heat treated (95°C for 15 min) in lysis buffer (10 mM Tris-HCl, 1 mM EDTA, 1% [v/v] Triton X100, 0.5% Tween-20, pH 8) before magnetocapture. NTC refers to no template control.

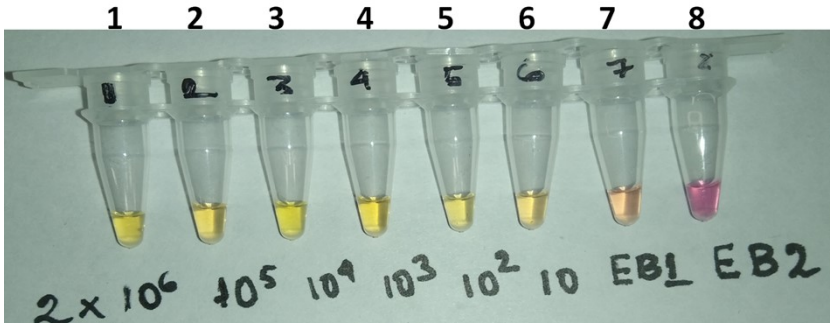


**Fig. S10.** Colorimetric LAMP assay using WarmStart LAMP Kit (NEB # E1700S) on magnetocapture extracted gDNA. DNA copies ranging from 2 x $10^1$ – $10^6$ copies in 25 μL 0.05 M MES buffer (pH 5.2) were subjected to magnetocapture using 2.5 mg CCCMP, and then eluted using 25 μL elution buffer (10 mM Tris-HCl (pH 8.5)). The follow-up 20 μL colorimetric LAMP reaction in tubes 1 – 6 then consisted of 10 μL 2X proprietary LAMP colorimetric mastermix, 8 μL elution, and 2 μL 10X *E. coli malB* primer mix (without loop primers). EB1 (tube 7) sample consisted of a CCCMP-mediated magnetocapture experiment without any genomic DNA that was eluted using elution buffer (a "mock" experiment), followed by colorimetric LAMP having the same reaction composition as above. EB2 (tube 8) sample contained the addition of 8 μL elution buffer (without any DNA from magnetocapture) to a colorimetric LAMP having the same reaction composition as above.

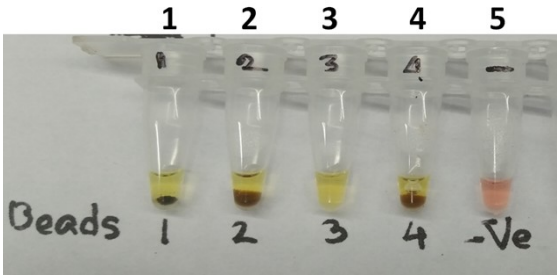


**Fig. S11.** Colorimetric LAMP assay using WarmStart LAMP Kit (NEB # E1700S) on magnetocapture extracted gDNA and "neutralized" magnetic particles. In tube 1, a mock CCCMP magnetocapture experiment using 2.5 mg CCCMP and 25 μL 0.05 M MES pH 5.2 was conducted but in the absence of any genomic DNA. At the elution step, the magnetic particles were incubated (10 min) and then resuspended in the 25 μL elution buffer (10 mM Tris-HCl pH 8.5) itself. The follow-up 20 μL colorimetric LAMP reaction then consisted of 10 μL 2X proprietary LAMP mastermix, 3 μL resuspended "neutralized" magnetic particles from above, and 2 μL 10X *E. coli malB* primer mix (without loop primers), and 4 μL water. For tube 2, $10^6$ copies of *E. coli* genomic DNA in 25 μL 0.05 M MES pH 5.2 buffer were subjected to 2.5 mg CCCMP magnetocapture. After elution, the particles were resuspended in 25 μL water and 8 μL particles were subjected to a 20 μL colorimetric LAMP as described above. In tube 3, 8 μL elution from the magnetocapture experiment described for tube 2 was subjected to a 20 μL colorimetric LAMP as discussed above. In tube 4, an identical magnetocapture experiment as described for tube 1 was performed but was not subjected to elution. Right after washing with 0.05 M MES pH 5.2, the magnetic particles were resuspended in 25 μL water. 8 μL particles were subjected to a 20 μL colorimetric LAMP as described above. In tube 5, 8 μL elution buffer (10 mM Tris-HCl pH 8.5, without any magnetocapture) was subjected to a 20 μL colorimetric LAMP as described above.

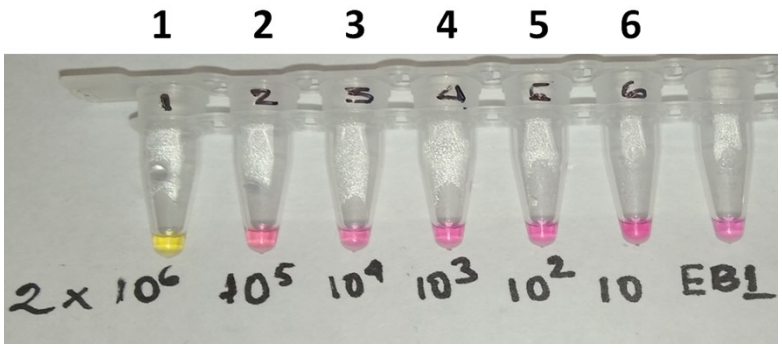


**Fig. S12.** Colorimetric LAMP assay using WarmStart LAMP Kit (NEB # E1700S) on magnetocapture extracted gDNA. DNA copies ranging from 2 x $10^1$ – $10^6$ copies in 25 µL 0.05 M MES buffer (pH 5.2) were subjected to magnetocapture using 2.5 mg CCCMP, and then eluted using 25 µL elution buffer having the composition of 25 mM Tris-HCl (pH 8.5)). The follow-up 20 µL colorimetric LAMP reaction in tubes 1 – 6 then consisted of 10 µL 2X proprietary LAMP colorimetric mastermix, 8 µL elution, and 2 µL 10X *E. coli malB* primer mix (without loop primers). EB1 (tube 7) sample consisted of a CCCMP-mediated magnetocapture experiment without any genomic DNA (a "mock" experiment) that was eluted using 25 mM Tris-HCl pH 8.5 elution buffer, followed by colorimetric LAMP having the same reaction composition as above.

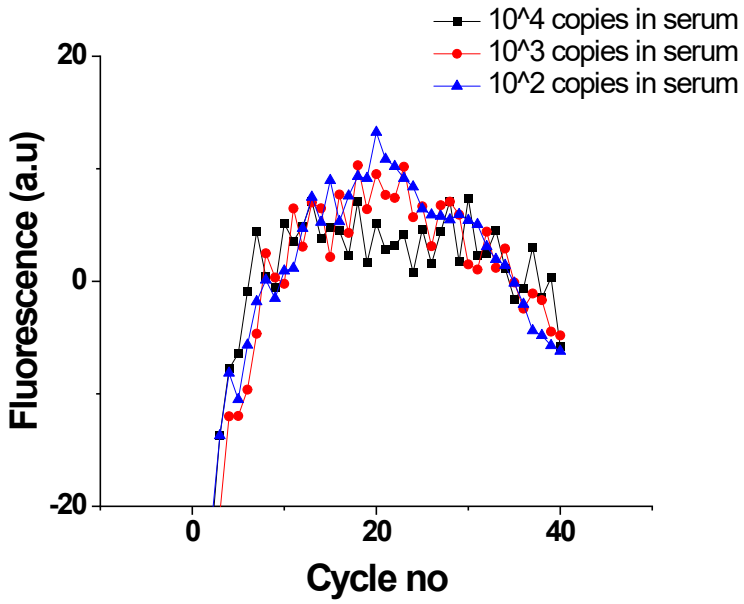


**Fig. S13.** Real-time PCR amplification of human genomic DNA in the presence of 50% fetal bovine serum.

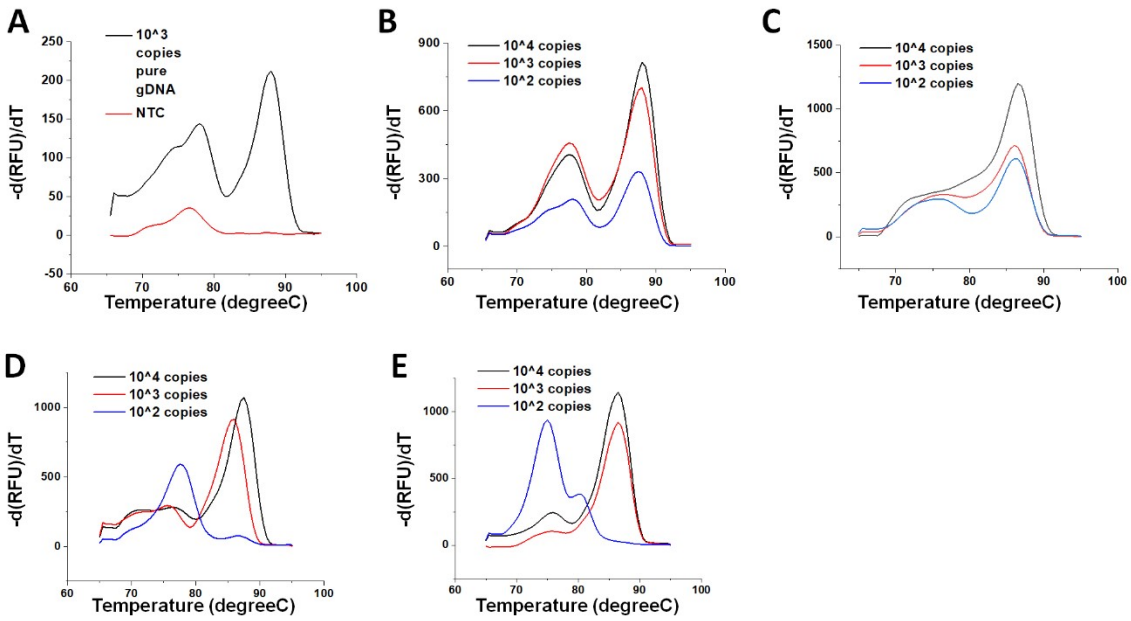


**Fig. S14.** Real-time PCR melt curve analysis for magnetocapture experiments on $10^2$ – $10^4$ copies of human genomic DNA in aqueous solution and serum. A, melt curve analysis for pure genomic DNA ($10^3$ copies) and no template control (NTC). B, melt curve analysis for CCCMP magnetocapture followed by real-time PCR on $10^2$ – $10^4$ copies of genomic DNA from the aqueous sample. C, CCCMP magnetocapture followed by real-time PCR on $10^2$ – $10^4$ copies of genomic DNA from serum. D, ECCMP magnetocapture followed by real-time PCR on $10^2$ – $10^4$ copies of genomic DNA from the aqueous sample. E, ECCMP magnetocapture followed by real-time PCR on $10^2$ – $10^4$ copies of genomic DNA from serum.

## References


1 B. G. Maciel, R. J. da Silva, A. E. Chávez-Guajardo, J. C. Medina-Llamas, J. J. Alcaraz-Espinoza and C. P. de Melo, *Carbohydr. Polym.*, 2018, **197**, 100–108.

2 P. T. Trieu and N. Y. Lee, *Anal. Chem.*, 2019, **91**, 11013–11022.

3 J. Hui, Y. Gu, Y. Zhu, Y. Chen, S.-J. Guo, S.-C. Tao, Y. Zhang and P. Liu, *Lab. Chip*, 2018, **18**, 2854–2864.

4 X. Qian, Q. Qu, L. Li, X. Ran, L. Zuo, R. Huang and Q. Wang, *Sensors*, , DOI:10.3390/s18061878.

5 V. Kamat, S. Pandey, K. Paknikar and D. Bodas, *Biosens. Bioelectron.*, 2018, **99**, 62–69.

6 I. A. Nanayakkara, W. Cao and I. M. White, *Anal. Chem.*, 2017, **89**, 3773–3779.

7 S. Chumwangwapee, A. Chingsungnoen and S. Siri, *Forensic Sci. Int. Genet.*, 2016, **25**, 19–25.

8 K. R. Pandit, I. A. Nanayakkara, W. Cao, S. R. Raghavan and I. M. White, *Anal. Chem.*, 2015, **87**, 11022–11029.

9 A. P. Tiwari, R. K. Satvekar, S. S. Rohiwal, V. A. Karande, A. V. Raut, P. G. Patil, P. B. Shete, S. J. Ghosh and S. H. Pawar, *RSC Adv.*, 2015, **5**, 8463–8470.

10 S. N. Tammam, M. A. F. Khalil, E. Abdul Gawad, A. Althani, H. Zaghloul and H. M. E. Azzazy, *Carbohydr. Polym.*, 2017, **164**, 57–63.

11 C. Jiang, S. Xu, S. Zhang and L. Jia, *Anal. Biochem.*, 2012, **420**, 20–25.

12 W. Cao, C. J. Easley, J. P. Ferrance and J. P. Landers, *Anal. Chem.*, 2006, **78**, 7222–7228.
13 K. A. Hagan, W. L. Meier, J. P. Ferrance and J. P. Landers, *Anal. Chem.*, 2009, **81**, 5249–5256.
14 T. S. Schlappi, S. E. McCalla, N. G. Schoepp and R. F. Ismagilov, *Anal. Chem.*, 2016, **88**, 7647–7653.
15 J. Hill, S. Beriwal, I. Chandra, V. K. Paul, A. Kapil, T. Singh, R. M. Wadowsky, V. Singh, A. Goyal, T. Jahnukainen, J. R. Johnson, P. I. Tarr and A. Vats, *J. Clin. Microbiol.*, 2008, **46**, 2800–2804.
16 F. Taghavifar, M. Hamid and G. Shariati, *Mol. Genet. Genomic Med.*, 2019, **7**, e00740.