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

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ABSTRACT: Nucleic acid extraction and concentration from complex biofluids is the prerequisite for nucleic acid amplification test (NAAT) applications in pathogen detection, biowarfare prevention, and genetic diseases. However, conventional spin-column mediated nucleic acid extraction is constricted by requirement for costly power-intensive centralized lab infrastructure, making them unsuitable for resource limited settings. Tremendous progresses on lab-on-a-chip devices or cartridges (e.g., Cepheid GeneXpert®) that integrate nucleic acid extraction and amplification has been made, but these approaches either require additional equipments or are costly. Similarly, their complexities made them difficult to fabricate in low resource settings by the end-user themselves. Conventional magnetic bead chemistry (e.g., silica beads) for nucleic acid extraction is relatively instrument-free, rapid, and amenable to automation, but relies on hazardous chaotrope chemistry and ethanol desalting that may limit their efficiency for downstream NAATs. Recent advances in several novel material coated magnetic bead-based extraction methods offer a possible solution to this problem, but even these novel materials also involve multistep synthesis impermissible at limited resource settings. To offer a possible magnetic particle based nucleic acid extraction doable at resource-limited settings, we investigated the nucleic acid capture ability of two chitosan coated magnetic particle synthesis that are preparable by minimally trained personnel using only water bath and magnetic stirrer within 6 – 8 h. We quantitatively probed the efficiency of the passive (without any electrical shaking or vortex-aided) magnetocapture using UV_{260}. To explore their suitability towards clinically relevant sensitive downstream NAATs, low copies (100 – 1000) of *E. coli* or human genomic DNA from aqueous solution, crude cell lysate, and fetal bovine serum were extracted by them and then successfully detected using quantitative real time loop mediated isothermal amplification (LAMP) or real time polymerase chain reaction (PCR). Alongside, their suitability with gel-based LAMP, colorimetric LAMP, and in situ (on beads) LAMP was also probed. With ease of preparation, reproducibility, and compatibility with downstream NAATs, we anticipate that these magnetic particles would be useful in expansion and decentralization of nucleic acid-based diagnosis for limited resource settings.

Keywords: Nucleic Acid Extraction; Limited Resource; Magnetic Particle Extraction; Real Time PCR; Real Time LAMP.

INTRODUCTION

According to the World Health Organization[1], the frequency of occurrence of infectious diseases are predicted to increase significantly in near future from the destruction of natural wildlife habitats[2], globalization[3], unplanned urbanization[4], and climate change[5]. In the last 20 years, this has already seen manifestation in the occurrence of several lethal viruses of particularly zoonotic origin such as severe acute respiratory syndrome (SARS), H1N1 influenza, middle east respiratory syndrome (MERS), Ebola, NIPAH, and the ongoing SARS-CoV-2[2]. These have led to several outbreaks, epidemics, and pandemics with significant loss of human life and disruption of economy. These diseases have so far appeared and are predicted to appear in short notices without giving significant lead timing[1], catching the health agencies around the world mostly underprepared to prevent such outbreaks. As it was seen in the early phase of SARS-CoV-2 pandemic, it would risk overwhelming the detection and therapeutic capability of a region[6]. In addition, such outbreaks are more likely to affect populations at remote and resource constrained settings due to their limited access to centralized labs, necessity of sample transport to faraway detection centers, and resulting loss of time for treatment initiation[7].

Nucleic acid amplification tests (NAATs) are the backbone analytical tools in pathogen identification, environmental monitoring, food contamination, genetic disease detection, and biowarfare prevention [8]. In the event of a sudden disease outbreak, NAATs are always the first type of diagnostic intervention. However, the traditional real time PCR, the mostly commonly used NAAT has remained predominantly confined to central labs due to its dependence on thermal cycler. Isothermal NAATs, (iNAATs) on the other hand, have helped decentralize nucleic acid detection based molecular diagnosis due to their independence of thermal cycler[9]. These has become further achievable thanks to invention of a diversity of readouts in iNAATs such as colorimetry, electrochemistry, turbidometry etc in addition to traditional real time fluorescence[9]. However, despite the automation of nucleic acid amplification and readout steps in NAATs or iNAATs, a critical step is the extraction of polymerase inhibitor-free nucleic acid from complex samples. The prevalent method for nucleic acid extraction is solid phase spin-columns suited for centralized high-speed centrifuges[10]. Commonly based on silica or cellulose, these spin columns capture the nucleic acid from complex biofluid using chaotropic salt[11,12]. It then removes the excess salt and remnants of complex biofluid components utilizing successive alcohol washes. Finally, low to moderate salt conditions are used to desorb the nucleic acid from solid phase support in the columns for downstream application. However, use of hazardous chaotropic salt and ethanol, both of which are known polymerase inhibitors, necessitates multiple wash steps, which in turn increases extraction time. Due to their restrictive ability to handle 0.5 – 1 mL liquid, the spin columns are also unsuitable for high dilution biofluid samples such as urine. In addition, silica column-based extraction is synonymous with use of high-speed centrifuges, which limits their application to centralized labs. In the event of a sudden outbreak causing the supply chain getting overwhelmed, these columns are difficult to fabricate by the end user themselves. Therefore, despite their wide and almost universal adoption, silica or cellulose based spin columns are unsuitable for NAAT based molecular diagnosis at limited resource settings.

To address these shortcomings, several research groups have come up with innovative non-silica membrane-based solutions that actuates the nucleic acid extraction without compromising its compatibility with downstream NAATs. These included several paper-based

microfluidic interventions from Whitesides group[13], origami based microdevice for simultaneous viable cell detection and isothermal NAAT[14], a nylon-cross-linked chitosan device capable of capturing tens of copies nucleic from very large sample volumes[15]. Despite the ingenuity of these devices, they would often require additional equipment to control the liquid flow for generating an analytically sensitive nucleic acid extraction. An alternative to these microdevices are magnetic bead-based nucleic acid extraction systems, which relies on the binding of nucleic acid to a coated magnetic bead for physically separating nucleic acids from rest of the solution. After removal of undesirable components such as polymerase inhibitors and cell debris, amplification-ready nucleic acid is released from the beads. While silica-based magnetic beads still suffer from the same shortcomings as their spin column counterparts, recent advancements have been made in alternative materials such as polyamines or chitosan-based magnetic particles[16,17]. These magnetic particles are able to capture nucleic acid without use of chaotropic salt, alcohol, or extreme pH, while the captured nucleic acid remains suitable for downstream NAATs. Some of the recent chitosan magnetic particle-based extraction systems have also documented in situ (on-bead/on-support) amplification, significantly reducing steps and time of the ultrasensitive nucleic acid detection[17]. However, most of these magnetic particle-based nucleic acid capture systems are yet to be commercialized. Their preparations involve multiple processing steps, sophisticated instruments[17], prior hydrolysis of the chitosan ingredient into chitosan oligosachharide[18], or long (12 – 24 h) preparation time[19,20]. Despite their proven utility in ultrasensitive nucleic acid capture/detection, they may not reach an end-user at limited resource settings in the event of a sudden outbreak. Especially in cases where such extraction devices may require deployment in short notice, they could be challenging to fabricate by the end-user themselves due to lack of sophisticated instruments or necessary time to improvise.

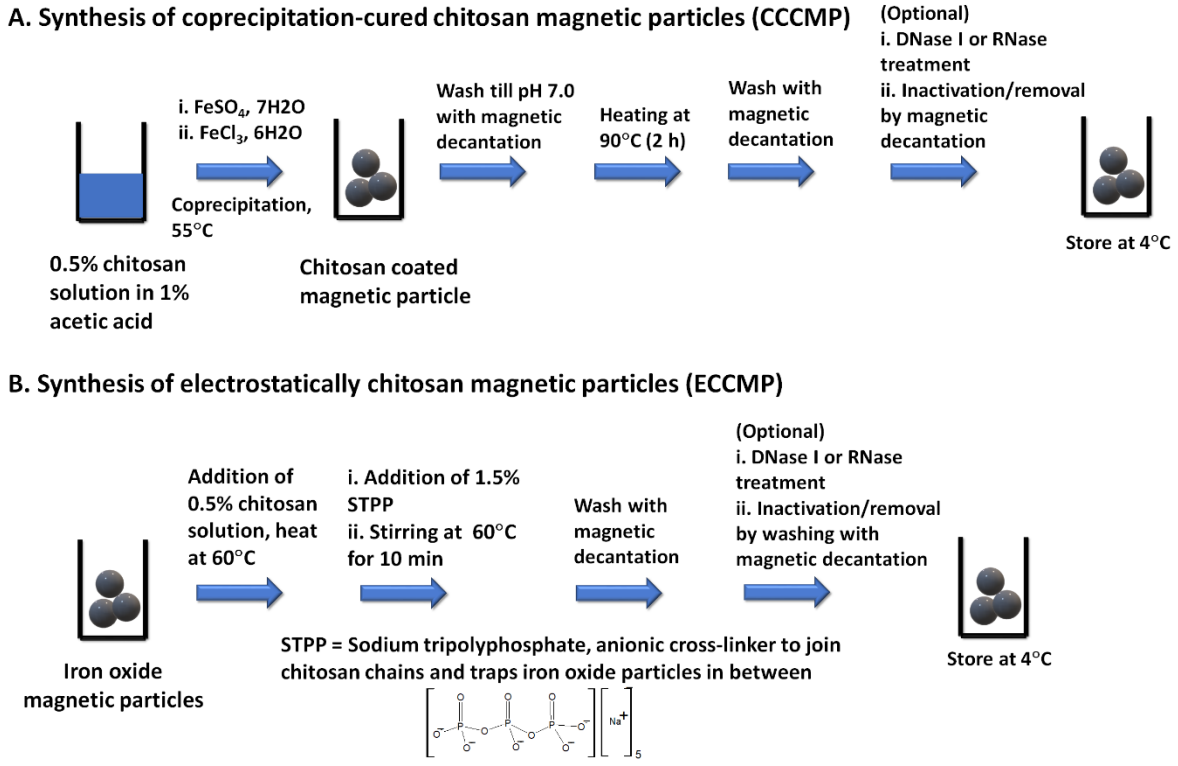
Manual handling of nucleic acid extraction or related reagent preparation is generally undesirable and inefficient. However, ease of preparation of critical molecular diagnosis components such as the nucleic acid extraction-capable magnetic particles, even if manual, would be considered critical and lifesaving in times of exigency. The primary motivation behind this work was thus to explore methods that can synthesize chitosan-coated magnetic particles in resource-limited settings within a few hours preferably by minimally trained personnel. The preparation time of such magnetic particles should be less than 8 h, so that it can be repeatedly prepared in hundreds of milligram scale within a short notice during an urgent situation (e.g., a pandemic). Despite the rapid synthesis, these particles should not compromise on the quality of the extracted nucleic acid for downstream procedures. Therefore, they should not only be able to extract nucleic acid from complex biofluids using nothing but magnetic separation, but the extracted nucleic acid should also be NAAT/iNAAT compatible. In this work, we investigated two such methods with synthesis and characterization, and then quantified their ability for electricity-free passive nucleic acid magnetocapture (i.e., benchtop incubation without instrument-enabled mixing). The compatibility of isolated DNA with downstream NAATs was investigated through magnetic extraction of low copy of extracted nucleic acid from aqueous solution, crude cell lysate, and complex biofluid (serum) coupled with follow-up gel-based loop mediated isothermal amplification (LAMP), in-situ (on-bead or on-particle) LAMP, real time LAMP with homemade master mix, commercial colorimetric LAMP mix, and real time PCR.

MATERIALS AND METHODS

For materials and methods, please see electronic supporting information.

RESULTS AND DISCUSSION

Preparation of chitosan magnetic particle. The primary motivation behind this work was to utilize magnetic particles that can extract amplification ready nucleic acid in resource-limited settings. To ensure that such magnetic particles become employable in a sudden health emergency (e.g., an outbreak), we opted for synthesis protocols that enables preparation of chitosan coated magnetic particles in 0.1 g scale with bare minimum equipments, within a short time (6 – 8 h), and by minimally trained personnel. Exploring the literature for available methods that would fulfil these criteria, we shortlisted and synthesized two types of magnetic nanoparticles, namely coprecipitation-cured chitosan magnetic nanoparticles (CCCMP) and electrostatically cross-linked chitosan magnetic particles (ECCMP). Interestingly, we could not find any report utilizing these magnetic particles for amplification-ready nucleic acid extraction from crude lysate or complex biofluid. For the synthesis of CCCMP, chitosan dissolved in acetic acid solution was subjected to alkaline coprecipitation with Fe (II)/Fe (III) salts followed by curing (90°C). For ECCMP preparation, gelation method involving ionic cross-linker sodium tripolyphosphate was utilized to trap pre-synthesized iron oxide nanoparticles within chitosan polymer. With molecular diagnosis in mind, the synthesized chitosan magnetic particles could be subjected to an optional DNase I or RNase treatment followed by heat inactivation and washing using magnetic decantation. Both synthesis protocols could produce



Scheme 1. Synthesis of chitosan coated magnetic particles.

chitosan coated magnetic particle using nothing but magnetic stirrer and a water bath (or a temperature controllable magnetic stirrer) and were completed within 5 – 6 h (Scheme 1, Figure S1).

Characterization of the magnetic particles. The characterization of CCCMP and ECCMP as well as that of coprecipitation synthesized bare iron oxide magnetic particles was carried out using scanning electron microscopy (SEM), EDX, FT-IR, and XRD. The direct evidence of

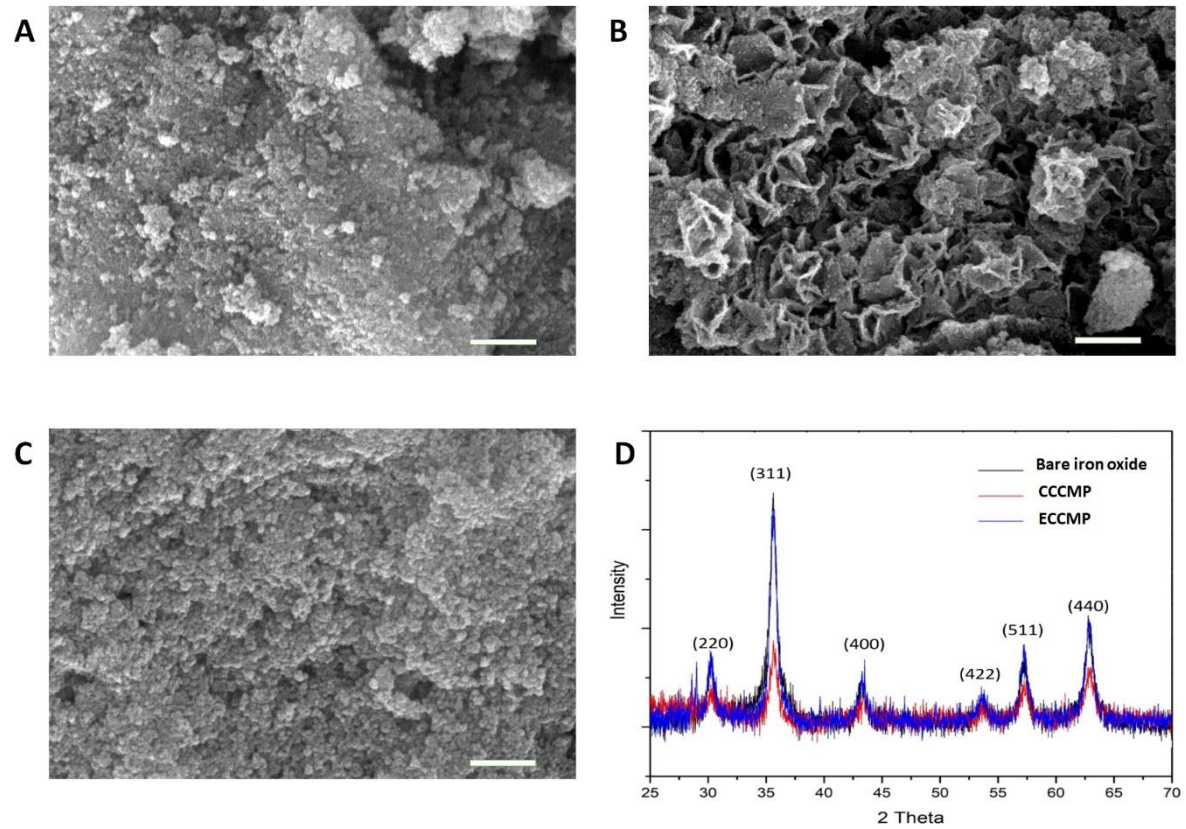
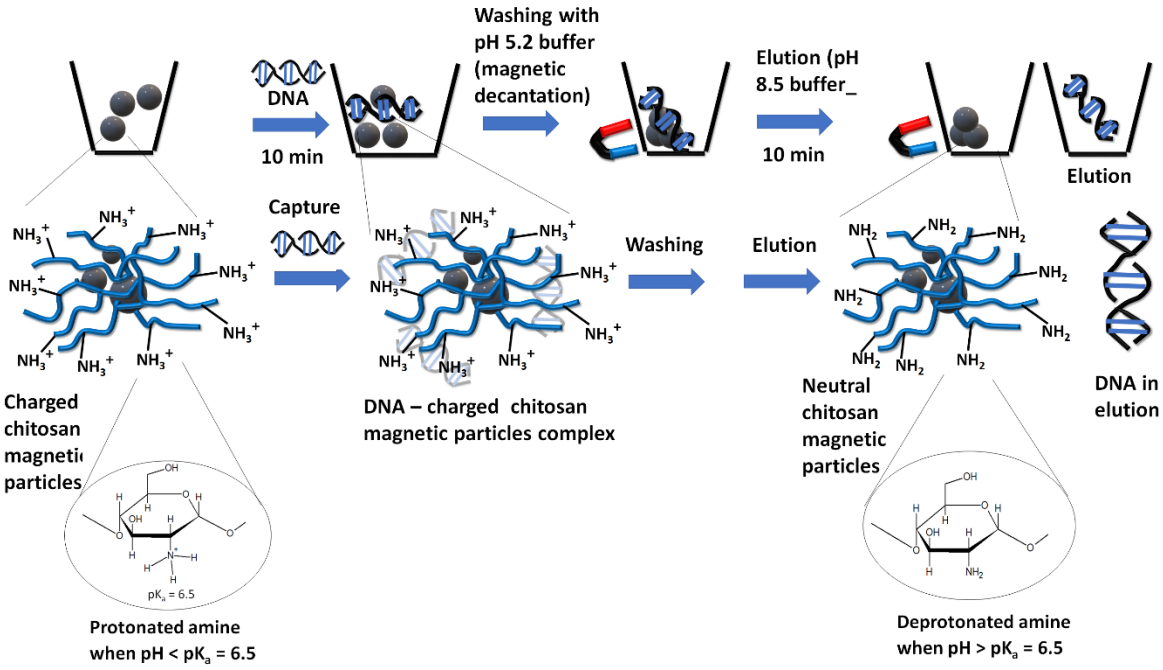


Figure 1. SEM and XRD characterization of chitosan-iron oxide materials. A, SEM of bare iron oxide. B, SEM of CCCMP. C, SEM of ECCMP. D, XRD of bare iron oxide, CCCMP, and ECCMP. The scale bar in the SEM images is 500 nm.

the chitosan coating came from EDX spectrum analysis which revealed the presence of carbon and nitrogen in CCCMP and ECCMP but not in bare iron oxide (Supporting Information Figure S2). Intense peaks at 538 cm^{-1} (in bare iron oxide) and 545 cm^{-1} bands (in CCCMP and ECCMP) characteristic of the Fe-O bending frequency were found in FT-IR investigations, confirming the presence of iron oxide in all three substrates. Broad peaks due to possible moisture absorbance were detected at 3260, 3267 and 3175 cm^{-1} in bare iron oxide, CCCMP, and ECCMP, respectively. In addition, peaks from C-O stretching in primary alcohols (1028 cm^{-1} in CCCMP and 1066 cm^{-1} in ECCMP), N-H bending in chitosan amine (1585 cm^{-1} in CCCMP and 1531 cm^{-1} in ECCMP), and CH_3 symmetrical deformation (1370 cm^{-1} in CCCMP and 1378 cm^{-1} in ECCMP) substantiated the chitosan coating on the iron oxide. The SEM images of the MPs showed agglomerated particles with some unusual filament-like structure present in some regions of CCCMP (Figure 1A – C). Magnification indicated presence of spherical shaped particles consistent with coprecipitation synthesized iron oxide nanoparticles. The crystalline structure of Fe_3O_4 nanoparticles were characterized by XRD as shown in Figure 1D. All three Fe_3O_4 nanoparticles showed diffraction peaks at 30.32º (220), 35.60º (311), 43.24º (400), 53.62º (422), 57.34º (511) and 62.83º (440) are associated with Fe_3O_4. The observed peaks of Fe_3O_4 were consistent with the database in ICDD (International Centre for Diffraction Data) PDF Card - 01-071-6337 and also revealed that the pure Fe_3O_4 phase is a spinal structure. The average diameter of the Fe_3O_4 nanoparticles calculated by using Debye-

Scherrer's equation to the (311) peak at 2θ=35.60° is about 15.5 nm, respectively. XRD result shows the chitosan coating does not alter the crystallinity of the Fe_3O_4 core (Figure 1D). Overall, the characterization of magnetic particles indicated successful coating of chitosan on iron oxide magnetic particles.



Scheme 2. 30 min DNA magnetocapture procedure to extract DNA from solution, wash (to remove non-nucleic acid molecules), and then release ("elute") the same into solution. The procedure uses protonation-deprotonation based charge switching of chitosan backbone amino groups.

DNA binding study. Next, we assessed the CCCMP and ECCMP for their ability to capture nucleic acid from solution. Due to the presence of the amino group with a pK_a ~6.4 in aqueous solutions, chitosan owes its nucleic acid capture capability on solution pH variation. Quantitatively, it relies on the Henderson-Hasselbach equation ($pH = pK_a + \log_{10}([A^-]/[HA])$ that correlates solution pH with the ratio of deprotonated (NH_2) to protonated (NH_3^+) form. As a result, a chitosan-based material would acquire a positive charge below pH 6 – 6.5 and a predominantly charge-neutral state above pH 7 – 7.5. It would therefore extract negatively charged nucleic acid at pH 6 – 6.5 from solution and release the same as elution at solution pH 8.5. Prior reports on chitosan based microdevices and magnetic devices as well as gene delivery studies have utilized this charge switching property for nucleic acid capture and transport[21]. The 25 – 30 min magnetocapture assay described in Scheme 2 was therefore comprised of a nucleic acid adsorption (hereafter referred as "capture") by the positively charged chitosan backbone under the influence of a pH 5.2 buffer (10 min), washing with the same buffer (5 – 10 min), and desorption of nucleic acid from chitosan-magnetic particles (hereafter referred as "elution") using a pH 8.5 buffer (10 min). Utilizing this assay, 1 – 5 mg wet CCCMP or ECCMP demonstrated a linear genomic DNA capture ability from aqueous solution with mean capture efficiency of 277.0 ± 21.2 and 233.3 ± 30.5 ng/mg, respectively (Figure 2). In contrast, the DNA capture ability of bare iron oxide magnetic particles was 11.2 ± 7.2 ng/mg. This was calculated solely based on DNA capture experiment by 5 mg bare iron oxide as the absorbance for 1 and 2.5 mg was below the limit of detection of nanodrop (data not shown). This

experiments thus justified the importance of chitosan coating on iron oxide for nucleic acid capture. It also quantified the DNA binding capacity of both the CCCMP and ECCMP materials while validating the signature nucleic acid binding capability of chitosan-based materials.

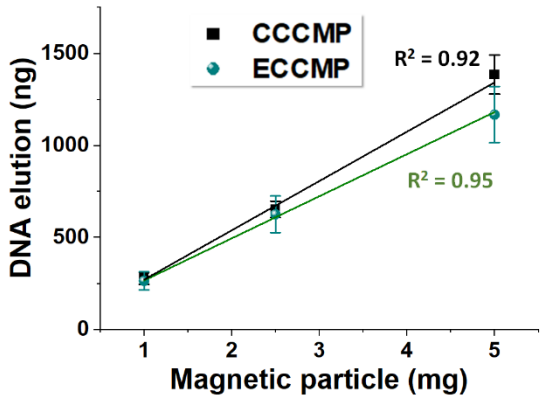


Figure 2. DNA capture and elution capability of 1, 2.5, and 5.0 mg of CCCMP and ECCMP. Error bars represent standard deviation. The lines are linear fit for the data points. Error bars represent standard deviations (n=3).

Detection of magnetocaptured DNA through isothermal amplification. Direct detection instruments (those using UV_{260}) or direct fluorescence (e.g., in qubit instrument)) can neither detect clinically relevant ultralow quantity (hundreds to thousands of copies) of target nucleic acid nor identify specific sequence elements in it. NAATs or iNAATs, in contrast, would specifically identify presence or absence of clinically relevant ultralow amount of biomarker nucleic acid sequence by utilizing primers while also acting as a billion-fold (or higher) signal amplifier element. However, their performance as specific and sensitive bioanalytical tools are contingent on the purity of extracted nucleic acid and may get attenuated because of presence of impurities such as PCR inhibitors[22]. An effective nucleic acid extraction assay is thus expected to rescue amplification-ready nucleic acid from complex biofluids through physical separation of polymerase inhibitors. The second key goal of this study was therefore to assess the compatibility of the CCCMP and ECCMP extracted nucleic acid (including those isolated from complex biofluids) for downstream NAAT/iNAAT applications. To test this qualitatively, we performed magnetocapture followed by loop mediated isothermal amplification (LAMP) of *E. coli* genomic DNA present in aqueous solution and crude lysate. The reason for selecting LAMP over other isothermal amplifications was its reported compatibility with a number of readout methods which includes but are not limited to turbidimetry, electrochemistry, colorimetry, surface plasmon resonance, lateral flow assay, and real time fluorescence[23]. To mimic conditions similar to those found in limited resource settings, we opted to utilize our own homemade LAMP master mix over the commercially available ones as the latter is not as commonly available as their real time PCR counterparts. While utilizing an earlier reported set of LAMP primers (*E. coli malB* gene[24]), non-specific LAMP amplification was encountered during agarose gel electrophoresis. Cross-checking our findings with that of an earlier publication by Whitesides group that used the same primer set[25], we inferred that the non-specific amplification could be due to absence of betaine in our homemade amplification mix causing primer-dimer formation (see Table S1 for primer sequence and Figure S4A). The non-specific amplification was mitigated after the removal of loop primers from the 10X primer mix and characteristic ladder-like patterns in gel electrophoresis were noted only in LAMP

experiments in presence of genomic DNA (Figure S4B). Without any further optimization in magnetocapture or LAMP protocol, 10^9 copies of genomic DNA in aqueous solution was subjected to magnetocapture followed by LAMP (Figure S5A). Similarly, 10^9 *E. coli* cells were heat lysed and then subjected to magnetocapture followed by LAMP. Elutions from CCCMP and ECCMP magnetocapture on both aqueous genomic DNA solution and crude lysate demonstrated successful LAMP amplifications in agarose gel electrophoresis (Figure S5B and S5D). We then investigated whether LAMP could also be performed on the DNA-bound magnetic particles prior to the elution step as it would reduce the assay timing by at least 10 min. To investigate this, we subjected the DNA-bound magnetic particles (following magnetic decantation wash but before pH 8.5 elution) themselves to LAMP. In agarose gel, ladder-like patterns characteristic of LAMP reactions were visualized for these experiments (Figure S5C and S5E). Overall, these assays qualitatively established the compatibility of magnetocapture elution from both CCCMP and ECCMP with LAMP experiments, showing that DNA from aqueous solution as well as crude lysate can be extracted. An interesting observation was that LAMP can even be performed "in-situ" on the DNA-bound magnetic particles themselves without elution. This could probably be attributed to the alkaline pH of the LAMP buffer (pH 8.8) causing some degree of elution, an in situ "on-bead/particle" amplification, or the combination of both. Although highly promising in terms of reducing assay time, this aspect was not pursued in this work any further and would be investigated separately in a follow up study.

Limit of Detection for magnetocapture coupled with real time LAMP. While gel electrophoresis mediated visualization of LAMP can qualitatively confirm an amplification experiment, this method of readout is neither sensitive enough to detect low copy target nucleic acid nor a quantitative technique, both of which are requirements in molecular diagnosis assays. We therefore opted for real time LAMP using our homemade mastermix to establish the limit of detection (LoD or analytical sensitivity) of magnetocapture followed by LAMP assays. While experimenting to establish proof-of-concept assays using 10^6 copies of target *E. coli* genomic DNA, we noted that our homemade real time LAMP mastermix was successfully able to distinguish target from no template control (NTC) experiments (Figure S6A). However, the NTC itself was producing large fluorescence despite the LAMP reactions at this point being devoid of loop primers (Figure S6A). We attributed the large fluorescence in NTC LAMP to possible minor primer-dimer formation that was otherwise undetectable in agarose gel electrophoresis. We deduced that a higher temperature LAMP initiation would further mitigate non-specific interactions like primer-dimer formation. Therefore, we modified the standard real time LAMP protocol (66°C 1 min for 60 cycles with fluorescence monitoring at each cycle) to a touchdown-like LAMP protocol (Figure S6B). Although it reduced the fluorescence in both positive control as well as NTC, the latter was attenuated over 6-fold. Next, we subjected 10^1 – 10^5 copies of *E. coli* DNA (as pure genomic DNA or in the form of crude lysate) to magnetocapture, while the elution was analysed with follow up real time LAMP (Figure 3A). For CCCMP magnetocapture with either genomic DNA in aqueous solution or as crude lysate, the LoD was 10^2 copies (established using characteristic "S" shaped amplification profile at Figure 3B and 3C with corresponding melt curve analysis in Figure S7A and S7B). In comparison, the LoD for ECCMP magnetocapture followed by real time LAMP turned out as 10^3 copies for aqueous genomic DNA and 10^2 copies for crude lysate (amplification profile at Figure 3D and 3E with melt curve analysis in Figure S7C and S7C). Encouragingly, the LAMP assays could reproducibly distinguish "yes/no" samples within 45 min, making the total

turnaround time for magnetocapture and amplification 1.5 – 2 h. The instrument-free nature of the magnetocapture extraction combined with overall low turnaround time is anticipated to accelerate the "yes/no" clinical decision making in limited resource settings. One limitation of our LAMP assays were that the cycle threshold (C_t) varied considerably to obtain a mean C_t with low standard deviation, preventing any possible pathogen load estimation. This could be due to our use of homemade LAMP mastermix, use of touchdown-like LAMP, minor but detectable primer-dimer formation mediated amplification (as seen in melt-curve analysis), or a combination of all these factors. It is also possible that use of a commercial LAMP mastermix may lead to generation of reproducible Ct suitable for pathogen load determination. Altogether, these assays established that the DNA extraction using both magnetic particles from either aqueous solution or crude lysate was compatible with real time LAMP with clinically relevant analytical sensitivity.

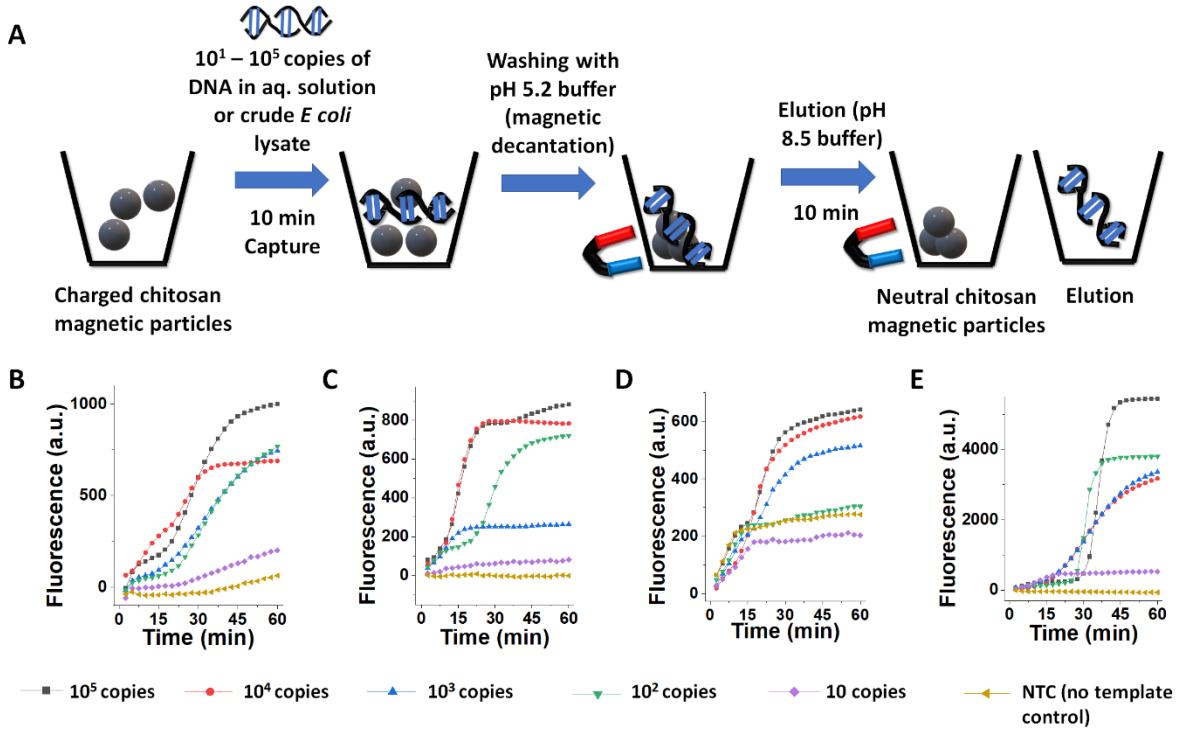


Figure 3. Limit of detection for magnetocapture, elution, and real time LAMP for detecting nucleic acid (DNA) from aqueous solution or crude cell lysate. A, Scheme of magnetocapture assay. B, elution from CCCMP magnetocapture assay on 10^1 – 10^5 copies of *E. coli* gDNA in aqueous solution subjected to real time LAMP. C, elution from CCCMP magnetocapture assay on 10^1 – 10^5 *E. coli* cells heat lysate subjected to real time LAMP. D, elution from ECCMP magnetocapture assay on 10^1 – 10^5 copies of *E. coli* gDNA in aqueous solution subjected to real time LAMP. E, elution from ECCMP magnetocapture assay on 10^1 – 10^5 *E. coli* cells heat lysate subjected to real time LAMP.

Investigation on magnetocapture followed by pH based colorimetric LAMP readout. Next, we investigated whether the colorimetric LAMP protocol can be integrated with magnetocapture assays. The polymerase activity during LAMP reaction would cause proton generation, leading to a pH drop, and would trigger the color change in a pH indicator dye present in the solution[26]. The proprietary WarmStart Colorimetric LAMP 2X Mastermix uses this principle to provide a fast and clear visual (pink to yellow) readout of positive LAMP reactions. The pH change (with resulting color change) also relies on low concentration (25 – 400 μM) of Tris providing very weak buffering ability. If the colorimetric LAMP could be

integrated with magnetocapture assays, we anticipated that together they would provide a highly utilitarian and rapid NAAT molecular diagnosis platform suitable for limited resource settings. We therefore investigated the CCCMP magnetocapture of 2 x 10^1 – 10^6 copies of *E. coli* genomic DNA from aqueous solution followed by colorimetric LAMP on the elution. Within 10 min of starting the LAMP reaction, we noticed perceivable color change to various degrees (Figure S8 tubes 1 – 6). The elution from the negative control "mock" magnetocapture (on buffer alone but no DNA) had a clear visual difference from those containing DNA (Figure S8 tube 7). Similarly, addition of elution buffer alone (without magnetocapture) retained the pink color of the commercial mastermix (tube 8). Although this apparently suggested a successful assay with LoD in the order of 10 copies, we experimented further with the conditions. Interestingly, when we added the neutralized magnetic particles from the "mock" magnetocapture to the colorimetric amplification mix, it also changed color from pink to yellow within 10 min of starting the amplification (Figure S9 tube 1). This suggested that the color change could be due to release of remnant protons attached to chitosan amines and might not be from the LAMP reaction. To test further, we carried out CCCMP magnetocapture on 10^6 copies of genomic DNA, eluted the same, resuspended the CCCMP particles to water (pH 7) post-elution, and subjected the particles as well as elution to colorimetric LAMP. Both the reactions become yellow within 10 min (tubes 2 for particles and 3 for elution, Figure S9). It again hinted that that protons bound that even so-called "neutralized" particles could be responsible for the color change. Similarly, "in situ" ("on-bead") LAMP on magnetic particles from another "mock" (no genomic DNA) CCCMP magnetocapture but without incubation with "neutralizing" elution buffer also demonstrated color change (Tube 4, Figure S9). The last experiment confirmed that the protons bound to chitosan itself could cause pH reduction, resulting in a color change in the indicator dye present in colorimetric master mix. Furthermore, the studies indicated that incubation with 10 mM Tris-HCl pH 8.5 would probably be incompletely neutralizing chitosan. It was causing color change (i.e., a false positive readout) in colorimetric LAMP even in the absence of eluted DNA (as in tube 1, Figure S7). A false positive readout might also originate from inadvertent transfer of a trace amount of chitosan particles into the LAMP reaction tube as the partially neutralized particles would release proton during LAMP. Overall, these reactions indicated that 10 mM Tris-HCl pH 8.5 as the magnetocapture elution buffer may not be suitable with colorimetric pH change-based LAMP reactions. We tried changing the concentration of neutralizing elution buffer to 25 mM Tris-HCl pH 8.5 without changing the incubation time of elution. This elution buffer, however, was probably too concentrated (therefore having higher buffering capacity) for its pH to be altered by LAMP proton release (tube 1, Figure S10). The experiments hinted at possible novel correlations about interactions between chitosan and the neutralizing "elution" buffer as well as a possible role of neutralization kinetics. It suggested the need for further optimization in terms of elution buffer composition as well as neutralization time for the magnetocapture for integration with colorimetric LAMP. We abstained from experimenting with colorimetric LAMP any further in this work and would pursue this in future studies.

Limit of Detection for magnetocapture followed by real time PCR. Real time PCR assays are still the mainstay of clinical NAAT molecular diagnosis and are used in several automated instruments such as GeneXPert®[27]. However, the target nucleic acid in clinical samples is often present in complex biofluids like urine, cough, swab, serum containing various PCR inhibitors which inhibit downstream real time PCR application. Traditional spin-column based procedures to extract nucleic acid from these biofluids relies on the centralized high-speed

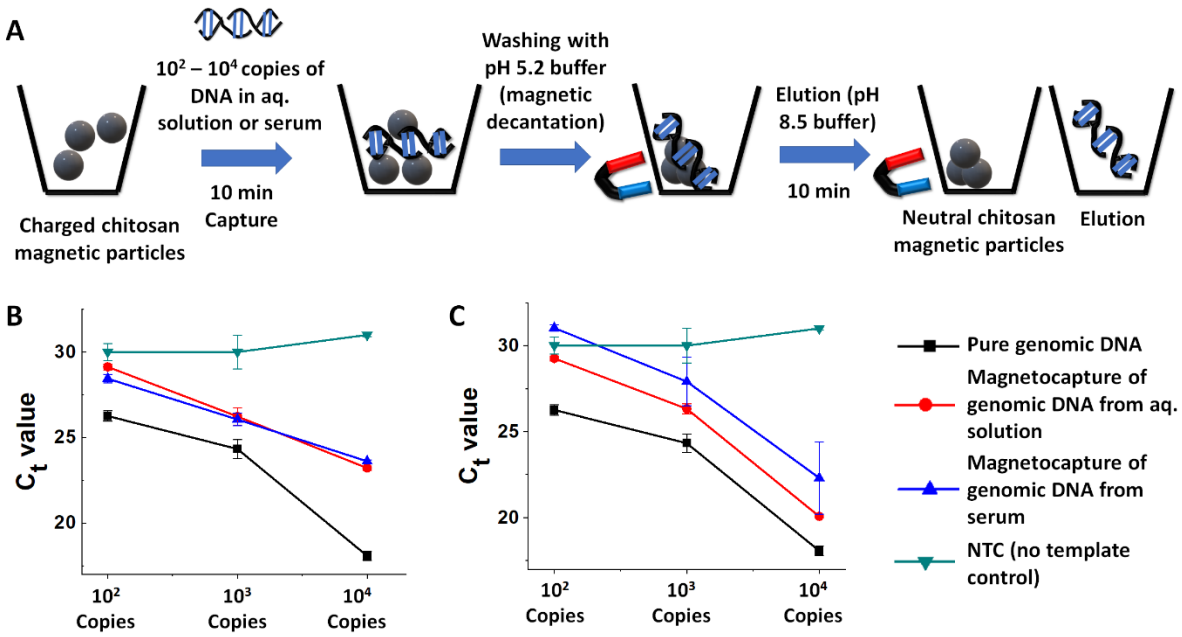


Figure 4. Limit of detection for magnetocapture, elution, and real time PCR for detecting nucleic acid (DNA) from aqueous solution or fetal bovine serum. A, Scheme of magnetocapture assay. B, cycle threshold (C_t) values for CCCMP magnetocapture followed by real time PCR on 10^2 – 10^4 copies of human genomic DNA in aqueous solution or serum. C, cycle threshold (C_t) values for CCCMP magnetocapture followed by real time PCR on 10^2 – 10^4 copies of human genomic DNA in aqueous solution or serum. In both cases, same dataset for pure genomic DNA and no template control (NTC) are plotted. Error bars represent standard deviation.

centrifuges. We hypothesized that charged CCCMP or ECCMP should be able to rescue nucleic acid from such complex biofluids in centrifuge free manner and make it suitable for downstream real time PCR analysis. Unlike real time LAMP using homemade mastermix, we employed commercial real time PCR mastermix due to their commonplace availability. The inhibitory role of complex biofluid was immediately apparent when real time PCR assays on 10^2 – 10^4 copies of purified genomic DNA spiked into 50% fetal bovine serum failed to generate amplification curve (Figure S11). Before utilising the CCCMP and ECCMP towards extracting serum spiked DNA, their amenability with real time PCR was first investigated through their ability to capture genomic DNA from aqueous solution (Figure 4A). When compared for cycle threshold (C_t) values, there was a consistent increase between same copies of pure genomic DNA before and after magnetocapture from aqueous solution (Figure 4B and 4C, melt curve analysis in Figure S12). The increase in Ct value could be attributed to reduction in analytical sensitivity from the loss of some DNA molecules during the wash steps or from the failure to get all bound DNA desorbed during elution. In the later, these DNA molecules probably failed to get released in the "neutralizing" elution buffer and might still remained electrostatically bound to partially neutralized chitosan (as shown in the colorimetric study), decreasing analytical sensitivity. When challenged with the "rescue" of 10^2 – 10^4 copies of DNA from serum, both CCCMP and ECCMP magnetocapture were able to restore real time PCR amplification albeit with slight further increase of Ct value. The increase of C_t value in this instance could be from minute presence of PCR inhibitors that could not be completely removed in the magnetocapture process as well as loss of genomic DNA. The magnetocapture with follow-up real time PCR together had a LoD in the order of 10^2 copies for CCCMP and 10^3 copies for ECCMP for both aqueous or serum samples and therefore is sufficiently sensitive to detect clinically relevant low copy number of target nucleic acid in molecular diagnosis

assays. Interestingly, the LoDs from the magnetocapture with real time PCR were similar to that for magnetocapture with follow up real time LAMP reactions, possibly validating the reproducibility of the methods. These experiments demonstrated that the magnetic particles can be utilized for identifying (through capture and amplification) nucleic acid from complex biofluid, and for physically separating it from PCR inhibitors present therein.

Future Studies. We found that both the CCCMP and ECCMP systems lost their ability to capture nucleic acid after 2 weeks under 4°C storage conditions. It could be due to the weak electrostatic nature of the interaction between chitosan and iron oxide. We did not check whether they are stable at lower storage temperature (e.g., -20°C). However, the inexpensiveness of the precursor chemicals, minimal equipment necessity for synthesis, and amenability to preparation in a short time would possibly outweigh this factor. The extraction system in its present form is incompatible with colorimetric LAMP and possibly other pH-based readout systems. It is likely that changing the elution buffer concentration, pH, or adoption of a different colorimetric pH indicator dye would resolve this issue. However, these factors were not explored in this work. In our experiments, the real time LAMP experiments in combination with melt curve analysis reproducibly detected the presence of analyte nucleic acid to as low as clinically relevant 100 – 1000 copies. Presumably due to the use of homemade LAMP mix, however, the standard deviation associated was quite high to report average C_t values with n = 3. Therefore, the magnetocapture followed by real time LAMP experiments at present can only act as "Yes or No" system without the ability of quantification (for e.g., finding bacterial load). Future studies would therefore comprise of optimizations to improve the stability of magnetic particles beyond 2 weeks, changing buffer conditions for colorimetric LAMP readout, and obtaining Ct values for real time LAMP. We would also be exploring the ability of magnetic particles to extract RNA, capability of reverse transcription and TaqMan probe-based detection. We also noticed but chose not investigate possibility of "in situ" (on-bead/on particle) amplification for LAMP experiments which would minimize the extraction timing by at least 10 min in this work. The experimental optimization for "in situ" optimization would also be taken up in future studies.

CONCLUSION:

In this work we evaluated the application of two chitosan-coated magnetic particles for extraction of amplification-ready nucleic acid from complex biofluids for application in limited resource settings. The magnetic particles are preparable within 6 – 8 h by minimally trained personnel using only water bath and magnetic stirrer using commonly available inexpensive chemicals. The particles were able to capture and physically separate genomic DNA from aqueous solutions, crude cell lysate, and fetal bovine serum without any mechanized shaking, mixing, or stirring. The extraction assay was completed within 30 min, purifying nucleic acid that can be detected using NAATs such as real time LAMP and real time PCR. Within a combined 1.5 – 2 h min turnaround time, the magnetocapture method in combination with real time LAMP was able to detect 10^2 – 10^3 copies of genomic DNA reproducibly from both aqueous solution and crude cell lysate. In combination with real time PCR assays, the magnetocapture methods were able to detect 10^2 – 10^3 copies of spiked human genomic DNA in 50% fetal bovine serum with a few cycle threshold values (C_t values) higher than that of pure genomic DNA. Assays involving the CCCMP consistently demonstrated slightly higher analytical sensitivity than ECCMP and could be due to the marginally higher DNA binding capacity of the former. The compatibility of the magnetocapture methods with real time LAMP

and real time PCR highlighted the potential adaptability of the method with rapid pathogen detection, biowarfare prevention, cell free nucleic acid detection, genetic disease identification, mutation screening, and digital PCR. Overall, the study eliminates the necessity for sophisticated instruments for magnetic particle synthesis and then demonstrates the utility of high efficiency magnetocapture of clinically relevant low copy of nucleic acids. Altogether, we anticipate that this study would help expand NAAT applications in resource limited settings and democratize nucleic acid-based diagnosis.

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Supporting information

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

MATERIALS AND METHODS

$FeCl_3 \cdot 6H_2O$ (#GRM165), $FeSO_4$,$7H_2O$ (#TCE119), 2X real time SYBR mastermix (#MBT074) were purchased from HiMedia while 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. DNA concentration estimations using UV260 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 Eppendorf master cycler.

Synthesis of coprecipitation-cured chitosan coated magnetic nanoparticles (CCCMP). The synthesis was carried out as described elsewhere with slight modification (CITE). 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 in the preheated (50°C) reactor 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) and 9.3 ml 1% medium molecular weight chitosan (SRL) 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 with 10 mL 0.05 M MES buffer for 5 times (in each step incubated with MES buffer for 10 minutes) and at last washed with 10 mL autoclaved water 5 times 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 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)

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 and placed in a preheated sand 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 (0.5% in water) was added with continuous stirring. The reaction carried out for 10 mins. Then the reaction mixture was washed with 10 mL of autoclaved water for 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, 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 presences

of elements in all types of magnetic particles were identified using energy dispersive X-ray spectroscopy (EDS) attached with FE-SEM. X-ray diffraction spectroscopic (XRD) analysis was carried out for the determination of crystalline structure of prepared magnetic particles. Fourier transform infrared spectra (FT-IR) of the samples were recorded on FTIR spectrometer (Perkin Elmer) from 500 to 4000 cm^{-1}.

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 adown at 4000 r.p.m for 5 minutes and 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. Supernatant fluid was transferred into a new sterile tube. Subsequently, cold isopropanol was added to the supernatant to final 50% concentration and kept at −20 °C for 20 min. After this stage, the solution was centrifuged at 12000 r.p.m and supernatant was discarded. The pellet is dissolved with 1 ml of cold 70% ethanol, centrifuged at 12000 r.p.m, and 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 uL 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 benchtop with occasional finger tapping. The supernatant was removed by the magnet. 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 benchtop with occasional finger tapping. 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 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 benchtop with occasional finger tapping. 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 magnet. 100 µl 0.05 M MES buffer (pH -5.2) was added and incubated for 10 mins (Charging Step) on benchtop with occasional finger tapping. The supernatant was removed by the magnet. Next, 25 ul 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 heat lysate from 10^1 – 10^5 cells (in case of crude lysate) was added to the particles and incubated for 10 mins on 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 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 benchtop with occasional finger tapping. The supernatant was collected as elution and subjected to NAAT procedure as described below. The magnetic particles left out is called as 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 three 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 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 and elution obtained from DNA binding assay.

Real time LAMP to determine LoD for detection of bacterial genomic DNA from 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 the three primer pairs in the following final concentrations: 0.2 µM outer primers, and 1.6 µM forward and backward inner primers. 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. Reaction mixture contains 0.2 µM outer primers, and 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 cycles; 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 for 30 times in a CFX Maestro or CFX connect real time PCR machine (BioRad). This was followed by default standard melt curve analysis protocol present in the instrument.

DNA extraction with CCCMP, ECCMP with mammalian genomic DNA in 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 100% 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 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 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[2]), 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 default program of melt curve analysis.

Table S1. 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*)[1]	
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*)[2]	
Forward primer	TGG CAC CAC ACC TTC TAC AAT
Reverse primer	GGT CTC AAA CAT GAT CTG GGT CA

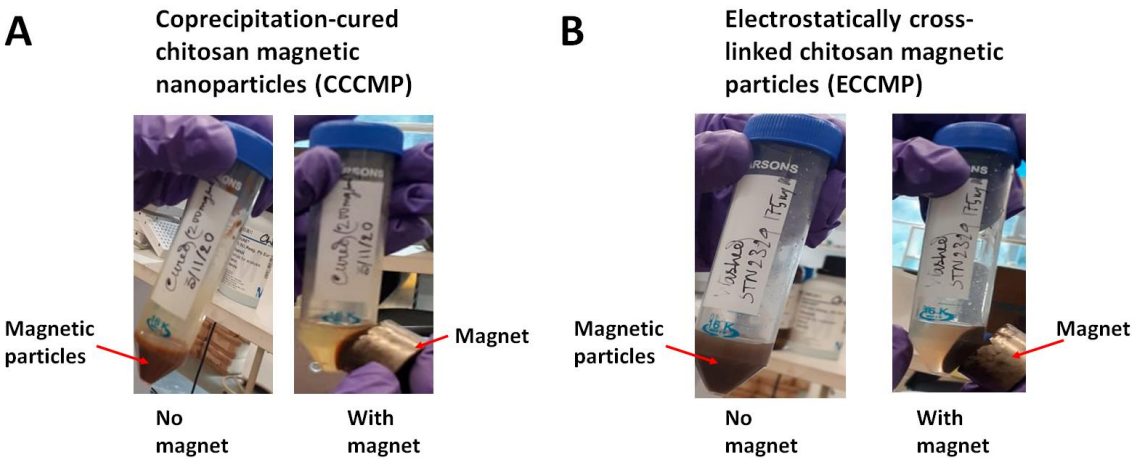

Figure S1. Images of CCCMP (A) and ECCMP (B) particles in the presence or absence of the magnet.

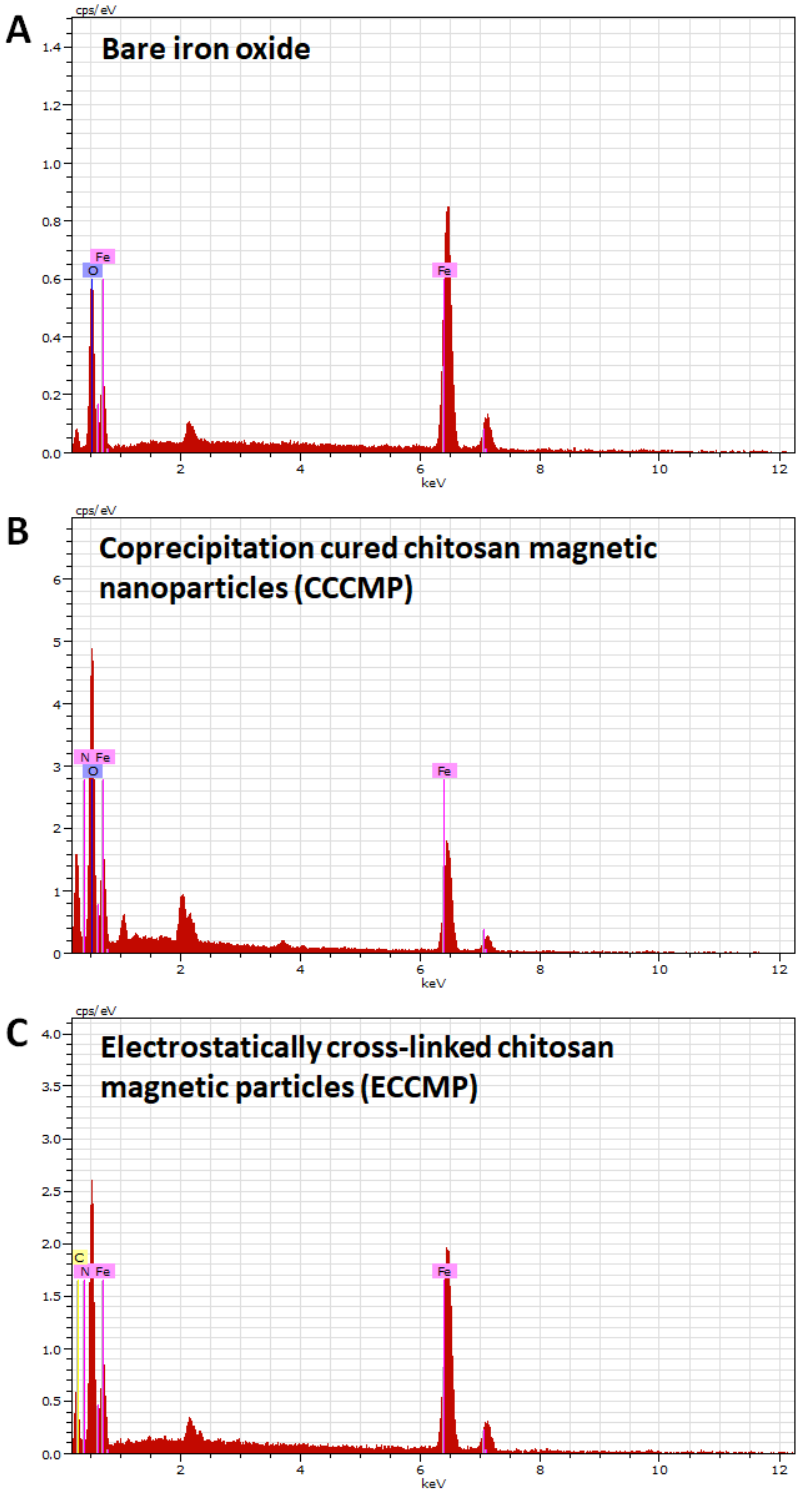

Figure S2. EDX characterization of bare iron oxide (A), CCCMP (B), and ECCMP (C) magnetic particles.

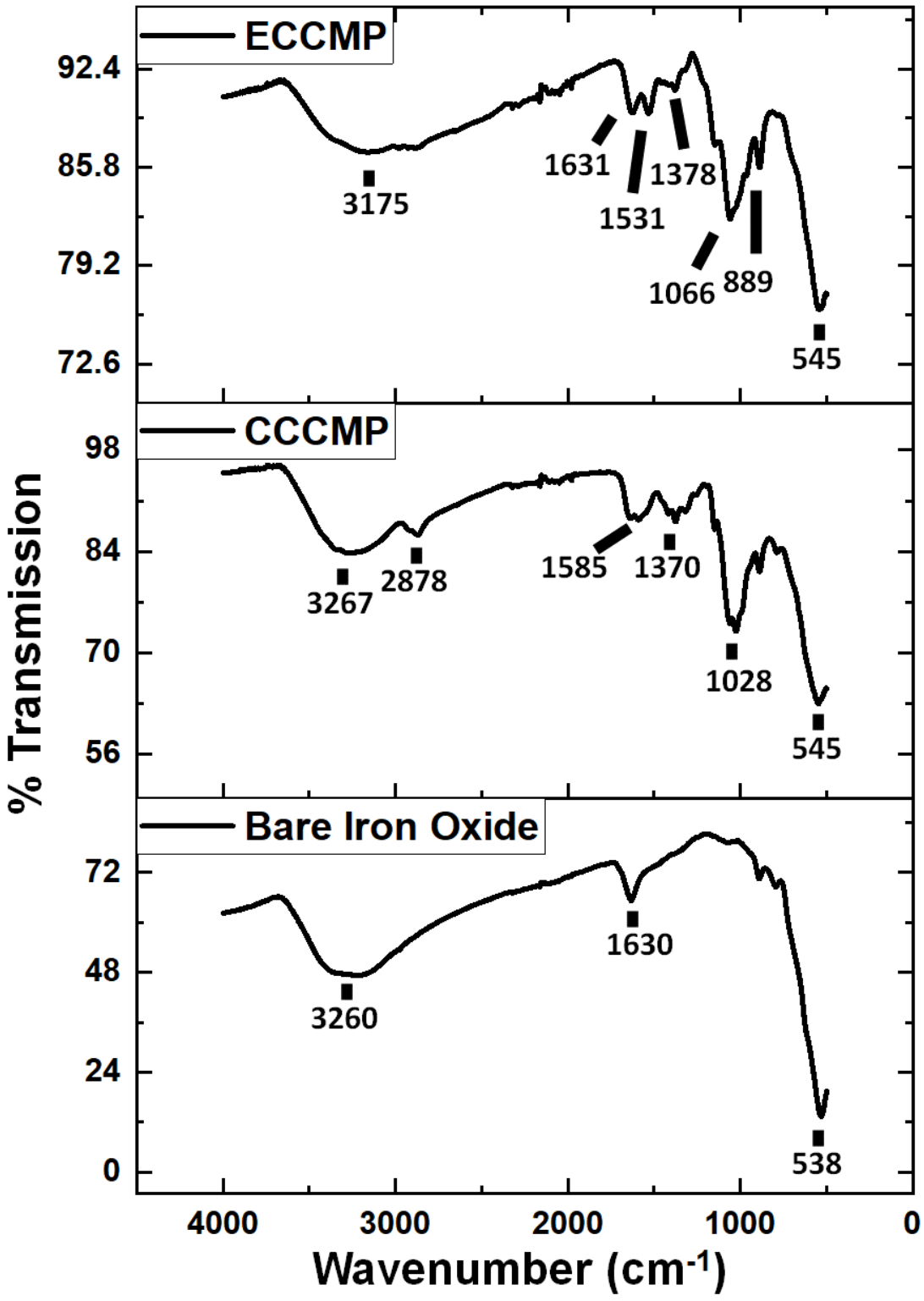

Figure S3. FT-IR characterization of CCCMP, ECCMP and bare iron oxide magnetic particles.

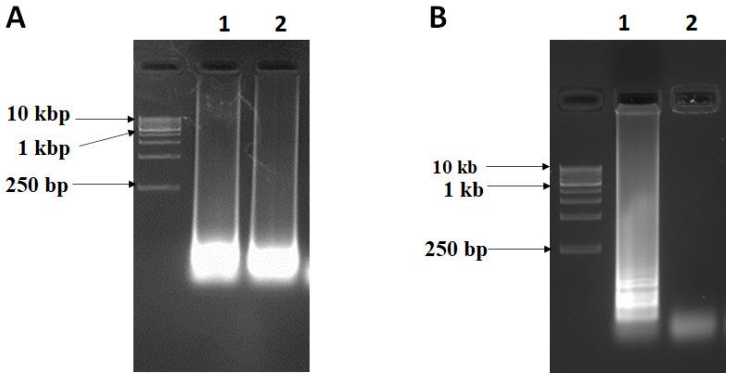

Figure S4. Loop mediated isothermal amplification for detecting *malB* gene in E coli. A, non-specific amplification in the presence of loop primers analysed in 1.5% agarose gel. Lane 1, in presence of *E. coli* genomic DNA. Lane 2, in the absence of *E. coli* genomic DNA. B, amplification in the absence of loop primers analysed in 1.5% agarose gel. Lane 1, in presence of *E. coli* genomic DNA. Lane 2, in the absence of *E. coli* genomic DNA. Leftmost lanes in both gels represent 10 kb ladder.

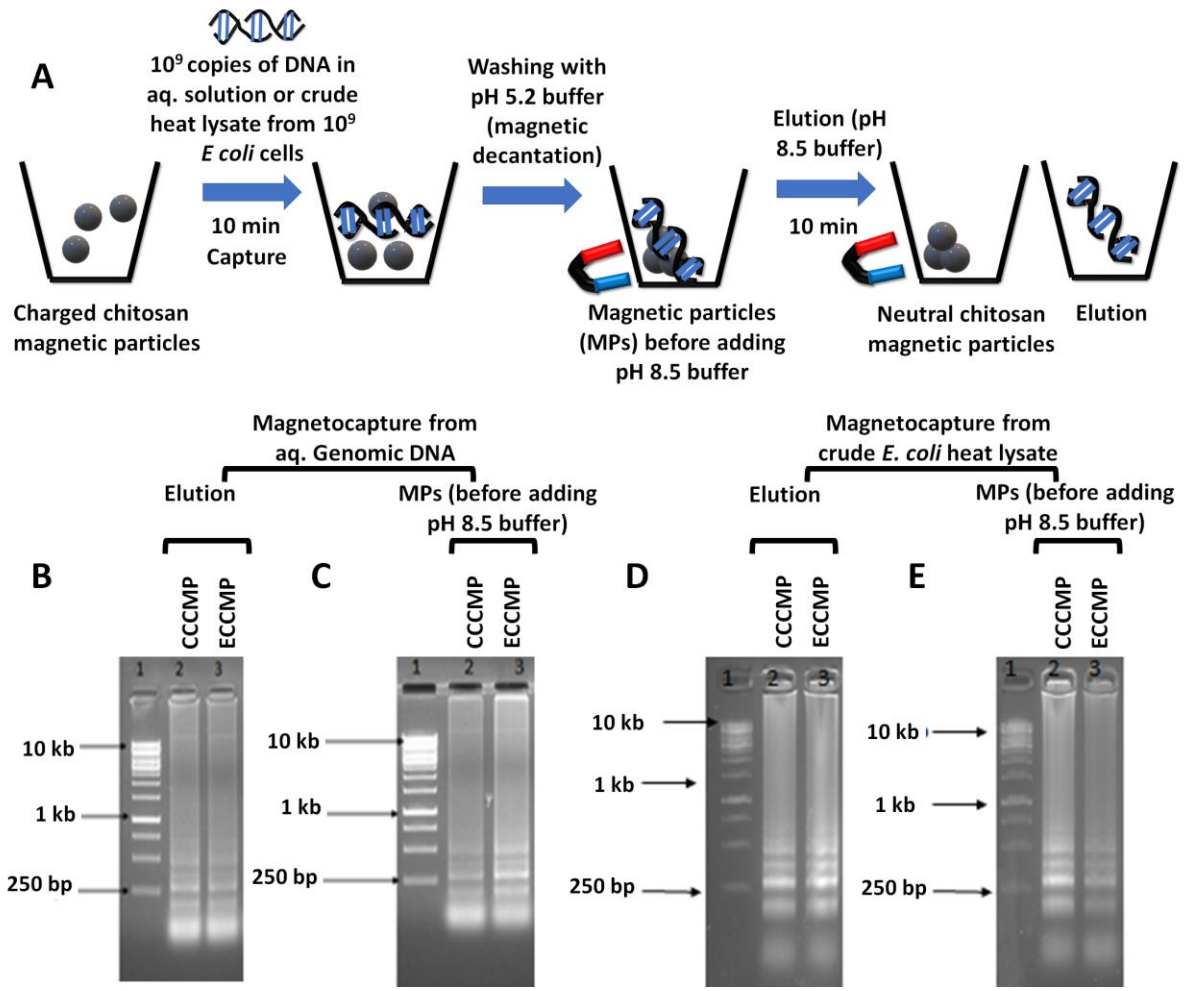

Figure S5. Magnetocapture, elution, and loop mediated isothermal amplification (LAMP) on 10^9 copies of *E. coli* genomic DNA (gDNA) from aqueous solution or crude lysate. A, Scheme of magnetocapture assay. B, pH 8.5 buffer elution from magnetocapture assay on gDNA in aqueous solution subjected to LAMP. C, magnetic particles after pH 5.2 buffer washing but before pH 8.5 buffer elution from magnetocapture assay on gDNA in aqueous solution subjected to LAMP. D, pH 8.5 buffer elution from magnetocapture assay on crude lysate subjected to LAMP. E, magnetic particles after pH 5.2 buffer washing but before pH 8.5 buffer elution from magnetocapture assay on crude lysate subjected to LAMP. 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) before magnetocapture. All experiments were analyzed in 2% agarose gel electrophoresis where leftmost lanes represent 10 kb ladder.

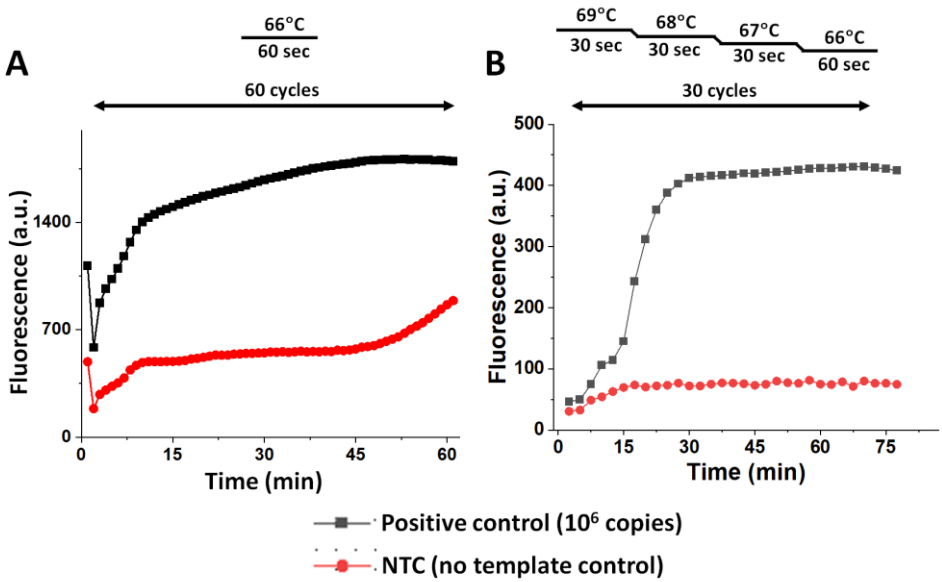

Figure S6. Comparison between real time loop mediated isothermal amplification (LAMP) (panel A) and touchdown real time LAMP (panel B) along with respective temperature cycling information. The experiments were conducted on 10^6 copies of *E. coli* genomic DNA and no template control (NTC).

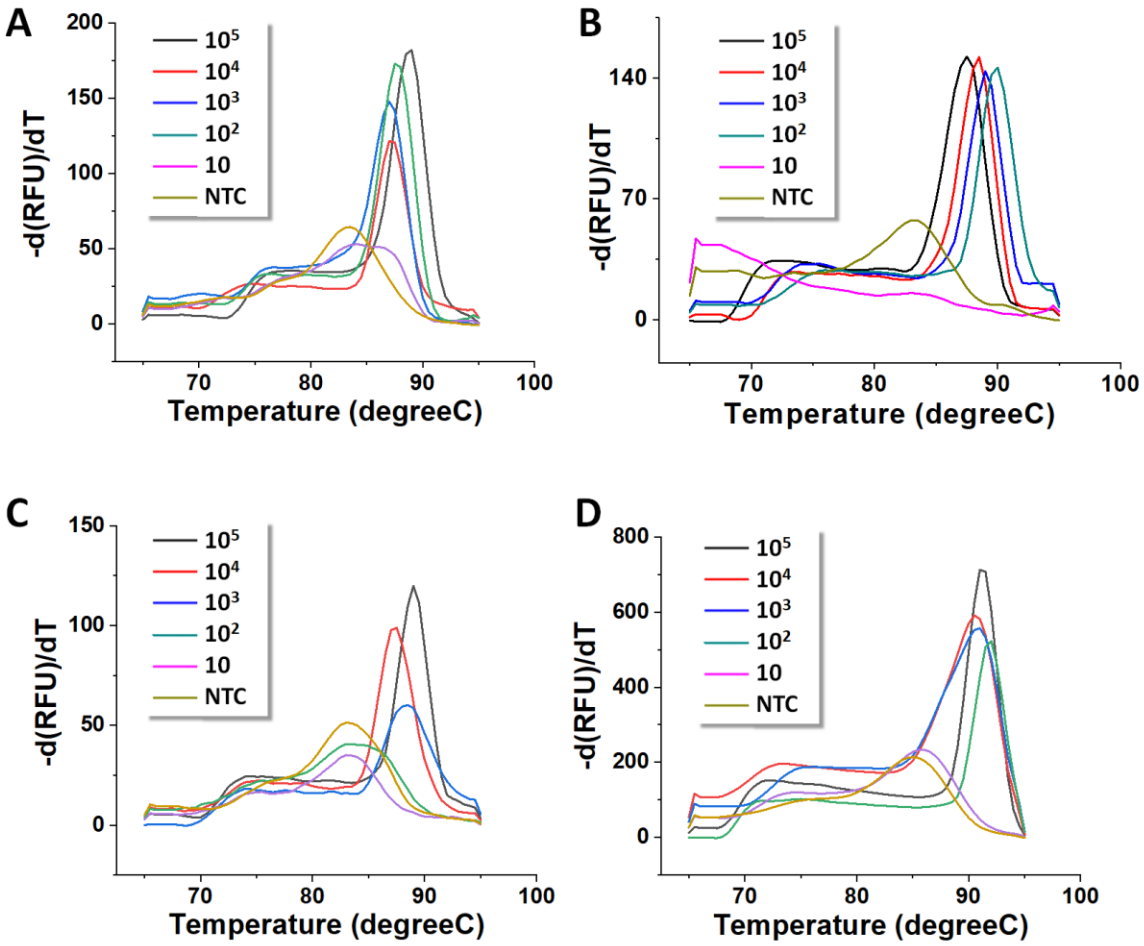

Figure S7. Derivative melt curve analysis for real time LAMP experiments on magnetocapture experiments elutions on 10^1 – 10^5 copies of *E. coli* genomic DNA in aqueous solution or crude lysate. A, real time LAMP on elution from CCCMP magnetocapture on aqueous gDNA. B, real time LAMP on elution from 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.

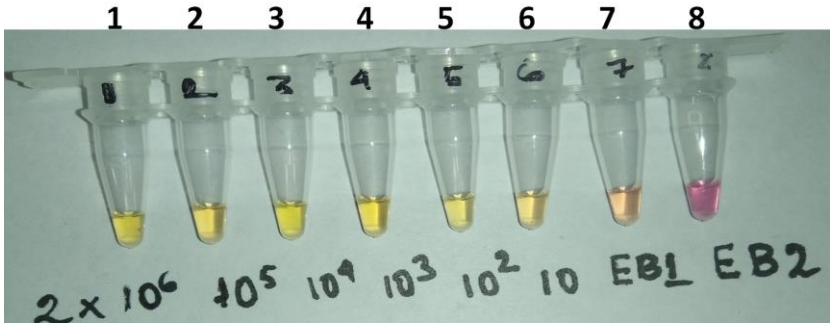

Figure S8. Colorimetric LAMP assay using WarmStart LAMP Kit (NEB # E1700S) on magnetocapture extracted gDNA. DNA copies ranging from 2 x 10^1 – 10^6 copies in 25 uL 0.05 M MES buffer were subjected to magnetocapture using 2.5 mg CCCMP, and then eluted using 25 uL elution buffer (10 mM Tris-HCl (pH 8.5)). The follow-up 20 uL colorimetric LAMP reaction in tubes 1 – 6 then consisted of 10 uL 2X proprietary LAMP colorimetric mastermix, 8 uL elution and 2 uL 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 addition of 8 uL elution buffer (without any DNA from magnetocapture) to a colorimetric LAMP having the same reaction composition as above.

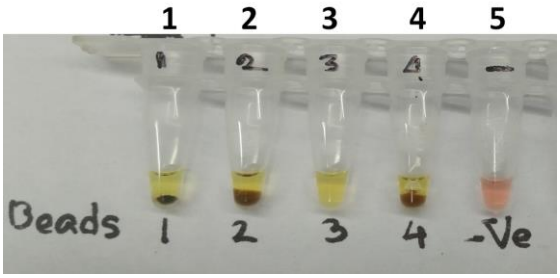

Figure S9. Colorimetric LAMP assay using WarmStart LAMP Kit (NEB # E1700S) LAMP kit on magnetocapture extracted gDNA and "neutralized" magnetic particles. In tube 1, a mock CCCMP magnetocapture experiment using 2.5 mg CCCMP and 25 uL 0.05 M MES pH 5.2 was conducted but in the absence of any genomic DNA. 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 uL colorimetric LAMP reaction then consisted of 10 uL 2X proprietary LAMP mastermix, 3 uL resuspended "neutralized" magnetic particles from above, and 2 uL 10X *E. coli malB* primer mix (without loop primers), 4 uL water. For tube 2, 10^6 copies of *E. coli* genomic DNA in 25 uL 0.05 M MES pH 5.2 buffer was subjected to 2.5 mg CCCMP. After elution, the particles were resuspended in 25 uL water and 8 uL particles was subjected to a 20 uL colorimetric LAMP as described above. In tube 3, 8 uL elution from the magnetocapture experiment described for tube 2 was subjected to a 20 uL 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 uL water. 8 uL particles was subjected to a 20 uL colorimetric LAMP as described above. In tube 5, 8 uL elution buffer (10 mM Tris-HCl pH 8.5, without any magnetocapture) was subjected to a 20 uL colorimetric LAMP as described above.

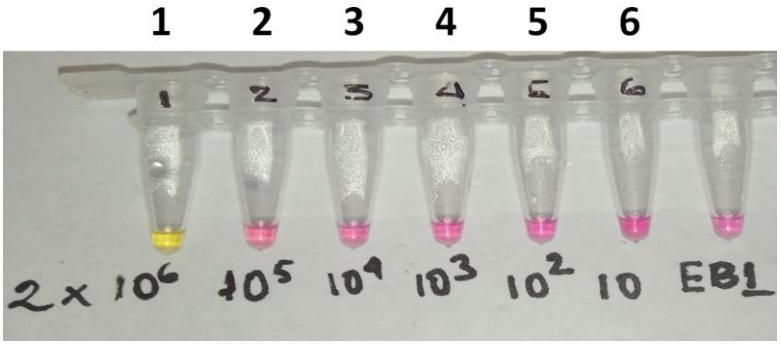

Figure S10. Colorimetric LAMP assay using WarmStart LAMP Kit (NEB # E1700S) on magnetocapture extracted gDNA. DNA copies ranging from 2 x 10^1 – 10^6 copies in 25 uL 0.05 M MES buffer (pH 5.2) were subjected to magnetocapture using 2.5 mg CCCMP, and then eluted using 25 uL elution buffer having composition of 25 mM Tris-HCl (pH 8.5)). The follow-up 20 uL colorimetric LAMP reaction in tubes 1 – 6 then consisted of 10 uL 2X proprietary LAMP colorimetric mastermix, 8 uL elution and 2 uL 10X *E. coli malB* primer mix (without loop primers). EB1 (tube 6) 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.

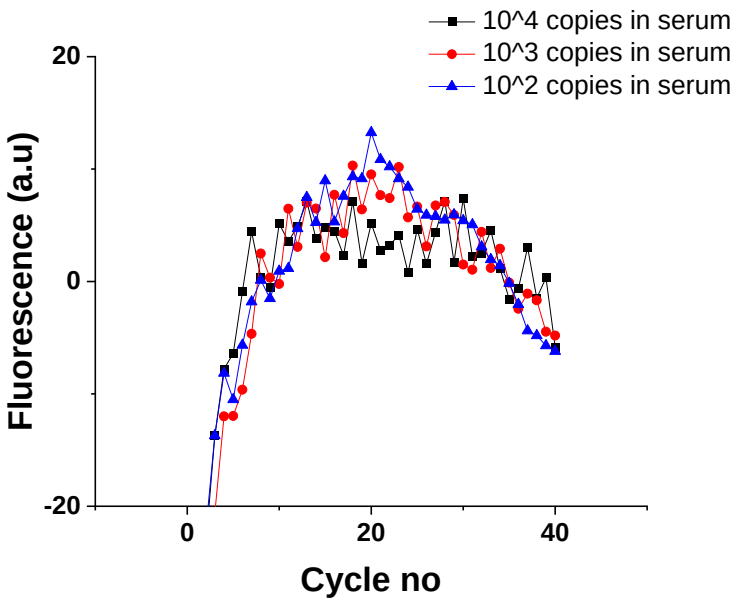

Figure S11. Real time PCR amplification of human genomic DNA in the presence of 50% fetal bovine serum.

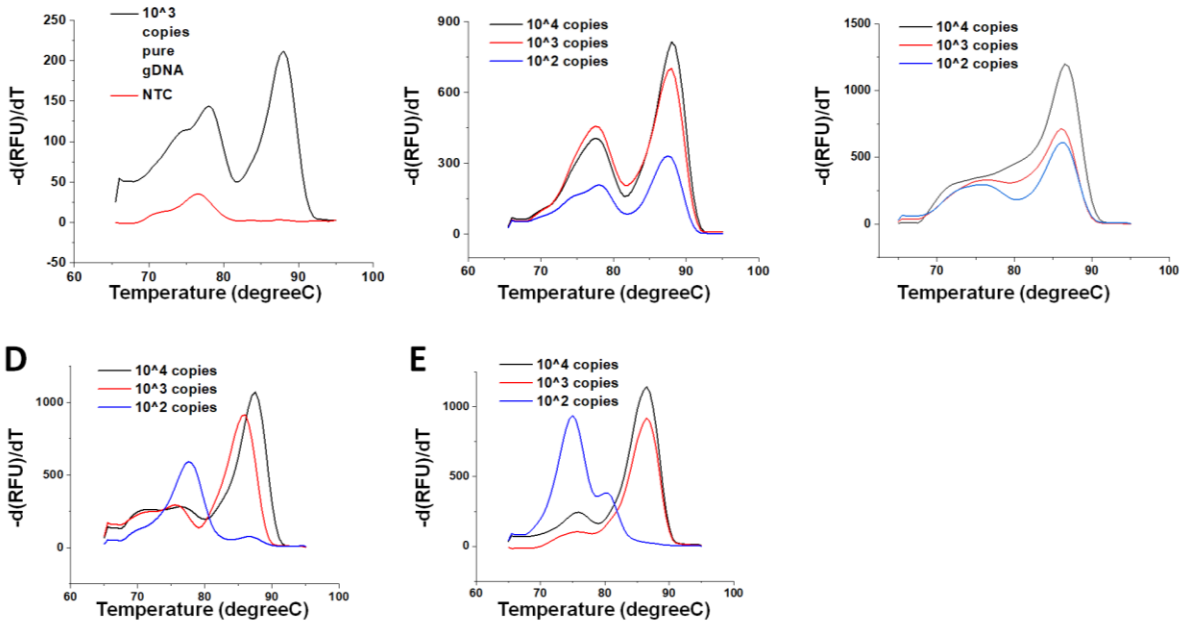

Figure S12. Real time PCR melt curve analysis for magnetocapture experiments on 10^2 – 10^4 copies of human genomic DNA in aqueous solution and serum. A, melt curve analysis for pure genomic DNA (10^3 copies) and no template control (NTC). B, melt curve analysis for CCCMP magnetocapture followed by real time PCR on 10^2 – 10^4 copies of genomic DNA from 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 aqueous sample. E, ECCMP magnetocapture followed by real time PCR on 10^2 – 10^4 copies of genomic DNA from serum.

References

(1) Hill, J.; Beriwal, S.; Chandra, I.; Paul, V. K.; Kapil, A.; Singh, T.; Wadowsky, R. M.; Singh, V.; Goyal, A.; Jahnukainen, T.; Johnson, J. R.; Tarr, P. I.; Vats, A. Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Common Strains of Escherichia Coli. J. Clin. Microbiol. 2008, 46 (8), 2800–2804. https://doi.org/10.1128/JCM.00152-08.

(2) Taghavifar, F.; Hamid, M.; Shariati, G. Gene Expression in Blood from an Individual with β-Thalassemia: An RNA Sequence Analysis. Mol. Genet. Genomic Med. 2019, 7 (7), e00740. https://doi.org/10.1002/mgg3.740.