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Paper-based visual detection of *Salmonella* bacteria using isothermal DNA amplification and magnetic bead aggregation

Sharmili Roy, Sim Xiao Wei, Ibrahim Abd Rahman and Minhaz Uddin Ahmed*

Biosensors and Biotechnology Laboratory, Chemical Science Programme, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE 1410 Brunei Darussalam.
Email: minhaz.ahmed@ubd.edu.bn; minhazua@gmail.com

ABSTRACT

Aims: The present study aimed to develop a new approach for detecting *Salmonella* species at picogram levels using magnetic bead (MB) aggregation through loop-mediated isothermal amplification (LAMP).
Methodology and results: For the first time to our knowledge, *Salmonella* LAMP amplicons were analyzed using MB aggregation. LAMPs were conducted with a simple heat block, and the results were compared with those obtained with conventional LAMP-MB techniques. Furthermore, the volume and concentration of MB solutions were optimized. Our method detected *Salmonella* genomic DNA at a low picogram level (1 pg/µL). The specificity of this method was also examined using other bacterial species. Owing to specific *Salmonella* primers, the use of LAMPs approach was time effective; because these amplicons could be utilized after 20 min instead of the 1 h needed for conventional methods. Furthermore, LAMP-positive amplicons were rapidly detected within 5 min.
Conclusion, significance and impact study: The determination of DNA in biological samples is a recent keystone in genomic analysis techniques. *Salmonella* is a foodborne pathogen that causes many diseases and, in extreme cases, death. Accordingly, detecting *Salmonella* has become a vital issue for food safety and security. Combining DNA and MBs on paper helped us to develop a new method for label-free, non-immobilized, naked eye detection of *Salmonella*. The process is very specific owing to the use of exact primers and does not require heavy or expensive instrumentation. In the future, this method could be applied to biosensors as well as in biomedical and molecular diagnostic fields.

Keywords: Food safety, loop-mediated isothermal amplification, magnetic beads, *Salmonella*

INTRODUCTION

Salmonella is a food-borne pathogen that can affect both humans and animals. According to the World Health Organization, the disease, salmonellosis, affects tens of millions of people worldwide every year, which can lead to a hundred thousand deaths. *Salmonella* comprises robust bacteria that can survive in a dry environment for weeks and in water for several months (WHO, 2013). It is necessary to quickly detect *Salmonella* in food before the germs are spread to humans and other animals. Generally, *Salmonella* is found in raw produce, especially raw chicken and eggs. The optimum survival temperature of the bacteria is 37 °C (Roszak *et al.*, 1987). At low temperature, the bacteria remain in the host without reproducing, yet the host may still be infected if *Salmonella*-infected produce was ingested. Conventional methods used for the detection and isolation of *Salmonella* includes immunomagnetic separation (Luxin *et al.*, 2007), enzyme immunoassay (EIA) (Isomaki *et al.*, 1989), and enzyme-linked immunosorbent assay (ELISA) (Carlsson *et al.*, 1972). However, all of these methods are expensive and time consuming compared with simple and direct methods such as fluorescence (Yang and Li, 2006), colorimetric detection (Safavieh *et al.*, 2014), simple lateral flow assays (Corstjens *et al.*, 2001), and loop-mediated isothermal amplification-magnetic bead (LAMP-MB) assays (Roy *et al.*, 2016).

Magnetic bead (MB) separation is a gentle, tube-based technique that has been newly redesigned as a quick paper-based detection technique. Previous studies have explained how MB separation, with its simple handling, rapidness, efficiency, and cleanliness, can replace filtration, centrifugation, and separation techniques (Lin *et al.*, 2013; Roy *et al.*, 2016). The MB method uses magnetism for efficient separation of cells as well as cell organelles including nucleic acids (NAs) (Palecek and Fojta, 2007). A magnetic field from an external source, usually a magnetic separation rack, is required to move the MBs. Unlike other isolation techniques, MB separation reduces handling steps, eliminates sample dilution, and reduces sample loss (Bruno and Kiel, 2002). On the basis of this principle, we attempted to detect *Salmonella* species through LAMP. LAMP is a method that allows for the rapid amplification of DNA with high specificity and efficiency under isothermal conditions. The LAMP reaction operates at a constant temperature using a strand displacement reaction (Notomi

***Corresponding author**

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et al., 2000). LAMP is advantageous because it can amplify DNA at isothermal conditions, usually around 60-65 °C, with greater efficiency than conventional methods without using a thermal cycler or heavy PCR instruments (Ahmed *et al.*, 2009). LAMP amplification can be performed with a simple heat block. For the LAMP amplification process, the denaturation of double-stranded DNA (dsDNA) is not needed to allow primer annealing followed by amplicon elongation because DNA polymerase possessing strand displacement activity is used (Craw and Balachandran, 2012). LAMP is also relatively cheaper in terms of reagents, and expensive equipment is not needed as it is for conventional PCR (Ahmed *et al.*, 2009), which is necessary for DNA denaturation, primer annealing, and elongation. A positive LAMP reaction can also be detected using 2% agarose gel electrophoresis that is stained and placed under UV light in a UVP machine. Overall, LAMP requires less time for amplification and detection of DNA compared with conventional techniques that reduce needed instrumentation, such as thermocyclers, and time (Roy *et al.*, 2016; Safavieh *et al.*, 2016). Some of the applications of LAMP include rapid diagnosis of viral, bacterial, and parasitic diseases in addition to food adulteration to identify genus and species-specific analytes (Safavieh *et al.*, 2014).

In this study, our main goal was to detect *Salmonella* species using the combined LAMP-MB method to establish visual detection of LAMP amplicons on paper. This experiment demonstrates an approach towards on spot detection of species-specific bacterial DNA without the use of massive and high-cost instruments. Utilizing paper makes the entire process cost effective, as paper is easily obtained worldwide, and allows for quick and easy detection. Moreover, this detection platform provides a cost effective, disposable analytical device and point of care (POC) diagnosis of bacterial diseases. Furthermore, the hydrophilic nature of paper helps to easily absorb the liquid solution (Maxwell *et al.*, 2013; Renault *et al.*, 2013).

MATERIALS AND METHODS

Reagents and instruments

Dynabeads (Myone™ streptavidin C1) were obtained from Invitrogen (Carlsbad, CA), and the beads were washed before use in actual experiments. Magnetic beads were mixed in diethylpyrocarbonate-treated water (Shanghai Beyotime, Shanghai, China). Whatman filter paper (grade 1, pore Ø 1 µM) was used throughout the experiments as the paper substrate. Tris buffer, which contained 2× binding and washing buffer, was used in the experiments; in addition, the Tris buffer also contained 2.0 mol/L NaCl, 1 mmol/L of EDTA, and 10 mmol/L Tris-HCL, and the pH was maintained at 7.5. Magnetic bead solutions can be stored at room temperature owing to their thermostability. All chemicals were of analytical grade.

Salmonella species were obtained from a commercially available detection kit via the LAMP reaction (Eiken Chemical Co., Ltd, Japan). Six primers were used to detect six different regions on the target gene. To generate a static magnetic field and create a strong aggregation with DNA and MBs, neodymium magnets were used. The concentration and purity of DNA were estimated by ultraviolet-visible spectrophotometry (Nanophotometer™, Implen GmbH, Munich, Germany). All images of aggregate spots on the papers were taken with a Nikon camera. All solutions were prepared and diluted using ultrapure water (18.3 MΩ/cm).

LAMP preparation

The LAMP reaction was controlled in a polypropylene tube containing 25 µL of the total reaction mixture. The LAMP reaction mixture contained the following reagents: 3 mmol/L $MgSO_4$ (New England Biolabs, Beverly, MA); 0.64 mol/L betaine (Sigma–Aldrich, St. Louis, MO); 0.4 mmol/L dNTP (New England Biolabs); 0.2 µmol/L each of F3 and B3; 1.6 µmol/L each of FIP and BIP; 0.8 µmol/L each of LF and LB; 16 U of *Bst* DNA Polymerase, Large fragment (New England Biolabs); 2.5 µL of 10× Thermopol buffer (New England Biolabs); and, 5 µL of *Salmonella* DNA. To stop evaporation, 10 µL of mineral oil was added onto the surface of the solution. The reaction was carried out at 65 °C, and LAMP amplicons were removed for analysis after different amplification times ranging from 20 to 60 min. Aliquots (2 µL) of the amplified LAMP products were analyzed with 2% agarose gel electrophoresis and stained with FloroSafe (1st Base, Singapore) to confirm amplification. Gels were visualized under UV light in a UVP machine (Upland, CA, USA). The amplicons were used for MB-DNA assays that could be detected by the naked eye on paper.

Aggregation assays with LAMP amplicons

Before beginning the experiments, the amount of MBs and DNA used in the LAMP reactions was optimized. The range studied during optimization was 0.5 µL to 2 µL and 0.5 µL to 5 µL for MB solutions and the amount of DNA, respectively. Before use, MBs were cleaned with washing buffer. The MB solutions (in tubes) were vortex-mixed for 10 s and placed in a magnet for 5 min. The cap was tilted in order to be washed and then twisted to tighten the pallets. Next, the cap was opened while ensuring any droplets on the cap were obtained. The supernatant was removed by pipetting or inverting, and a piece of paper was used to catch any water that remained. Then, 5.5 µL of 2× washing buffer was added to the tube and the tube was removed from the magnet to re-suspend the magnetic beads. The magnet beads were then washed by rotating the tube 180°. The tube was placed back onto the magnet for 5 min, and the process was repeated for a total of three washes. The supernatant was removed and 5.5 µL of Diethylpyrocarbonate (DEPC)-treated water was added (Figure 1). Finally, the tube was removed from the magnet. The magnetic beads were re-suspended with DEPC water and stored at 4 °C. After performing LAMP amplification, those LAMP amplicons were added to the

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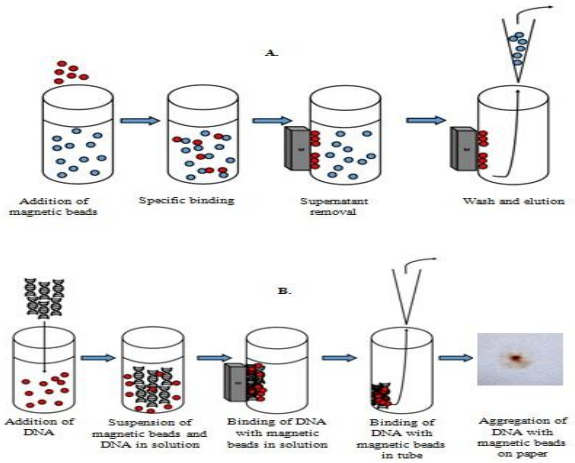

Figure 1: A, Washing protocol for the magnetic beads. B, Aggregation process of magnetic beads with DNA on a paper substrate.

washed MB solutions, and the visual detection of *Salmonella* species was performed.

Image processing

For visual detection, the aggregates with LAMP amplicons were transferred to the paper, and pictures were taken with the digital camera. Then, all those pictures were saved in Image J software (National Institutes of Health, USA), and relative intensities were calculated for all samples in comparison with the blank (buffer). The intensity of the blank was calculated with respect to the brightness of the paper. Signals obtained from the images were transformed in the Image J software for intensity measurement of dark spot and bright background regions. With higher DNA concentrations, the MB-DNA binding affinity was stronger, and the beads began to aggregate more on the paper than they did for lower DNA concentrations. Therefore, for the intensity analysis, darker areas showed lower intensity relative to brighter background areas. High intensities suggested weak binding of the MBs with DNA and that the MBs were dispersed over the paper. Water and buffer placed on the paper were used as negative controls. Intensity values were calculated relative to the background and to the controls. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Magnetic bead separation speed is determined by the resistive force generated by the viscosity of the buffer. To further develop this alternative method, researchers have recently been giving attention to the binding capacity of MBs with dsDNA, which are typically used in engineering applications (Tsouti *et al.*, 2011). Magnetic beads have good binding capacities because of their large total surface areas. Under equilibrium conditions, beads were observed using a microscope, which showed that they exhibit magnetic and hydrodynamic forces with each other (Smith *et al.*, 1992). Moreover, they are highly established in solution and respond quickly to an external magnetic field. This whole process offers faster and less time-consuming procedures compared with conventional methods (Roy *et al.*, 2016). Magnetic methods depend on separation of micrometer-sized paramagnetic or ferromagnetic particles from biological or chemical media. Superparamagnetic particles have been shown to be very active under a strong magnetic field but hold no residual magnetism in the absence of a magnetic field (Lee *et al.*, 2012). These particles do not react with each other; they only bind together when a magnetic field is present. However, they have been observed to separate when external magnetic fields are removed from the magnetism solution (Lin *et al.*, 2013). Various processes exist to prepare MBs. Generally, beads are disposed to magnetism, such as iron oxide, coated with biological or synthetic polymers or made up of streptavidin antibodies (Gijs, 2004). Here, we used streptavidin Dynabeads throughout our experiments.

Optimization of the MB-LAMP DNA assay protocol

The MBs are simple to use in the handling of biotinylated nucleic acids, antibodies, and other targets. Owing to their homogenous, uniform, and stable sizes and shapes, they give robust and reproducible results (Saiyed *et al.*, 2003). MBs with DNA have been studied with a single polypropylene tube placed in an external magnet (Lin *et al.*, 2013), but we optimized the entire assay using paper. The beads were prepared with a monolayer that consisted of recombinant streptavidin, which was covalently coupled to the smooth surfaces (Olsvik *et al.*, 1994). The main advantage of biotin binding is the strong affinity for binding of biotinylated ligands (Maki *et al.*, 2008). They are hydrophilic in nature and showed rapid liquid-phase reaction kinetics for binding with DNA.

To optimize the best volume of MB solution, different amounts of MB solutions were used. The utilized range was from 0.5 µL to 5 µL for MB solutions with LAMP amplicons. A clearly visible dark spot was obtained from 1 µL of MBs. Based on this test, the amount of MBs used was 1 µL throughout the experiment. Furthermore, the amount of LAMP amplicons was also optimized. The optimization range was from 0.5 µL to 5 µL. The best results were obtained using 1 µL of positive-LAMP amplicons, which were mixed with 1 µL of MBs. Furthermore, the optimum amount of MBs and LAMP DNA aggregation were transformed into a paper. Intensity was measured and analyzed using the Image J software. With this software, a bright intensity showed the dispersion of MB solution that did not bind with DNA on the paper. In contrast, a low intensity indicated strong binding capacity between DNA and MBs, which caused good aggregation. On the basis of this principle, the particle aggregations were determined, and the intensity of the dark spots was calculated.

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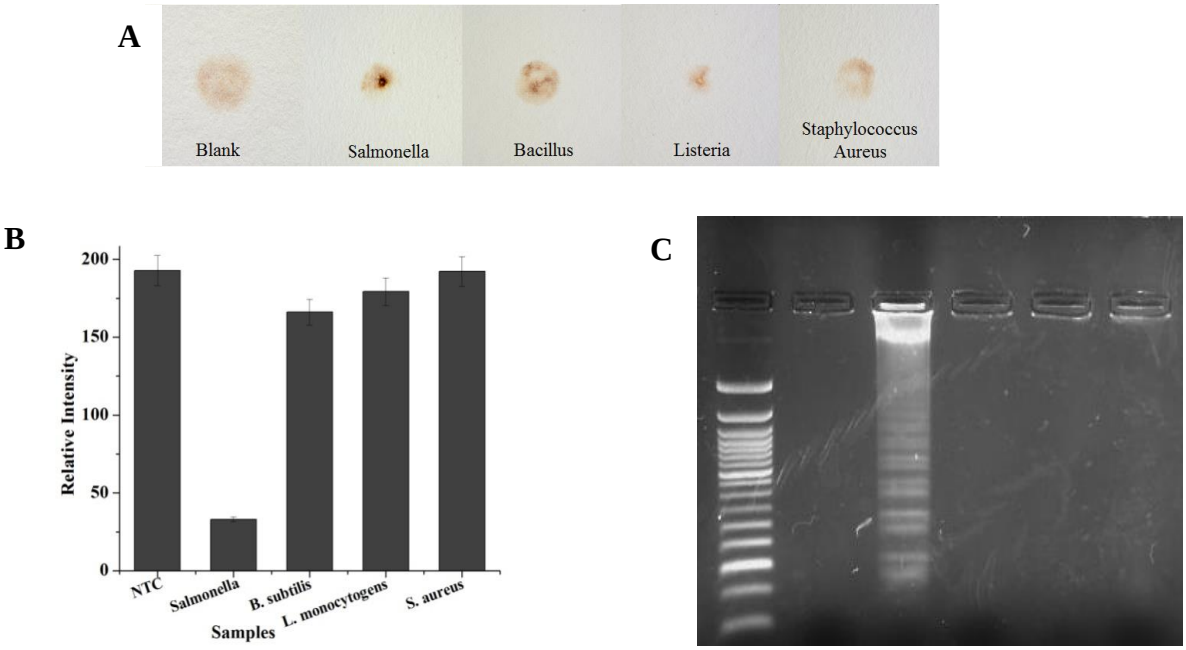

Figure 2: Detection of LAMP amplicons and cross-reactivity test with other species. A, Aggregation of different species after LAMP amplification with magnetic beads on paper. B, Relative intensities were calculated based on aggregation on the paper with *Salmonella* primer. Tris-EDTA buffer was used as negative control (NTC). C, Gel electrophoresis analysis of LAMP amplicons. The analysis was performed with *Salmonella* primers and run on a 2% agarose gel. Lane M, 50 bp ladder was used as a marker; lane 1, control with Tris-EDTA buffer; lane 3, LAMP product from *Salmonella* gDNA; lanes 3, 4, and 5, LAMP products from *Bacillus subtilis*, *Listeria monocytogenes*, and *Staphylococcus aureus* gDNA (non-target control), respectively. The gel was stained with FloroSafe.

Specificity of LAMP-MB aggregation with *Salmonella*

To determine whether the *Salmonella* primer cross-reacted with other bacterial species, all species underwent LAMP reaction in the presence of *Salmonella* primers. The amplicons were then suspended with MBs in a magnetic field to allow them to aggregate (Figure 2). Firstly, LAMP amplicons were performed with *Salmonella* primers from gDNA of *Salmonella*, *Bacillus subtilis*, *Listeria monocytogenes*, and *Staphylococcus aureus* to check the specificity of the primers at 63 °C with a simple heat block. Later, the LAMP amplicons were used for the MB aggregation process and placed on the paper for analysis with Image J software. Figure 2A represents the aggregations that occurred because the use of positive *Salmonella* LAMP amplicons and others showed the dispersion of non-target samples. Figure 2B shows the analytical measurements of LAMP-MB aggregations that were placed on the paper. To confirm the cross-reactivity of samples, they were analyzed using a 2% agarose gel (Figure 2C), which indicated the correlation between LAMP-MB aggregation with conventional techniques. The main reason that there was no aggregation for other species except *Salmonella* was because the primers were very specific only for *Salmonella* and not the other species.

Sensitivity of LAMP-MB aggregation assay

The length of DNA strands is an important factor for the formation of dark spots on the paper, and LAMP amplification produces long strands (1-4 kb) compared with other isothermal amplifications (Nelson *et al.*, 2014; Ahmed *et al.*, 2010). Moreover, LAMP amplification is more effective and sensitive than other conventional techniques such as PCR, EIA, and ELISA or other isothermal amplifications (Notomi *et al.*, 2000; Mori *et al.*, 2001; Njiru, 2012; Safavieh *et al.*, 2014). In this study, we achieved LAMP-MB aggregation with *Salmonella* species for the first time to our knowledge. This study also shows that the LAMP-MB method was more sensitive than conventional gel electrophoresis. To reduce the time and amount of laborious work required, the LAMP-MB method was applied, and proved to be a good example of a simple, cost effective, on spot detection strategy. In the present study, the limit of detection of the LAMP-MB method was 1 pg/µL. In previous studies, LAMP DNA had long strands, which allowed for stronger binding to MBs (Eldelstein *et al.*, 2000). The utilized concentration range of the gDNA template of *Salmonella* was 1000 to 1 pg/µL. Aggregation occurred with all of these LAMP amplicons, and the sensitivity was determined to be 1 pg/µL (Figure 3A and 3B). To compare this result with a conventional method, we analyzed LAMP amplicons with

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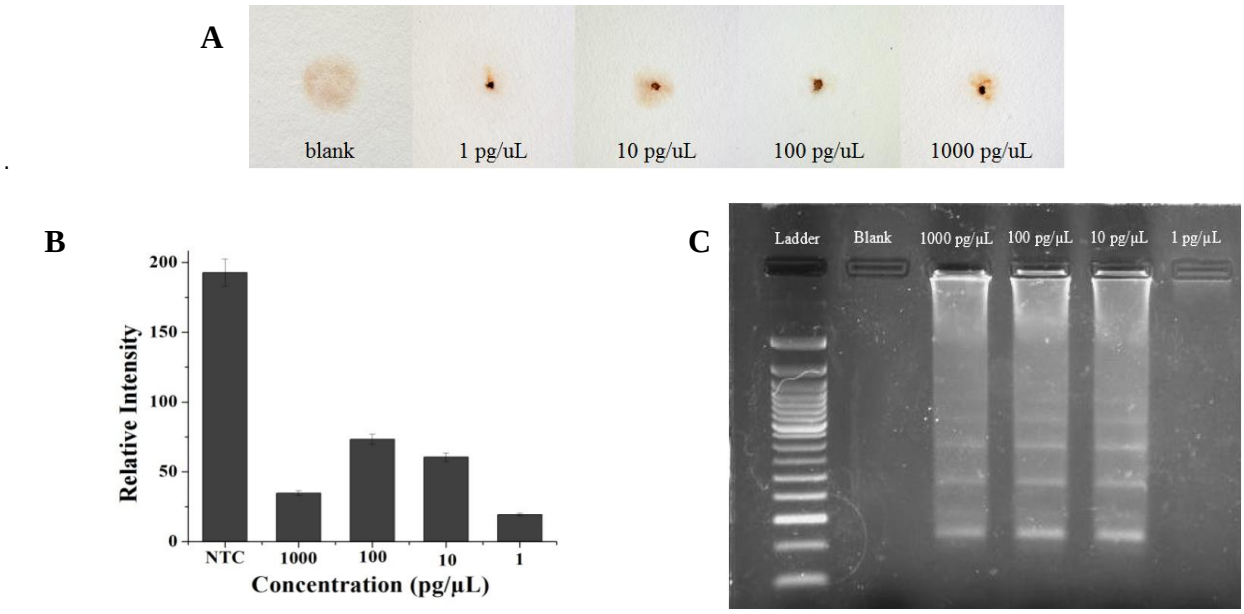

Figure 3: Detection of LAMP products after the amplification and study of LAMP-MB binding capacity. Sensitivity was determined for *Salmonella* species and compared with a conventional method. A, Aggregation of different concentrations of LAMP amplicons after amplification of *Salmonella* DNA templates (1, 10, 100, 500, and 1000 pg/µL) with magnetic beads on paper. B, Relative intensity was calculated based on aggregation on the paper with *Salmonella* LAMP amplicons. Tris-EDTA buffer was used as a negative control (NTC). C, Comparison of LAMP-MB technique with conventional method of gel electrophoresis of LAMP amplicons. Lane M, 50 bp ladder used as a marker; lane 1, control with Tris-EDTA buffer; lane 2, 3, 4, and 5, LAMP products from *Salmonella* gDNA concentrations from 1000, 100, 10, and 1 pg/µL, respectively. The gel was stained with FloroSafe.

gel electrophoresis. In comparison, the LAMP-MB mechanism proved to be more sensitive (Figure 3C). The sensitivity test performed using the same LAMP amplicons with the MB assay determined that 1 pg/µL was the optimum concentration; the aggregation appeared as a dark spot on the paper at the same concentration, whereas, using gel electrophoresis, the sensitivity was 10 pg/µL. Figure 3C showed the positive amplification started from 1000 to 10 pg/µL but the product did not show positive amplification for 1 pg/µL of LAMP. This investigation showed that the LAMP-MB assay is simpler and more sensitive than the tedious conventional gel electrophoresis method.

Effect of the amplification time of LAMP-MB assays

Current research has focused on reducing analysis time, which is a challenging issue. The development of new techniques helps to reduce the steps and time for analyses compared with conventional methods. To reduce the time, we investigated the optimal LAMP amplification time between 0 min and 60 min. After 20 min of LAMP reaction, positive amplicons were observed (Figure 4A and 4B). As a negative control, Tris buffer was used without any *Salmonella* DNA template. The positive amplicons were compared with gel electrophoresis where positive amplicons were observed after 40 min of LAMP reaction. Simultaneously, these data also helped to prove that the LAMP-MB assay is more sensitive and requires less time than conventional techniques. This strategy is applicable for on spot detection to make POC analytical devices (Safavieh *et al.*, 2016). In contrast, to achieve a significant and clear dark spot on the paper, the incubation time was optimized. Firstly, single polypropylene tube tests were performed until the external magnet was placed. Upon its removal, the LAMP-MB complexes began binding, but they bound more strongly with longer incubation time. Based on this observation, we varied the incubation time from 0 min to 60 min in 5-min intervals. This investigation showed that after 5 min of incubation time, strong binding occurred and, after transferring the complex to the paper, it showed a visibly darker spot than at 0 min of dispersion. In contrast with the results of Lin *et al.*, instead of 60 min dispersion time, the detection process can be completed in 20 min, indicating the reduced complexity of the process. The magnetic field provided by the magnetic rack was strong enough to keep LAMP-MB complexes stable in the static field but did not affect the hydrodynamic structure of the complex. The stability of the superparamagnetic bead suspension associated with DNA was a function of DNA attaching density, length of chain, and strength of the magnetic field (Li *et al.*, 2009). However, a certain incubation period is needed to stabilize the complex in the solution before transferring to the paper; the effect of permanent DNA bonding occurred

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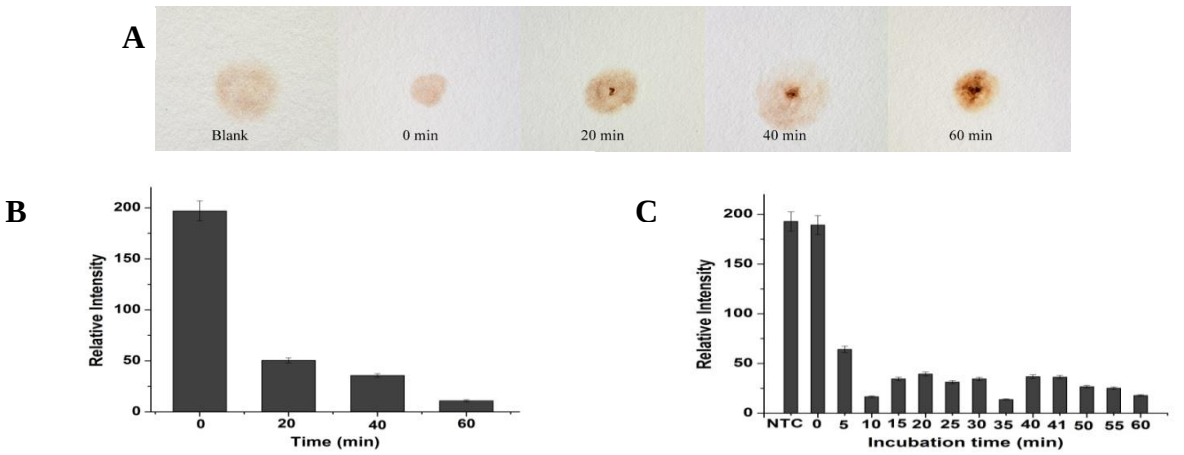

Figure 4: Determination of LAMP products at different time intervals. A, Aggregation of LAMP amplicons at different time intervals after amplification of *Salmonella* DNA with magnetic beads on paper. B, Relative intensity was calculated based on aggregation on the paper with LAMP amplicons. Tris-EDTA buffer was used as a negative control (NTC). C, Aggregation of MBs with LAMP amplicons after different incubation times on paper (0-60 min).

with the MB particles even after removing the external magnetic field.

CONCLUSION

With the progress of our investigation, it can be concluded that the LAMP-MB technique is a sensitive, specific, cost-effective, and time-efficient method. It was more sensitive than any of the conventional methods; the limit of detection of this method was 1 pg/µL, and there were no non-specific adsorption reactions. Owing to rapid LAMP amplification, positive amplicons can be detected within 20 min. Moreover, a visible, significant dark spot only took 5 min to appear with paper detection. This method required only minimal and very simple instrumentation and less laborious work for completion of the entire procedure. Another advantage of this technique is that centrifugation or vacuum manifolds are not needed. Moreover, this process is level and an immobilization-free technique. Previously, in a few studies, researchers have used tedious methods, such as immobilization and leveling methods, which require more chemical modifications to fabricate the entire detection analysis (Castaneda *et al.*, 2007). Owing to the use of these methods, experiments have become more expensive and lengthy, which is not suitable for rapid research. Therefore, in the future, our technique could potentially be employed in health screening and in POC devices. To avoid food contamination and food allergies with microbes, we believe that this paper-based detection technique of *Salmonella* with MBs will be the most efficient and easiest technique for on spot detection.

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REFERENCES

Ahmed, M. U., Saito, M., Hossain, M. M., Rao, S. R., Furui, S., Hino, A., Takamura, Y., Ahmed, M. U., Saito, M., Hossain, M. M., Rao, S. R., Furui, S., Hino, A., Takamura, Y., Takagi, M. and Tamiya, E. (2009). Electrochemical genosensor for the rapid detection of GMP using loop-mediated isothermal amplification. *Analyst* **134, 966-972.**

Ahmed, M. U., Hasan, Q., Hossain, M. M., Saito, M. and Tamiya, E. (2010). Meat species identification based on the loop mediated isothermal amplification and electrochemical DNA sensor. *Food Control* **21(5), 599-605.**

Bruno, J. G. and Kiel, J. L. (2002). Use of magnetic beads in selection and detection of biotoxin aptamer by electrochemiluminescence and enzymatic methods. *BioTechniques* **32, 178-183.**

Carlsson, H. E., Lindberg, A. A. and Hammarstrom, S. (1972). Titration of antibodies to *Salmonella* O antigens by enzyme-linked immunosorbent assay. *Infection and Immunity* **6(5), 703-708.**

Castaneda, M., Alegret, S. and Merkoci, A. (2007). Electrochemical sensing of DNA using gold nanoparticles. *Electroanalysis* **19, 743-753.**

Corstjens, P., Zuiderwijk, M., Brink, A., Li, S., Feindt, H., Niedbala, R. S. and Tanke, H. (2001). Use of up-converting phosphor reporters in lateral-flow assays to detect specific nucleic acid sequences: A rapid, sensitive DNA test to identify human papillomavirus type 16 infection. *Clinical Chemistry* **47(10), 1885-1893.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Craw, P. and Balachandran, W. (2012). Isothermal nucleic acid amplification technologies for point-of-care diagnostics: A critical review. *Lab on a Chip* **14(12), 2469-2486.**

Eldelstein, R. L., Tamanaha, C. R., Sheehan, P. E., Miller, M. M., Baselt, D. R., Whitman, L. J. and Colton, R. J. (2000). The BARC biosensor applied to the detection of biological warfare agents. *Biosensors and Bioelectronics* **14, 805-813.**

Gijs, M. A. M. (2004). Magnetic bead handling on-chip: new opportunities for analytical applications. *Microfluid Nanofluid* **1, 22-40.**

Isomaki, O., Vuento, R. and Granfors, K. (1989). Serological diagnosis of *Salmonella* infections by enzyme immunoassay. *The Lancet* **333(8652), 1411-1414.**

Lee, J. T., Abid, A., Cheung, K. H., Sudheendra, L. and Kennedy, I. M. (2012). Superparamagnetic particle dynamics and mixing in a rotating capillary tube with a stationary magnetic field. *Microfluid Nanofluidics* **13(3), 461-468.**

Li, D., Rogers, J. and Biswal, S. L. (2009). Probing the stability of magnetically assembled DNA-linked colloidal chains. *Langmuir* **25(16), 8944-8950.**

Lin, C., Zhang, Y., Zhou, X., Yao, B. and Fang, Q. (2013). Naked-eye detection of nucleic acids through rolling circle amplification and magnetic particle mediated aggregation. *Biosensors and Bioelectronics* **47, 515-519.**

Luxin, W., Yong, L. and Azlin, M. (2007). Rapid and simultaneous quantitation of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* in ground beef by multiplex real-time PCR and immunimagnetic separation. *Journal of Food Protection* **6, 1366-1372.**

Maki, W. C., Mishra, N. N., Cameron, E. G., Filanoski, B., Rastogi, S. K. and Maki, G. K. (2008). Nanowire-transistor based ultra-sensitive DNA methylation detection. *Biosensors and Bioelectronics* **23(6), 780-787.**

Maxwell, E. J., Mazzeo, A. D. and Whitesides, G. M. (2013). Paper-based electroanalytical devices for accessible diagnostic testing. *Materials Research Society* **38, 309-314.**

Mori, Y., Nagamine, K., Tomita, N. and Notomi, T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical Biophysical Research Communications* **289, 150-154.**

Nelson, D. A., Strachan, B. C., Sloane, H. S., Li, J. and Landers, J. P. (2014). Dual-force aggregation of magnetic particles enhances label-free quantification of DNA at the sub-single cell level. *Analytical Chimica Acta* **819, 34-41.**

Njiru, Z. K. (2012). Loop-mediated isothermal amplification technology: Towards point of care diagnostics. *PLOS Neglected Tropical Diseases* **6, e1572.**

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28(12), e63.**

Olsvik, O., Popovic, T., Skjerve, E., Cudjoe, K. S., Hornes, E., Ugelstad, J. and Uhlen, M. (1994). Magnetic separation techniques in diagnostic microbiology. *Clinical Microbiology Reviews* **7, 43-54.**

Palecek, E. and Fojta, M. (2007). Magnetic bead as versatile tools for electrochemical DNA and protein biosensing. *Talanta* **74(3), 276-290.**

Renault, C., Li, X., Fosdick, S. E. and Crooks, R. M. (2013). Hollow-channel paper analytical devices. *Analytical Chemistry* **85, 7976-7979.**

Roszak, D. B. and Colwell, R R. (1987). Survival strategies of bacteria in the natural environment. Microbiological Review **51(3), 365-379.**

Roy, S., Rahman, I. A., Santos, J. H. and Ahmed, M. U. (2016). Meat species identification using DNA-redox electrostatic interactions and non-specific adsorption on graphene biochips. *Food Control* **61, 70-78.**

Roy, S., Rahman, I. A. and Ahmed, M. U. (2016). Paper-based rapid detection of pork and chicken using LAMP-magnetic bead aggregates. *Analytical Methods* **8, 2391-2399.**

Safavieh, M., Ahmed, M. U., Sokullu, E., Ng, A., Braescu, L. and Zourob, M. (2014). A simple cassette as point of care diagnostic device for naked-eye colorimetric bacteria detection. *Analyst* **139(2), 482-487.**

Safavieh, M., Ahmed, M. U., Ng, A. and Zourob, M. (2014). High-throughput real-time electrochemical monitoring of LAMP for pathogenic bacteria detection. *Biosensors and Bioelectronics* **58, 101-106.**

Safavieh, M., Kanakasabapathy, M. K., Tarlan, F., Ahmed, M. U., Zourob, M., Asghar, W. and Shafiee, H. (2016). Emerging loop-mediated isothermal amplification-based microchip and microdevice technologies for nucleic acid detection. *ACS Biomaterials* **2(3), 278-294.**

Saiyed, Z. M., Telang, S. D. and Ramchand, C. N. (2003). Application of magnetic techniques in the field of drug discovery and biomedicine. *Biomagnetic Research and Technology* **1, 2.**

Smith, S. B., Finzi, L. and Bustamante, C. (1992). Direct mechanical measurements of the elasticity of single DNA molecules by using magnetic beads. *Science* **258(5058), 1122-1126.**

Tsouti, V., Boutopoulos, C. and Chatzandroulis, S. (2011). Capacitive microsystems for biological sensing. *Biosensors and Bioelectronics* **27(1), 1-11.**

WHO, World Health Organization (2013). *Salmonella* (non-typhoidal). Available from: http://www.who.int/mediacentre/factsheets/fs139/en/ [Retrieved on 29 February 2016].

Yang, L. and Li, Y. (2006). Simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella Typhimurium* using quantum dots as fluorescence labels. *Analyst* **131(6), 394-401.**

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