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ARTICLE

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Culture-free biphasic approach for sensitive detection of *Escherichia coli* O157:H7 from beef samples

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Abstract

Foodborne illnesses are a major threat to public health also leading to significant mortality and financial and reputational damage to industry. It is very important to detect pathogen presence in food products early, rapidly, and accurately to avoid potential outbreaks and economic loss. However, "gold standard" culture methods, including enrichment of pathogens, can take up to several days. Moreover, the food matrix often interferes with nucleic acid amplification methods of detection, requiring DNA extraction from the sample for successful molecular detection of pathogens. Here, we introduce a "biphasic" amplification method that can achieve high sensitivity detection with background noise from ground beef food samples without culture or other extraction methods in 2.5 h. Homogenized ground beef is dried resulting in an increase in porosity of the dried food matrix to allowing amplification enzymes and primers to access the target DNA and initiate the reaction within the dried food matrix. Using Loop Mediated Isothermal Amplification, we demonstrate the detection of 1–3 cfu of *Escherichia coli* bacteria in 30 mg of dried food matrix. Our approach significantly lowers the time to result to less than a few hours and have a pronounced impact on reduction of instrumentation complexity and costs.

KEYWORDS
biphasic, complex food matrix, culture independent, *E. coli*, porous matrix

1 | INTRODUCTION

Rapid and accurate detection and identification of foodborne pathogens from complex matrices remains a challenge. Foodborne illnesses, caused by pathogenic contamination of food and water by bacteria, fungi, viruses, and toxins, have not only become a major threat to public health, but also can lead to mortality and increase economic burden. According to the Center for Disease Control and Prevention, 2011 estimates show that each year 48 million Americans are stricken ill due to foodborne pathogens, out of which 128,000 are hospitalized and 3000 eventually die of foodborne diseases ("Burden of Foodborne Illness: Findings"). Moreover, these illnesses cause economic loss of $77.7 billion annually due to medical costs, productivity losses, and mortality (Scharff, 2012). Financial and reputational damage faced by companies can also be significant due to foodborne outbreaks. In 2018, approximately 125 recalls were made, encompassing more than 20 million pounds of ready-to-eat and raw meat products due to bacterial contamination and undeclared allergens and extraneous material found in the products ("Recall Summaries 2019"). It is important to know that foodborne outbreaks can be highly underestimated due to misdiagnosis, under-reporting, and improper sample testing (Vidic et al., 2019). Thus, it becomes increasingly important that pathogen presence in food products is detected easily, rapidly, and accurately to avoid potential outbreaks and economic loss.

Contamination of food products can occur through water and air, as well as contact with the food processing environment, soils and fertilizers, and raw materials. There are 31 known pathogens that cause foodborne illnesses, out of which the leading causes are *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Campylobacter* spp., *Salmonella* spp., and other Shiga toxin-producing *E. coli* strains ("Burden of Foodborne Illness: Findings"). In fact, *E. coli* is the leading cause of death in young children (Vidic et al., 2019). Because most bacterial outbreaks express common symptoms such as fever, vomiting, and diarrhea, it becomes increasingly difficult to identify the responsible pathogenic agent (Vidic et al., 2019). Hence, food safety is a challenge and early detection becomes highly essential. Conventional culture methods such as enrichment of pathogens on agar plates have been considered as the "gold standard." In these methods, food samples are homogenized, and subsequently the microorganisms are allowed to multiply in selective media broth or agar plates (Cai et al., 2007; Yun Wang & Duncan, 2017). Thereafter, a sample from the broth or plates is taken where now enough pathogens exists for them to be identified using molecular DNA identification tests. The culture methods allow the growth of even a few pathogens to amplify by a billion-fold or more in 24 h as doubling times are in the 20-40 min (Kubitschek, 1969; Sezonov et al., 2007). According to regulatory protocols, 25 g of solid food sample is mixed with 225 ml of media both and homogenized and allowed to culture (Andrews & Hammack, 1998; Detection Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* STECs from Meat Products and Carcass and Environmental Sponges, 2019). Even 1 pathogen can grow to over 16×10^6 organisms in 12 h in the resulting 250 ml. Even if 20 µl, the typical volume for a polumerase chain reaction (PCR) reaction, is sampled from this volume of 250 ml, over 1000 pathogens will still be found in that 20 µl. In addition, the selective culture mediums provide the desirable environmental conditions for the growth of target pathogen, increasing their concentration and diluting inhibitory compounds from the food products (Fratamico et al., 2011; Gracias & McKillip, 2004). Not only that, enrichment is also useful in reviving injured cells that may be damaged during food processing (Li et al., 2014). Often, more than one enrichment step may be required to specifically detect and identify the pathogen (Gracias & McKillip, 2004). Nonetheless, the largest limitation of the culture method is that it is highly laborious as it requires several replicates to obtain reliable results and time consuming, possibly taking up to a week to successfully determine the causative agent (López-Campos et al., 2012; Stevens & Jaykus, 2004; Taskila et al., 2012; Vidic et al., 2019; Yun Wang & Salazar, 2016). Moreover, some pathogens can enter a viable but non-culturable state due to which false-negative results are prevalent and can impair the detection (Li et al., 2014; Vidic et al., 2019). Reduction in detection time becomes critical for foodborne illness control; hence, faster methods of detection with high sensitivity must be utilized to identify contaminated food and sources of outbreaks quickly.

The food matrix, being a complex assortment of components including inorganic particles, biochemical compounds, and indigenous microflora, often interferes with nucleic acid amplification methods. Complex food matrices such as fruits, vegetables, and meats can release enzymes and antimicrobial components that interfere with downstream analysis and detection. Pathogens can also adhere to these matrices, affecting their separation from other components, resulting in a significant diagnostic challenge in these complex food matrices (Law et al., 2014).

To overcome these limitations of culture-based methods, alternative methods such as nucleic acid-based assays (e.g., PCR) and immunoassays have been used to reduce detection time to hours. It should be noted that the detection limit of immunoassays ranges in 10^4 to 10^5 cfu/g resulting in the need to have a very high number of pathogens available if pathogen enrichment was not to be used in the original samples (Yun Wang & Salazar, 2016). Similarly, the sensitivity of PCR is also between 10^3–10^4 cfu/ml (~1–10 cfu/µl) after high yield and good quality DNA nucleic acid extraction has been done (Yun Wang & Salazar, 2016). Traditional extraction methods can fail to yield good quality DNA due to ion and molecule complexes found in food matrix that inhibit the enzymes for amplification and chemical and thermal treatments cause fragmentation and random breaks of long DNA strands (Vidic et al., 2019). These extraction methods have strong impact on the detection limit of PCR. Hence, it has not been possible to eliminate the amplification of pathogens via the gold standard culture method.

Other techniques to identify the pathogen after the culture include ELISA and lateral flow immunoassays, which are based on antibody-antigen interactions. Some of these techniques rely on micro and nanoparticles which help capture and concentrate the target and allow for colorimetric detection. Though these techniques are low cost, have long-term stability, have short detection time, and are suitable for in-field screening, the critical factor is that the antibodies are required to be sensitive and specific. Lateral flow assays show higher false-positive rates than PCR (Bohaychuk et al., 2005; Yun Wang & Salazar, 2016), and culture is still required as a step before the identification.

Due to the requirement of high signal to noise ratio of the pathogen to background food matrix and inhibitory compounds, conventional culturing methods such as broth or agar plate enrichment have remained the gold standard (López-Campos et al., 2012; Stevens & Jaykus, 2004; Taskila et al., 2012; Yun Wang & Salazar, 2016). However, non-culture methods have been developed to separate target bacterial pathogens from food samples, removing inhibitors from matrices and concentrating the pathogens to a detectable level (Yun Wang & Salazar, 2016). Physical separation methods such as centrifugation and filtration have been commonly used due to their simplicity and the possibility of processing large-volume samples. However, these methods often require a series of concentration and washing steps which results in a decrease in target bacteria recovery (Fukushima et al., 2007; Joshi et al., 2009; Stevens & Jaykus, 2004; Yun Wang & Salazar, 2016; Wu, Duan, Shi, Fang, & Wang, 2014) and are not able to isolate very few pathogens from complex media. Immunomagnetic separation methods have the advantage of specific removal of the target from the food matrices and reaction inhibitors with a low separation time, but high affinity and

specificity of ligands to the target is necessary and the size, surface area, and magnetic properties of the particles can affect molecular attachment and the efficacy of the separation(Horák et al., 2007; Irwin et al., 2002; Jiang et al., 2011; Stevens & Jaykus, 2004; Tu et al., 2003; Yun Wang & Salazar, 2016). Not only that, in complex matrices, limit detection of pathogen remains in the 10^3–10^4 cfu/g range (Yun Wang & Salazar, 2016). Apart from PCR and immunoassays, oligonucleotide microarray technology has been advantageous due high sensitivity and specificity to target sequences as well as multiplex detection capabilities (El-Boubbou et al., 2007; Horák et al., 2007; Irwin et al., 2002; Jiang et al., 2011; Stevens & Jaykus, 2004; Tu et al., 2003). However, these assays also require production of oligonucleotide probes and fluorescent labeling of target DNA sequences, which are expensive (Yun Wang & Salazar, 2016). Microfluidic technology has been important as portable diagnostic tools for detection studies, but these technologies also require target bacteria or DNA extraction, purification, and concentration into small volumes before PCR or any other detection mechanism is utilized (Bhunia, 2014; Dwivedi & Jaykus, 2011; Fluit et al., 1993; Golsteyn Thomas et al., 1991; Lui et al., 2009; Mairhofer et al., 2009; Rossen et al., 1992; Sharma & Mutharasan, 2013; Yixian Wang et al., 2013; Yun Wang & Salazar, 2016). Other biosensors such as surface plasmon resonance (SPR), impedance, capacitive, voltammetric, and potentiometric biosensors have been utilized to detect pathogens in food matrices without cultural enrichment; however, sensitivity for target, reliability of results, and stability of biomaterials are still much of a concern with these technologies (Yun Wang & Salazar, 2016).

Due to the increasing complexities and limitation of culture and non-culture methods, there is still an unmet need of a diagnostic platform that can achieve high sensitivity with background noise from food samples without culture or other extraction/separation methods in a rapid turnaround time. As recommended by the World Health Organization, biosensor diagnostic tools must be developed to be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable (Vidic et al., 2019).

To address these challenges, here we introduce a new reaction method called the "biphasic" amplification method, in which pathogen detection occurs directly from complex solid food matrices without pre-enrichment or culture of target pathogens before the identification. We have used this technique for bacterial detection in ground beef samples. In this process, solid ground beef samples are homogenized as per the standard process. An aliquot of the resulting meat matrix is heated at 95°C and dried, resulting in a solid phase at the bottom of the reaction tube. Isothermal DNA amplification reagents are introduced creating a liquid phase above the solid phase. Our technique results in a two-phase reaction, a "biphasic" amplification reaction, in which the solid phase at the bottom is of the dried food matrix that does not remix with the top supernatant phase where the fluorescent amplicons are concentrated, allowing for a high signal to noise ratio and greater fluorescence change. We demonstrate our platform by efficiently amplifying cell-free *E. coli* O157:H7 with a detection limit of 2.5 copies in 30 mg of meat sample (~83.3 copies/g) as well as *E. coli* O157:H7 bacteria with a detection limit of 1 cfu/30 mg of meat sample (~33 cfu/g). When compared to the standard control of the loop mediated isothermal amplification (LAMP) reaction with 30 mg of food matrix, our platform showed a sensitivity that was three orders of magnitude higher than that of the control.

2 | METHODS

2.1 | DNA and bacteria

To evaluate the detection capabilities of using the biphasic approach, bacterial DNA or pathogen was directly spiked into homogenized food samples. Purified genomic DNA of *Escherichia coli* (O157:H7), NR-4629, was obtained from BEI Resources. Genomic DNA vials were aliquoted and stored at −80°C until use. Appropriate stock volumes were either used for direct experimentation or diluted to the required concentration in 1x phosphate-buffered saline (PBS). For experiments using pathogenic bacteria, *E. coli* (O157:H7), NR-4356 was obtained from BEI Resources. These bacterial glycerol stocks were stored at −80°C until use, and the culture protocol of bacteria is explained in the next section.

2.2 | Bacterial culture

Luria-Bertani broth and agar was acquired from the University of Illinois Urbana-Champaign Cell Media Facility, and used for *E. coli* culture. The bacteria were inoculated in broth and grown for 16 h overnight in 37°C, after which PBS stocks were prepared.

PBS stocks of bacterial pathogens were prepared and stored as stated in the work of Liao and Shollenberger (Liao & Shollenberger, 2003). Briefly, from the overnight culture, 250 µl was centrifuged at 5000*g* for 10 min to create a bacterial pellet. After washing the pellet twice with 1 × PBS, it was finally diluted in 1 ml of PBS, aliquoted, sealed with parafilm, and stored in the dark at room temperature. PBS stocks were not used for more than 4 days post-culture. Before experimentation, appropriate PBS dilutions of the stock were plated on agar to know the exact bacterial concentration in the stocks. Based on the counts, the dilutions of the bacterial stocks were made in 1 × PBS buffer for the biphasic experiments.

2.3 | Meat sample preparation and drying

Ground beef (85% lean, 15% fat) was obtained from a local grocery store. Twenty-five grams of sample was weighed and added into homogenizer bags and stored in −20°C until usage for experiments. Before experimentation, frozen samples were thawed in 4°C for 6 h before further processing. Each sample was homogenized with 225 ml of PBS for 1 min in a conventional blender. The sample was then divided into 25 parts of 10 ml homogenate. For each experiment, the 10 ml homogenate was centrifuged at 3200*g* for 3 min. This concentrated the meat debris at the bottom of a 15 ml tube, after which the supernatant was discarded. Ten-

fold serial PBS dilutions of DNA or bacterial stocks were prepared for the experiments. After spiking the sample with DNA or bacterial pathogen, 200 µl nuclease-free water was added to dilute the debris such that addition of 66 µl of meat mixture into PCR reaction tubes would result in 30 mg of dried meat weight. The spiked and diluted sample was then distributed into 0.2 ml PCR reaction tubes. A total of ~20 PCR tubes would be required to distribute the 1.5 ml wet food matrix in the 15 ml tube. These samples were then dried in a heater at 95°C for 20 min.

2.4 | LAMP reactions

The LAMP assays were designed to target the malB gene for *E. coli*. The primer sequences for the assays were synthesized by Integrated DNA Technology (IDT). Primer sequences targeting the conserved malB gene were acquired from Hill et al. (2008).

The optimized LAMP assay is comprised of the following components: 1.025 mM dNTPs (New England Bioabs), 4 mM $MgSO_4$ (New England Biolabs), 1× isothermal amplification buffer (New England Biolabs), and 0.29 M Betaine (Sigma-Aldrich), 0.47 U/µl Bst 2.0 WarmStart DNA Polymerase (New England Biolabs), 2 mg/ml bovine serum albumin (BSA) (New England Biolabs), and 0.74× EvaGreen Dye (Biotium), a dsDNA intercalating dye. These individual components were stored according to manufactural instructions and a mix including all components was created fresh before each reaction. A 0.74 × primer mix was also added such that the final concentration of individual primer components in the reaction were 0.15 µM F3 and B3, 1.17 µM FIP and BIP, and 0.59 µM LF and LB primers. The final reaction volume was 96 µl.

All LAMP reactions were performed in 0.2 ml PCR reaction tubes using the Eppendorf Mastercycler® realplex Real-Time PCR System. The tubes were incubated at optimized temperature of 70°C for 60 min in the thermocycler, and fluorescence data were recorded every minute during the reaction. Four replicates were done for each experimental condition.

2.5 | Optimization reactions

Several optimizations were done to develop the biphasic protocol for the detection of bacterial cell-free DNA in unpurified meat samples. To understand the amount of meat debris our biphasic reactions can tolerate, we conducted a series of reactions, in which the amount of meat sample (debris) dried was titrated from 1 mg to 50 mg of meat sample per reaction. For these reactions, post spiking the DNA in the meat sample, different volumes of nuclease-free water were to dilute the debris such that the wet amount aliquoted into each 0.2 ml PCR tube was equivalent to 1 mg to 50 mg dried weight, as required for the experiment. The aliquoted debris was then heated at 95°C until the debris was dried.

The second set of optimizations required titrations of reaction volume. Considering 30 mg of meat sample debris dried in our reaction tubes, the reaction volumes were tittered between 48 and 80 µl. The final reaction composition and concentrations of all reagents were the same as mentioned above, but the final reaction volume was varied.

Further optimizations were done with 30 mg of meat sample debris and 96 µl of reaction volume. Keeping all other reagent concentrations as mentioned above, optimization reactions were done to vary $MgSO_4$ concentration (6–10 mM Mg final concentration), primer concentration (0.5× or 0.74×), and BSA concentrations (1–2 mg/ml final in the 96 µl reactions). The 0.5× primer composition was 0.10 µM F3 and B3, 0.80 µM FIP and BIP, and 0.40 µM LF and LB. Finally, a series of reactions were also conducted in which incubation temperature of the reactions was varied between 65°C and 70°C.

Mixed meat sample and debris LAMP reactions were conducted as a standard control. The format of these reactions was as follows: In reaction tubes with the wet amount equivalent to 30 mg of dried meat sample, LAMP reaction reagents were added and mixed in the final concentrations mentioned above with a total volume of 96 µl.

Ten-fold serial dilutions of the template or bacteria in 1 × PBS buffer were amplified using the optimized protocol to determine the working range of our developed assays.

2.6 | Amplification data analysis

The raw fluorescence curves were plotted using GraphPad Prism and analyzed using a MATLAB script to develop the threshold bar graphs. The threshold time for each curve was taken as the time required for the curve to reach 10% of the total intensity. The amplification threshold bar graphs are shown as a mean and standard deviation of amplified replicates for each sample.

2.7 | Scanning electron microscopy (SEM) analysis

Dried meat samples processed with and without thermal lysis were fixed in PCR tubes using 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate-buffered saline. The samples were rinsed with PBS post-fixation and prepared for critical drying in 100% ethanol. These samples were kept in 4°C until the critical drying process. After critical drying, the meat samples were prepared for SEM imaging by first sputter coating with gold–palladium using the Desk-II TSC instrument. The FEI Quanta 450 ESEM imaging instrument was used to take SEM images at different magnification for each of the samples. Image segmentation of SEM images were done using a constant threshold to calculate the porosity of the sample in MATLAB.

2.8 | Simulation data

To perform the simulations for the biphasic reactions, we modeled the system as a continuum. Dividing the experimental procedure into two main steps, the model consisted of:

- Diffusion of the BST enzyme and the raw materials such as dNTP in the porous dried meat matrix through the microchannels to reach the target DNA.

- Amplification reaction of replicating the target DNA sequence of the pathogen by adding dNTPs in the presence of BST polymerase.

The continuum simulations modeling the system reflected the dimensions of the experimental setup in the order of millimeters. We first created different geometries modeled after the different drying patterns of the meat matrix in the standard 0.2 ml PCR tube for the biphasic reaction. To model the porous network within the matrix, image segmentation in MATLAB of SEM images of the dried matrix in PCR tubes were used to calculate the porosity of the samples. To characterize the difference in porosity, a constant threshold was used for image segmentation of pre and post thermal lysis sample SEM images. Thereafter, using the continuum transport equation (Equation 1), we simulated the flow and dynamics of the system in OpenFoam setting a mesh size of 1 µm. Equation (1) shows the time-dependent convection-diffusion-reaction equation where c_i is the concentration of species i, U is the velocity of the fluid in consideration, D_i is the diffusion coefficient of species i and rate of the reaction, R_i. Equation (2) shows that the rate of the reaction is dependent on the time constant, τ, of the reaction:

$$\frac{dc_i}{dt} - \nabla \cdot (U \cdot c_i) - \nabla \cdot (D_i \cdot \nabla c_i) = R_i, \quad (1)$$

$$R_i = \frac{\ln 2}{\tau} \times c_i \times 2^{\tau}. \quad (2)$$

For the simulations, pressure was maintained at 1 atm and the velocity of the fluid (U) was maintained at zero due to the fact that there are no pressure gradients, and the electro-osmotic velocity is negligible. To model the experimental reaction, the temperature was maintained at 65°C for 60 min, and consisted of two species, including the BST polymerase enzyme and the target pathogen DNA. We assumed these species to be in aqueous medium. Though the actual experimental setup consists of a buffer solution, we assumed that the charged ions in the solution do not affect the diffusion of the enzyme and dNTP into and through the matrix. The Debye length, which measures the persistence the of charged species' electrostatic effect, was calculated to be close to 5 nm for this system. However, the pores in the dried meat matrix formed post-lysis were seen to be in the order of micrometers (µm) from the SEM images. So, the electrostatic effects were considered negligible.

3 | RESULTS AND DISCUSSIONS

3.1 | Biphasic assay design for cell-free DNA detection of *E. coli* in ground beef sample

The process flow for sample processing and biphasic amplification from cell free DNA in ground beef samples begins by sampling 25 g of ground beef. This sample is homogenized with 225 ml PBS in a commercial blender, according to the standard procedure conducted by the USDA (Andrews & Hammack, 1998; Detection Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* STECs from Meat Products and Carcass and Environmental Sponges, 2019). The homogenized sample is divided into 25 parts (10 ml aliquots). A single 10 ml aliquot of homogenized sample is then centrifuged at 3200*g* for 3 min, after which the supernatant is discarded. The homogenized sample is spiked with pathogen DNA and 200 µl of H_2O for ease of processing. The total volume of the wet food matrix at this stage is 1.5 ml which is distributed into ~20 standard 0.2 ml PCR tubes (66 µl each) followed by rapid drying of the sample in a heater (95°C, 20 min) (Figure 1a). SEM characterizations of the dried meat matrix in PCR tubes were done and analyzed through image segmentation to calculate the matrix porosity. SEM images show that after drying, the dried food matrix has a porosity of ~55.3% (Figure 1b). Next, LAMP amplification buffer, primers and polymerase are added, and the reaction is performed at 70°C for 60 min, and fluorescence change due to amplification of the target is measured in a thermocycler. The solid dried food matrix allows for a biphasic system where the food matrix remains as a substrate, further allowing high signal to noise and large fluorescence change to be observed in the clear supernatant phase during amplification. It is important to note that any additional thermal lysis steps were not included in this process. As can be seen from Figure c,d, adding a thermal lysis step before the reaction gave similar porosity results (57.6%), in comparison to that we observed without thermal lysis. It is important to notice that the porosity of the matrix remains high without any thermal lysis. This is potentially an intrinsic property of the matrix itself.

3.2 | Biphasic assay tolerance of complex food matrix

Towards designing the biphasic assay, we first validated our selected primers for *E. coli* bacteria amplification. For LAMP detection of *E. coli*, we used previously published LAMP primers to amplify a conserved gene (mal B gene) found in a majority of infectious *E. coli* strains (Hill et al., 2008). Supplementary Figure 1 shows the amplification and the threshold times for *E. coli* DNA in 16 µl buffer reactions, and the limit of detection was found to be 2 copies per reaction. Because the primers showed high sensitivity for low copy detection, we proceeded to characterize the amount of meat sample for which we could achieve clear amplification signals. To start, we spiked and aliquoted homogenized meat sample such that each reaction contained 1, 5, 10, 30, or 50 mg of sample with 2500 copies of *E. coli* DNA. The purpose of this was to experimentally determine the amount of sample we can process without negative amplifications or causing low signal to noise ratios. After drying at 95°C for 20 min, the biphasic reaction was performed, and the amplification curves and threshold times are shown in Figure 2a,b. We observed that 2500 copies of *E. coli* could be detected in up to 30 mg of meat sample without increasing the amplification time while also delaying negative amplifications to later than 43 min.

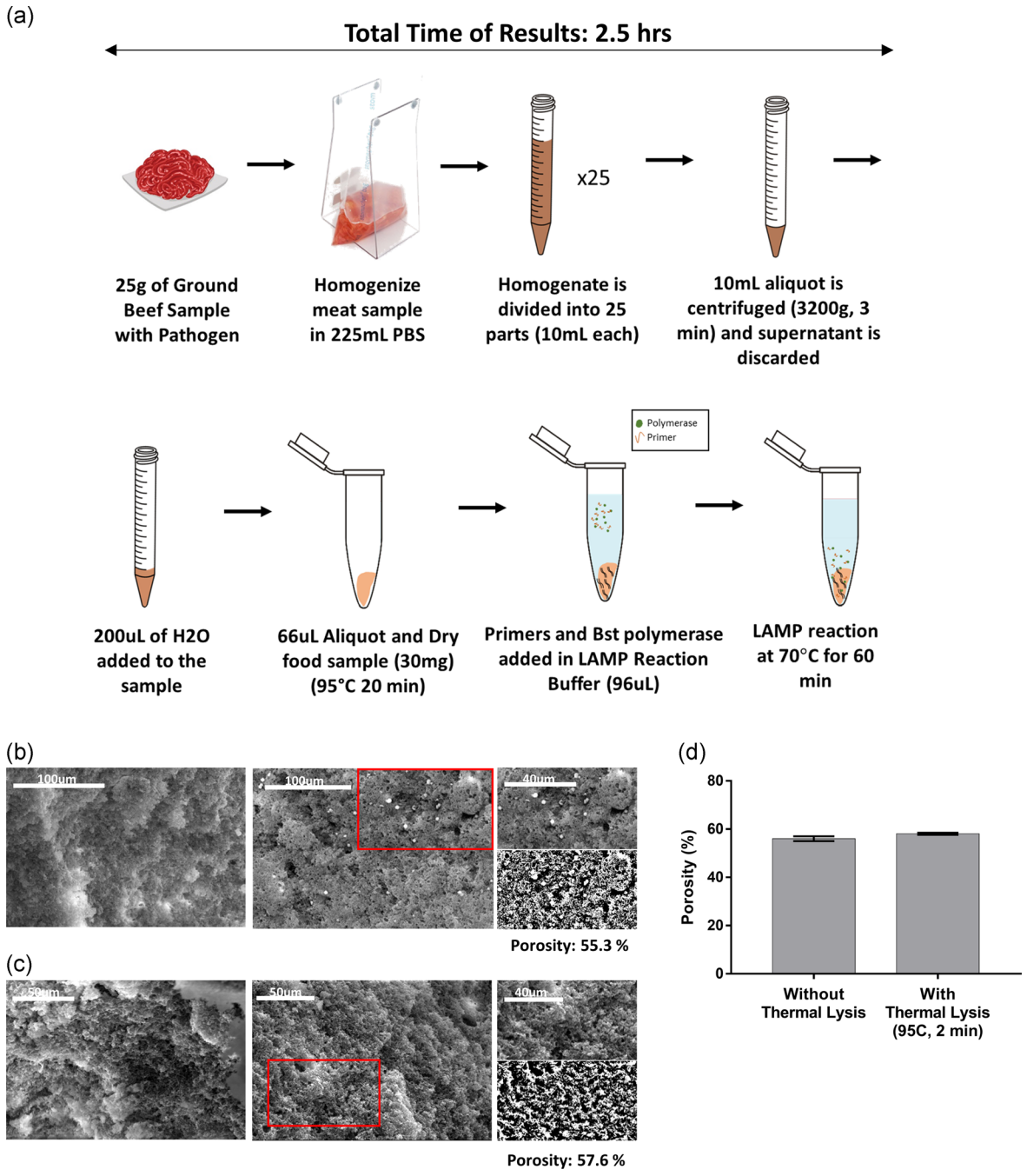

FIGURE 1 Biphasic reaction schematic and analysis of food matrix pre- and post-thermal lysis. (a) Process flow schematic of food matrix biphasic reaction. Twenty-five grams of ground beef sample is homogenized in 225 ml PBS and divided into 10 ml aliquots. Each aliquot is centrifuged at 3200*g* for 3 min to concentrate the pathogen-contained debris before the supernatant is discarded. Two hundred microliters of H_2O is added to the sample to make a final volume of 1.5 ml of wet food matrix and then into distributed (6 µl) into standard 0.2 ml PCR tubes and dried at 95°C for 20 min. Finally, LAMP reaction reagents including primers and polymerase are added for the final biphasic reaction to occur at 70°C for 60 min. (b) SEM at various magnification of the dried food matrix. Image segmentation data shows the porosity of the resulting solid cake is 55.3%. (c) SEM of the food matrix post a thermal lysis performed at 95°C for 2 min before adding primers and polymerase. Image segmentation of each sample shows porosity of the food matrix due to thermal lysis increased to 57.6%. (d) Dried food matrix porosity with and without thermal lysis. The bar graphs show mean and standard deviation (n = 3 samples). LAMP, loop mediated isothermal amplification; BS, phosphate-buffered saline; PCR, polumerase chain reaction; SEM, scanning electron microscopy

To increase sensitivity of reactions, we considered the possibility of adding a thermal lysis step to increase the porosity of the dried meat sample. We hypothesized that by rehydrating the sample with buffer and heating it to 95°C, air bubbles trapped within the matrix would expand, allowing for an increase the porous network. So, we performed a reaction with an added thermal lysis step at 95°C for 2 min post drying of the meat sample. Figure 2c,d shows the amplification and threshold curves, where we see that the added thermal lysis step caused false positive amplification as soon as 23 min for 1 mg of meat sample and 35 min for 30 mg of meat sample. Because the thermal lysis step does not improve matrix porosity (Figure 1) and this step also increases false positive amplification (Figure 2), we decided not to add the additional thermal lysis step for our final protocol. False positive amplifications most probably occurred with the extra thermal lysis step, as it could release high amounts of background DNA from the meat sample itself. It is important to consider that complex meat matrices often interfere with nucleic acid amplification not only due to its inclusion of inorganic particles, biochemical compounds such as fats, proteins, polysaccharides, and indigenous microflora, but also the high

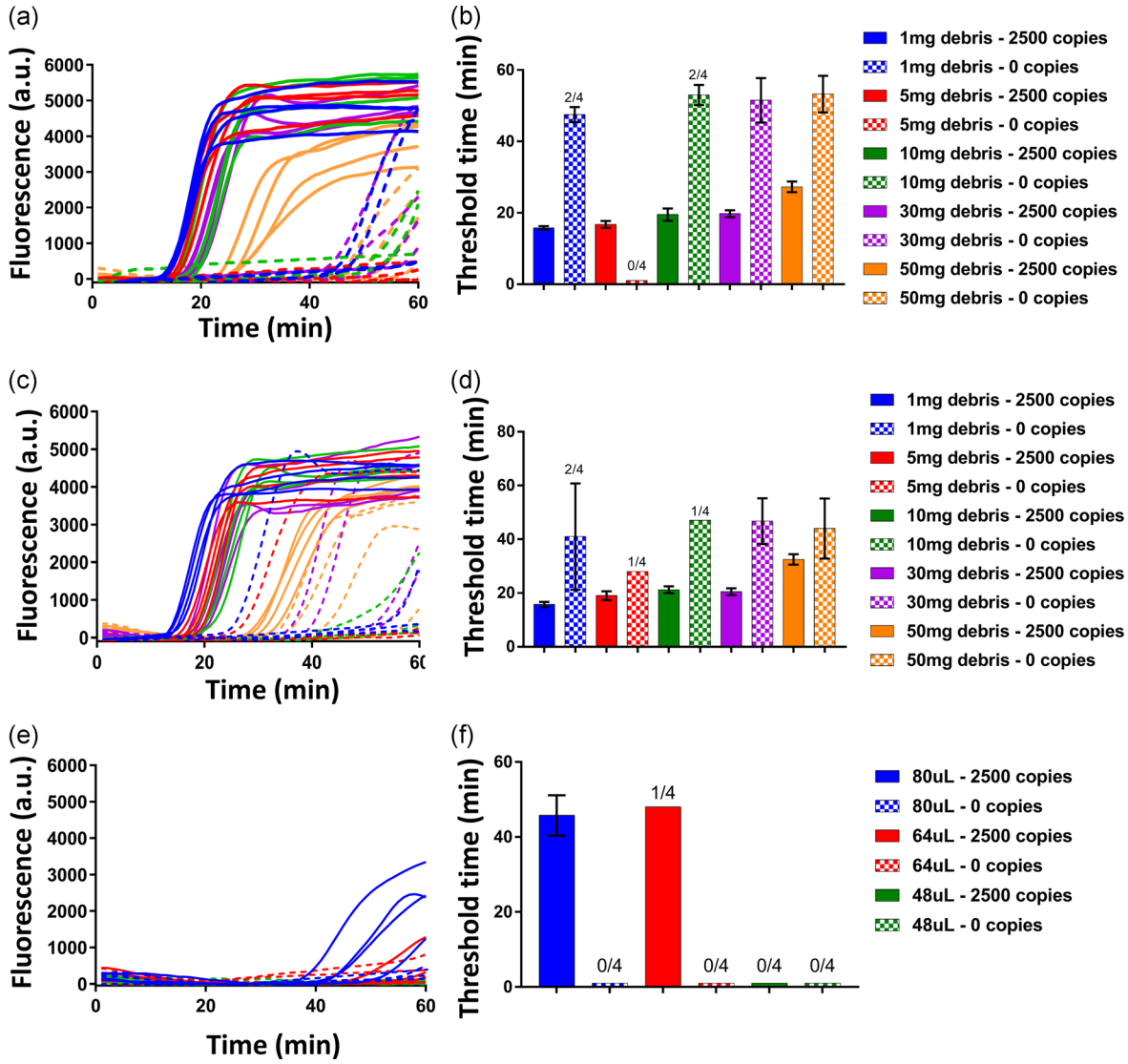

FIGURE 2 Debris and reaction volume characterization in biphasic format. (a,b) Raw fluorescence data and amplification threshold times of biphasic reactions with *Escherichia coli* DNA spiked in varying amounts of debris. Biphasic LAMP reactions did not include thermal lysis at 95°C, and reaction volumes increased based on debris amount. Reactions for 30 and 50 mg debris were done with 96 µl of reaction volume. (c,d) Raw fluorescence data and amplification threshold times of biphasic reactions with *E. coli* DNA spiked in varying amounts of debris. Biphasic LAMP reactions did include thermal lysis step at 95°C for 2 min before primers and polymerase were added to the reaction. Total reaction volumes varied based on debris amount. (e,f) Raw fluorescence data and amplification threshold times of biphasic reactions with *E. coli* DNA spiked in 30 mg of meat sample debris. Biphasic LAMP reactions did not include thermal lysis step at 95°C for 2 min. Total reaction volumes were varied to see if reactions were possible at higher reaction to debris ratios. Reactions done with 96 µl of total reaction volume are best with 30 mg of debris. The bar graphs show mean and standard deviation. LAMP, loop mediated isothermal amplification

amount of background DNA that is inherent in meat samples (Chen et al., 2013; Cho & Ku, 2017). Meat consists mainly of skeletal muscle tissue and contains DNA. To be more specific, 40%–45% of body weight is muscle mass for most mature mammals. Literature shows that there are 4E10 cells per kilogram of muscle mass (Cheek et al., 1971) equivalent to about 4E4 copies of DNA per milligram of meat to act as background noise against our *E. coli* DNA spiked samples. Our biphasic assay is robust and can allow for detection of *E. coli* DNA within a background noise of 1.2E6 copies of DNA from the 30 mg of meat itself.

As a last step in evaluating the signal to noise ratio of our biphasic assay, we titrated the final reaction volume to sample ratio. Our final reaction volume was 96 µl in the above assays. Figure 2e,f and shows the amplification curves and threshold results of reactions performed with 48 to 80 µl of final reaction volume without compromising the final concentrations of the reagents. We hypothesized that decreasing the final reaction volume could potentially decrease negative amplification that occurs due to primer dimerization, increasing the possibility of specific interactions between the primers and target. We observed that 80 µl final volume reactions showed delayed positive amplifications starting at 40 min for 2500 copies of *E. coli* DNA per reaction. Detection capabilities of 2500 copies of *E. coli* DNA decreased as the sample mass to reaction volume ratio increased. Hence, we maintained 96 µl as the final reaction volume of our assay.

To further understand the mechanism of biphasic amplifications in the food matrices, we performed simulations of the biphasic LAMP reaction on the dried food matrix. We recreated the drying patterns and porosity of food matrix as captured in SEM images (porosity 55.3%) and simulated the diffusion of Bst enzymes through the porous network to access the DNA and start amplification. The location of the pathogenic DNA was varied, and the simulation was performed for different distances between the enzyme and DNA location (Figure 3). We observed that only the enzyme and other materials such as dNTPs and primers diffuse into the matrix and reach the DNA, but the pathogen DNA does not diffuse out, confirming our experimental results. This is because the diffusion coefficient of DNA (2.82E−14 m^2/s) is three order of magnitude smaller than that of enzymes (5.63E−11 m^2/s) and other components of the reaction (Lukacs et al., 2000) (Figure 3). Mathematically, we can calculate the distance that DNA and other components can diffuse through the porous network during the time of the reaction. The equation $L = \text{sqrt}(D^*t)$ can be used, where L is the distance in meters that DNA can diffuse, t is the reaction time in seconds, and D is the diffusion coefficient. Given this equation, we can see that in a 60 min LAMP reaction, DNA would only diffuse ~10 µm through the matrix. In comparison, the enzymes (which are higher in concentration as well) diffuse ~450 µm through the matrix. In the simulation, we assume that that enzyme begins at 1420 µm from the DNA. However, experimentally, since enzymes and primers are equally distributed above the matrix, distance traveled by the enzyme is less. Nevertheless, it is clear that the diffusion of DNA would be very minimal during the reaction and the amplification would start in the food matrix itself. The concentration versus time curves for 1 copy and different locations within the matrix is shown as well (Figure 3).

3.3 | Optimization of biphasic LAMP reactions

For optimizations of the biphasic LAMP reactions to detect *E. coli* DNA in complex food samples and to prevent false positive amplification, we titrated the magnesium concentration, the reaction incubation temperature, and the primer and BSA concentration of our reaction. Divalent Mg ions are cofactors to DNA polymerases in an amplification reaction and are required for nucleotide transfer and catalysis of the 3′ to 5′ exonuclease activity associated with replicative DNA polymerases (Kumar Vashishtha & Konigsberg, 2018). We titrated the final magnesium ion concentration from 6 to 10 mM and the amplification results are shown in Figure 4a,b. We observed that as magnesium ion concentration increased, the threshold time for amplification also increased, with the biphasic assay with 10 mM Mg ions showing positive amplification of 2500 copies at approximately 38 min. The biphasic reaction with 6 mM Mg ions showed positive amplification occurring before 20 min and negative amplification after 40 min. The next parameter optimized was the LAMP reaction temperature. Our hypothesis was that increasing the temperature may increase the specificity of the primer annealing to the target region and subsequently decrease negative amplification. Figure 4c,d shows the amplification results of reactions that were performed at 65°C, 68°C, and 70°C incubation temperatures. The amplification curves and threshold times show that our biphasic reactions perform best at 70°C with positive amplification occurring at around 25 min and decreased negative amplifications. Only one of four replicates of the negative control amplified around 52 min. At lower temperatures, all four replicates of negative control amplified for each case. Finally, four combinations of primer and BSA concentrations in the reaction were optimized. Increased primer concentration can cause primer dimerization which can result in negative amplifications (Meagher et al., 2018). Figure 4e,f shows the amplification curves and threshold times of reactions where primers were reduced from 0.74× final concentration (0.15 µM F3 and B3, 1.17 µM FIP and BIP, and 0.59 µM LF and LB primers) to 0.5× final concentration (0.10 µM F3 and B3, 0.80 µM FIP and BIP, and 0.40 µM LF and LB primers). Decreasing primer concentration was effective in eliminating negative amplifications but also caused positive amplification threshold time to increase by at least 5 min. BSA is a common reagent used to decrease nonspecific binding. Increasing BSA concentration from 1 to 2 mg/ml also eliminated negative amplification but caused positive amplification of 2500 copies to increase to about 28 min. From these results, we decided that our final reactions would be performed with 0.74× concentration of primers and 2 mg/ml BSA.

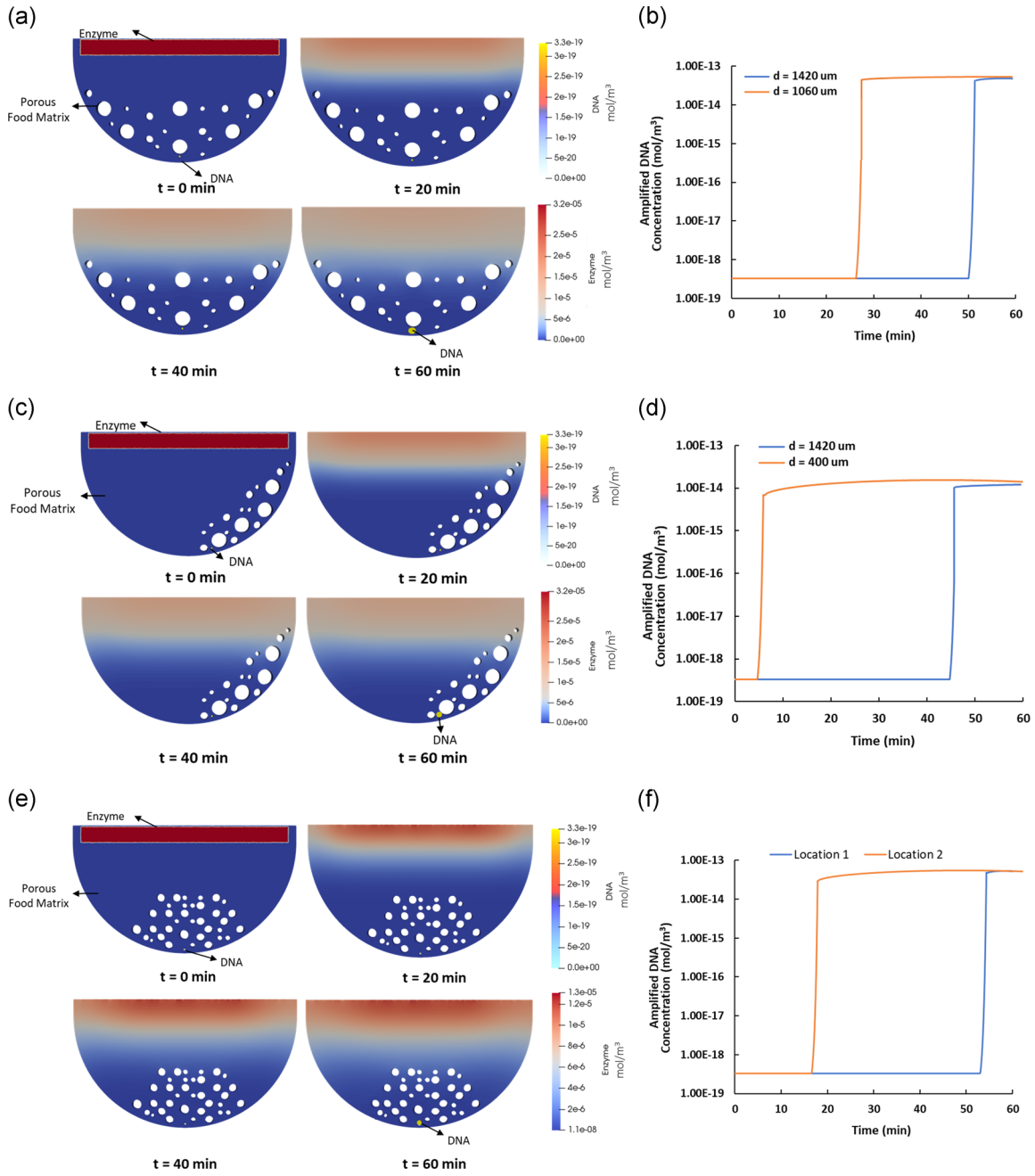

FIGURE 3 Simulation characterization of different matrix drying patterns. (a,c,e) Simulation of BST polymerase reaching target DNA in food matrix, where the target is 1420 µm from the enzymes at the starting of the reaction. The enzyme diffuses into the food matrix and reaches the DNA after 40 min. The reaction is complete by 60 min. (b,d,f) Concentration of DNA as a function of time using the empirical rate equation for 1 copy of DNA, where the DNA is located at two different distances from the enzyme at the starting of the reaction. BSA, bovine serum albumin

3.4 | Detection limit of *E. coli* DNA in 30 mg of ground beef sample

Based on the optimizations, for a 30 mg meat sample, the LAMP reaction protocol included conducting the reaction with 6 mM final Mg ion concentration, 0.74× primer concentration, and 2 mg/ml BSA at an incubation temperature of 70°C. Using the optimized protocol and to evaluate the range and limit of detection of our biphasic assay for cell free DNA, we spiked serial dilutions of *E. coli* DNA in homogenized ground beef samples and performed reactions. The amplification fluorescence curves, and the threshold times demonstrate a detection limit of 2.5 copies/30 mg of meat sample (Figure 5a,b). It should be noted that only one of four amplifications occur for the 2.5 copies. The number of positive amplification occurrences is equivalent to the number of samples measured. Due to Poisson sampling statistics, there is a possibility of not sampling 2.5 copies in every replicate. So, for the detection of low copy numbers, our number of positive amplifications is a function of the sampling.

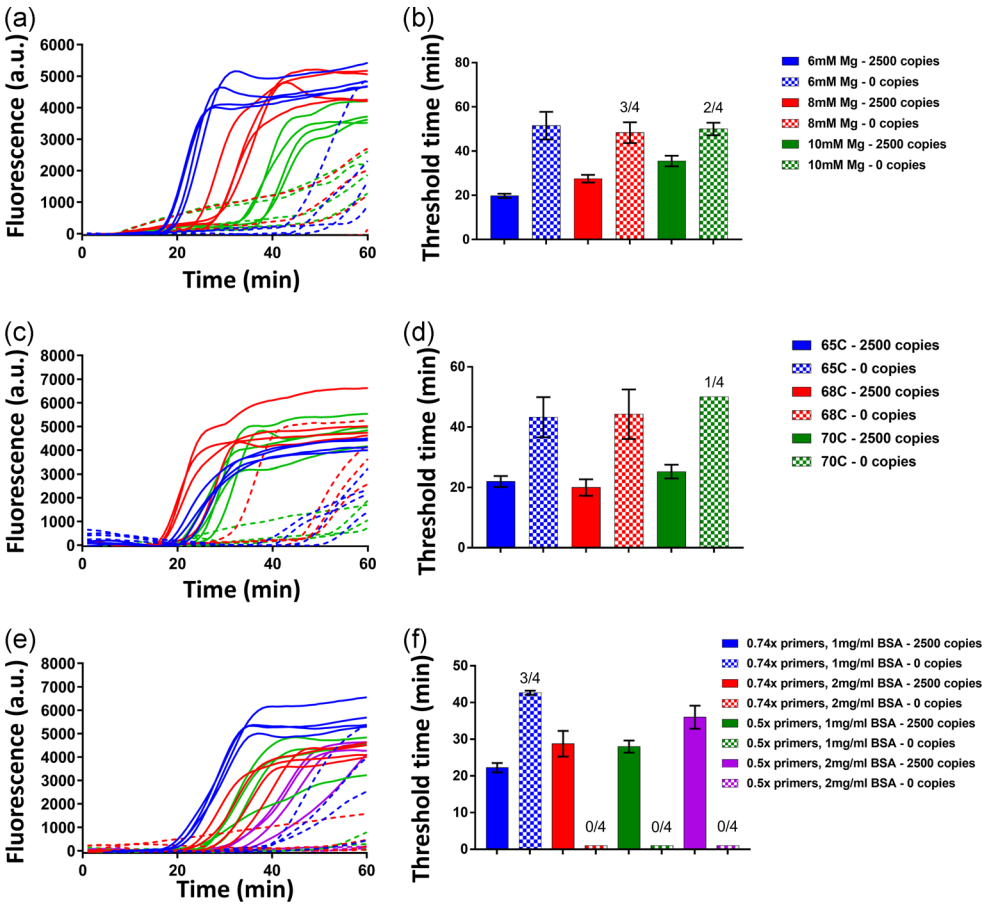

FIGURE 4 Optimizations of biphasic reactions with *Escherichia coli* DNA spiked in 30 mg debris. (a,b) Raw fluorescence data and amplification threshold times of biphasic reactions done with three different concentrations of Mg in the final reaction mix. Increasing Mg concentration delayed positive amplifications and decreased the time difference between positive and negative amplification. Reactions with 6 mM concentration showed best difference between positive and negative amplification reactions. These reactions were conducted at 0.74× final concentration of primers, 1 mg/ml final concentration of BSA and at 65°C. (c,d) Raw fluorescence data and amplification threshold times of biphasic reactions conducted at three different temperatures. These biphasic LAMP reactions were conducted with 6 mM Mg and 0.74× final concentration of primers and 1 mg/ml BSA. Reactions conducted at 70°C showed later negative amplification than the 65°C or 68°C reactions, without much delay of positive amplifications. (e,f) Raw fluorescence data and amplification threshold times of biphasic reactions with varied primer and BSA concentrations. In reactions were conducted with 6 mM Mg at 70°C. In reactions with 0.74× primer concentration combined with 2 mg/ml BSA, negative amplifications were delayed after 60 min. In reactions with 0.5× primer concentrations, negative amplifications were delayed after 60 min. Positive amplification times increased with increased BSA concentration. All bar graphs show mean and standard deviation. BSA, bovine serum albumin; LAMP, loop mediated isothermal amplification

Thus, our LOD can be considered to be 2.5 copies/30 mg of meat sample (~83 copies/g of meat sample).

Next, we compared the sensitivity of our biphasic assay with the standard meat processing methodology using LAMP protocol without the biphasic protocol. This reaction was performed with 30 mg of meat sample with *E. coli* DNA spiked at different concentrations and LAMP reaction mix (final reaction volume 96 µl) to allow for a direct sensitivity comparison with our biphasic reactions. The amplification fluorescence curves and threshold times in Figure 5c,d shows that the detection limit is greater than 2500 copies per 30 mg of sample. This is essentially three orders of magnitude greater than the detection limit of our biphasic reaction format. This comparison highlights that the LAMP reaction can be typically inhibited by the proteins, fats, and other inorganic particulates in the food matrix when they are dispersed in the final mixed reaction. This also demonstrates that the drying of the sample food matrix causes an inactivation of the inhibitory factors and thus is a very important advantage of our biphasic reactions.

3.5 | Detection of *E. coli* pathogens in food matrix in the biphasic assay

Finally, to translate our biphasic reaction module to detect pathogens in food matrix, we carried out experiments in which *E. coli* bacterial pathogens were spiked in the ground beef sample. Culture and growth protocols for pathogens can be found in the methods section. Figure 6a,b shows that the limit of detection for *E. coli* pathogens is

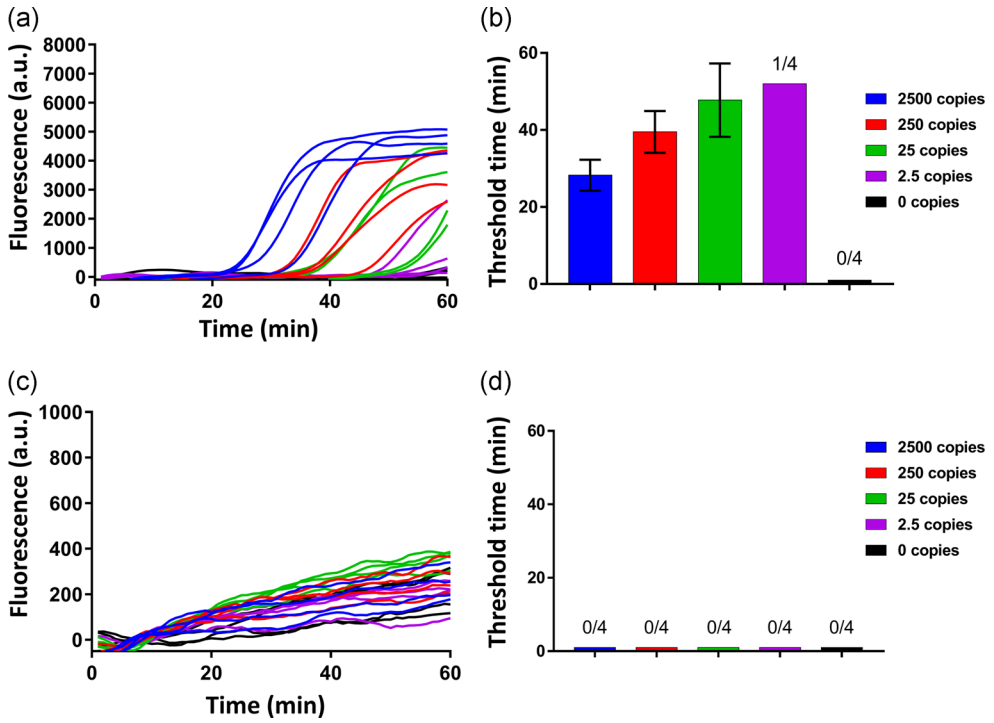

FIGURE 5 Detection limit of *Escherichia coli* DNA spiked in 30 mg debris using optimized reaction protocol in biphasic and mixed reaction control format. (a,b) Raw fluorescence data and amplification threshold times of biphasic reactions with *Escherichia coli* DNA. This reaction included 6 mM Mg final, 0.74× primers, and 2 mg/ml BSA final concentration sand was conducted at 70°C. (c,d) Raw fluorescence data and amplification threshold times of mixed reactions with *E. coli* DNA. Debris was not dried before adding LAMP reaction mix. The LAMP reaction was conducted with the same parameters mentioned above and final reaction mix was mixed well with the debris. Fluorescence data shows that amplification could not be detected even when 2500 copies of DNA were available in the reaction. BSA, bovine serum albumin; LAMP, loop mediated isothermal amplification

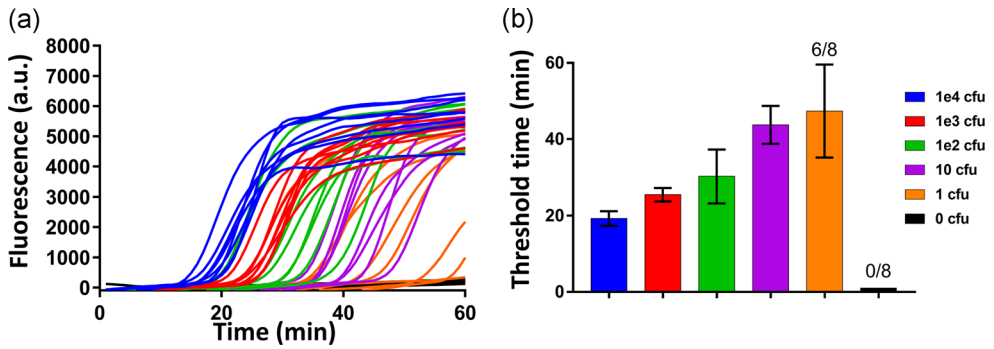

FIGURE 6 Detection limit of *Escherichia coli* pathogens spiked in 30 mg debris using optimized biphasic reaction. (a,b) Raw fluorescence data and amplification threshold times of biphasic reactions with *E. coli* pathogens. This reaction included 6 mM final, 0.74× primers, and 2 mg/ml BSA final concentration sand was conducted at 70 °C. Pathogen lysis occurs during the drying process of the debris at 95 °C for 30 min. Detection limit seen is 1 cfu/30 mg of debris which is equivalent to ~33 cfu/g when scaled up. BSA, bovine serum albumin

1 cfu/30 mg of meat sample. This is equivalent to 33 cfu/g of sample. It should be noted that 6/8 amplification occurred for 1 cfu pathogens due to sampling. We also note that an extra thermal lysis was not required for bacterial lysis in our biphasic reaction as the drying of the sample with bacteria occurred at 95°C for 20 min, enough for lysis of the bacterial to occur.

4 | CONCLUSION AND FUTURE DIRECTIONS

Our approach presented here provides an alternative to conventional pathogen detection in food, where we rapidly dry the food matrix with pathogen DNA to create a dried food matrix and utilize

the generated porous network to direct amplification enzymes and primers to diffuse into the matrix, access the pathogen target, and initiate the amplification from inside the dried food matrix. This precludes any need for conventional bacterial enrichment or nucleic acid purification. The drying of the food matrix allows for two phases to be formed, our "biphasic" approach. The dried solid phase inactivates the inhibitory compounds and indigenous microflora and prevents them from physically interfering from in the reaction. This allows for fluorescent amplicons post-amplification to accumulate in the clear supernatant phase, permitting a high signal to noise and fluorescence change in our reactions. We have demonstrated this biphasic reaction approach using *E. coli* DNA in ground beef samples and show that 2.5 copies can be detected in 30 mg of dried food matrix per reaction. We compared our biphasic reaction protocol to a mixed food matrix reaction without separation of the inhibitory components or purification of target nucleic acid and found that these reactions can have more than three orders of magnitude higher limit of detection. Further in our biphasic format, we showed a detection limit of 1 cfu/30 mg of dried food matrix for *E. coli* bacteria.

Our reaction modality allows for several advantages. First, it is a culture independent method that takes less than a few hours to get results. Minimal sample processing assures the integrity of DNA or bacteria, and recovery of target is not of a concern. Furthermore, we chose the LAMP amplification reaction for its advantages over PCR as it allows for isothermal amplification, high specificity due to usage of 4–6 primers, and higher levels of amplified product within 1 h. Our biphasic method allows for high sensitivity detection of pathogens at low concentrations without the conventional steps of culture or nucleic acid purification. Since we directly dry the food matrix, we can capture and retain few bacterial pathogens that can often be lost in food matrix separation. The current sample to result time in our platform is 2.5 h. This platform can reduce cost and time for food companies so that their lot storage time is reduced and resources can be managed (Nugen & Baeumner, 2008). This would also reduce the number of potential recalls that would occur due to foodborne pathogen contamination.

While in this paper we demonstrated that 30 mg of food sample could be processed per reaction, we need to consider how this mass could be scaled up in future manifestations of the technology. As noted in Figure 1, following the process used by USDA for foodborne pathogen testing, we homogenized 25 g of solid food in 225 ml of fluid. We then used 66 µl of the homogenized sample which, when dried, resulted in 30 mg of solid phase in our biphasic reaction. To scale this to larger mass of starting samples, we would need to process many more aliquots of the 66 µl samples in parallel, essentially partitioning the homogenized liquid sample into many parallel reactions. This is similar to the concept of droplet amplification, where a microliter scale sample is divided into picoliter volumes and even a single copy of nucleic acid could be detected in that small volume, allowing for detection of a single molecule in a larger volume. To process 250 ml using 66 µl aliquots would need about 3800 (array of 62 × 62) parallel reactions. While we performed our reactions in 200 µl PCR plastic vials, we propose to create using injection molding of plastics or etching in a silicon wafers a "cassette" with an array of 62 × 62 reaction chambers each with a volume of 200 µl. If the distance from center of one reaction chamber to next is 5 mm then the size of this square cassette will be about 12 in on a side, which is practically very feasible. The 250 µl homogenized samples spread or spun across the wells and dried. Then, our LAMP amplification reagents could be added for the reaction to occur at 65°C. Amplification and fluorescence change in any one of the wells would indicate detection of target pathogen. Our future work would include optimizations to improve the signal to noise ratio of dried sample to reaction mix in this system and as well as reduce the number of manual steps needed to perform the assay.

SIGNIFICANCE

Food safety and early detection become a challenge when conventional methods of enrichment and pathogen culture are used to detect pathogens causing foodborne diseases and outbreaks. Such culture methods are used to increase pathogen concentration in food samples, allowing for easier sample processing and detection. Moreover, complex compounds in food matrices can often interfere in amplification reactions, requiring separation/extraction of the pathogen from the matrix. However, these processes can take up to several days to obtain reliable results and successfully determine the causative agent. Here, we present a culture-independent "biphasic" approach to detecting *E. coli* pathogens directly from unprocessed meat samples. We dry meat homogenate at high temperature to create micro fluidic networks inside the dried food matrix and allow for DNA amplification. We show a sensitivity of 1 cfu *E. coli* in 30 mg of dried meat sample with sample-to-result time being less than 2.5 h.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Ariana Mostafa, Anurup Ganguli, and Rashid Bashir conceived the idea and designed the study. Ariana Mostafa, Jacob Berger, Carlos Saavedra performed the experiments. Ariana Mostafa, Archith Rayabharam, and Narayana R. Aluru designed and performed simulations. Ariana Mostafa, Archith Rayabharam, and Rashid Bashir wrote the manuscript. All edited the manuscript.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

Andrews, W. H., & Hammack, T. S. (1998). Food sampling/preparation of sample homogenate. Food and Drug Administration (Bacteriological Analytical Manual). Retrieved March 12, 2021, from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-1-food-samplingpreparation-sample-homogenate

Bhunia, A. K. (2014). August 1 One day to one hour: How quickly can foodborne pathogens be detected? *Future Microbiology*, *9*, 935–946. https://doi.org/10.2217/fmb.14.61

Bohaychuk, V. M., Gensler, G. E., King, R. K., Wu, J. T., & McMullen, L. M. (2005). Evaluation of detection methods for screening meat and poultry products for the presence of foodborne pathogens. *Journal of Food Protection*, *68*(12), 2637–2647. https://doi.org/10.4315/0362-028X-68.12.2637

Burden of Foodborne Illness: Findings. (n.d.). Center for Disease Control and Prevention. Retrieved December 1, 2020, from https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

Cai, S., Singh, B. R., & Sharma, S. (2007). Botulism diagnostics: From clinical symptoms to in vitro assays. *Critical Reviews in Microbiology*, *33*, 109–125. https://doi.org/10.1080/10408410701364562

Cheek, D. B., Holt, A. B., Hill, D. E., & Talbert, J. L. (1971). Review article cell size growth retardation developmental physiology muscle mass DNA protein malnutrition skeletal muscle cell mass and growth: The concept of the deoxyribonucleic acid unit. In. *Pcdiatric Research*, *5*, 312–328.

Chen, T. Y., Loan, P. T., Hsu, C. L., Lee, Y. H., Tse-Wei Wang, J., Wei, K. H., Lin, C. T., & Li, L. J. (2013). Label-free detection of DNA hybridization using transistors based on CVD grown graphene. *Biosensors and Bioelectronics*, *41*(1), 103–109. https://doi.org/10.1016/j.bios.2012.07.059

Cho, I. H., & Ku, S. (2017). October 1 Current technical approaches for the early detection of foodborne pathogens: Challenges and opportunities. *International Journal of Molecular Sciences*, *18*, 2078. https://doi.org/10.3390/ijms18102078

Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges. (2019). Retrieved from https://s27415.pcdn.co/wp-content/uploads/2020/01/64ER20-7/Microbial/5-USDA-FSIS-5C.00-Detection-Isolation-and-Identification-of-STEC.pdf

Dwivedi, H. P., & Jaykus, L. A. (2011). Detection of pathogens in foods: The current state-of-the-art and future directions. *Critical Reviews in Microbiology*, *37*, 40–63. https://doi.org/10.3109/1040841X.2010.506430

El-Boubbou, K., Gruden, C., & Huang, X. (2007). Magnetic glyco-nanoparticles: A unique tool for rapid pathogen detection, decontamination, and strain differentiation. *Journal of the American Chemical Society*, *129*(44), 13392–13393. https://doi.org/10.1021/ja076086e

Fluit, A. C., Widjojoatmodjo, M. N., Box, A. T. A., Torensma, R., & Verhoef, J. (1993). Rapid detection of salmonellae in poultry with the magnetic immuno- polymerase chain reaction assay. *Applied and Environmental Microbiology*, *59*(5), 1342–1346. https://doi.org/10.1128/aem.59.5.1342-1346.1993

Fratamico, P. M., Bagi, L. K., Cray, W. C., Narang, N., Yan, X., Medina, M., & Liu, Y. (2011). Detection by multiplex real-time polymerase chain reaction assays and isolation of shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 in ground beef. *Foodborne pathogens and Disease*, *8*(5), 601–607. https://doi.org/10.1089/fpd.2010.0773

Fukushima, H., Katsube, K., Hata, Y., Kishi, R., & Fujiwara, S. (2007). Rapid separation and concentration of food-borne pathogens in food samples prior to quantification by viable-cell counting and real-time PCR. *Applied and Environmental Microbiology*, *73*(1), 92–100. https://doi.org/10.1128/AEM.01772-06

Golsteyn Thomas, E. J., King, R. K., Burchak, J., & Gannon, V. P. J. (1991). Sensitive and specific detection of Listeria monocytogenes in milk and ground beef with the polymerase chain reaction. *Applied and Environmental Microbiology*, *57*(9), 2576–2580. https://doi.org/10.1128/aem.57.9.2576-2580.1991

Gracias, K. S., & McKillip, J. L. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology*, *50*, 883–890. https://doi.org/10.1139/w04-080

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. (2008). Loop-mediated isothermal amplification assay for rapid detection of common strains of *Escherichia coli*. *Journal of Clinical Microbiology*, *46*(8), 2800–2804. https://doi.org/10.1128/JCM.00152-08

Horák, D., Babič, M., Macková, H., & Beneš, M. J. (2007). July Preparation and properties of magnetic nano- and microsized particles for biological and environmental separations. *Journal of Separation Science*, *30*, 1751–1772. https://doi.org/10.1002/jssc.200700088

Irwin, P., Damert, W., Brewster, J., Gehring, A., & Tu, S. -I. (2002). Immuno-magnetic bead mass transport and capture efficiency at low target cell densities in phosphate-buffered saline. *Journal of Rapid Methods and Automation in Microbiology*, *10*(2), 129–147. https://doi.org/10.1111/j.1745-4581.2002.tb00018.x

Jiang, X., Wang, R., Wang, Y., Su, X., Ying, Y., Wang, J., & Li, Y. (2011). Evaluation of different micro/nanobeads used as amplifiers in QCM immunosensor for more sensitive detection of E. coli O157:H7. *Biosensors and Bioelectronics*, *29*(1), 23–28. https://doi.org/10.1016/j.bios.2011.07.059

Joshi, R., Janagama, H., Dwivedi, H. P., Senthil Kumar, T. M. A., Jaykus, L. A., Schefers, J., & Sreevatsan, S. (2009). Selection, characterization, and application of DNA aptamers for the capture and detection of *Salmonella enterica* serovars. *Molecular and Cellular Probes*, *23*(1), 20–28. https://doi.org/10.1016/j.mcp.2008.10.006

Kubitschek, H. E. (1969). Growth During the Bacterial Cell Cycle: Analysis of Cell Size Distribution. In. *Biophysical Journal*, *9*, 792–809. https://doi.org/10.1016/S0006-3495(69)86418-0

Kumar Vashishtha, A., & Konigsberg, W. H. (2018). The effect of different divalent cations on the kinetics and fidelity of *Bacillus stearothermophilus* DNA polymerase. *AIMS Biophysics*, *5*(2), 125–143. https://doi.org/10.3934/biophy.2018.2.125

Law, J. W. F., Mutalib, N. S. A., Chan, K. G., & Lee, L. H. (2014). Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Frontiers in Microbiology*, *5*(DEC), 770. https://doi.org/10.3389/fmicb.2014.00770

Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, *5*, 1–1. https://doi.org/10.3389/fmicb.2014.00258

Liao, C. H., & Shollenberger, L. M. (2003). Survivability and long-term preservation of bacteria in water and in phosphate-buffered saline. *Letters in Applied Microbiology*, *37*(1), 45–50. https://doi.org/10.1046/j.1472-765X.2003.01345.x

López-Campos, G., Martínez-Suárez, J. V., Aguado-Urda, M., & López-Alonso, V. (2012). Detection, identification, and analysis of foodborne pathogens, *Microarray detection and characterization of bacterial foodborne pathogens* (pp. 12–32). Springer. https://doi.org/10.1007/978-1-4614-3250-0_2

Lui, C., Cady, N. C., & Batt, C. A. (2009). Nucleic acid-based detection of bacterial pathogens using integrated microfluidic platform systems. *Sensors*, *9*, 3713–3744. https://doi.org/10.3390/s90503713

Lukacs, G., Haggie, P., Seksek, O., Lechardeur, D., Freedman, N., & Verkman, A. S. (2000). Size-dependent DNA mobility in cytoplasm and nucleus. *Journal of Biological Chemistry*, *273*(3), 1625–1629. Retrieved from https://pubmed.ncbi.nlm.nih.gov/10636854/

Mairhofer, J., Roppert, K., & Ertl, P. (2009). Microfluidic systems for pathogen sensing: A review. *Sensors*, *9*, 4804–4823. https://doi.org/10.3390/s90604804

Meagher, R. J., Priye, A., Light, Y. K., Huang, C., & Wang, E. (2018). Impact of primer dimers and self-amplifying hairpins on reverse transcription loop-mediated isothermal amplification detection of viral RNA. *Analyst*, *143*(8), 1924–1933. https://doi.org/10.1039/c7an01897e

Nugen, S. R., & Baeumner, A. J. (2008). Trends and opportunities in food pathogen detection. *Analytical and Bioanalytical Chemistry*, *391*(2), 451–454. https://doi.org/10.1007/s00216-008-1886-2

Recall Summaries. (2019). (n.d.). Retrieved June 2, 2020, from https://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries

Rossen, L., Nørskov, P., Holmstrøm, K., & Rasmussen, O. F. (1992). Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *International Journal of Food Microbiology*, *17*(1), 37–45. https://doi.org/10.1016/0168-1605(92)90017-W

Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the united states. *Journal of Food Protection*, *75*(1), 123–131. https://doi.org/10.4315/0362-028X.JFP-11-058

Sezonov, G., Joseleau-Petit, D., & D'Ari, R. (2007). *Escherichia coli* physiology in Luria-Bertani broth. *Journal of Bacteriology*, *189*(23), 8746–8749. https://doi.org/10.1128/JB.01368-07

Sharma, H., & Mutharasan, R. (2013). Rapid and sensitive immunodetection of Listeria monocytogenes in milk using a novel piezoelectric cantilever sensor. *Biosensors and Bioelectronics*, *45*(1), 158–162. https://doi.org/10.1016/j.bios.2013.01.068

Stevens, K. A., & Jaykus, L. A. (2004). Bacterial separation and concentration from complex sample matrices: A review. *Critical Reviews in Microbiology*, *30*, 7–24. https://doi.org/10.1080/10408410490266410

Taskila, S., Tuomola, M., & Ojamo, H. (2012). Enrichment cultivation in detection of food-borne Salmonella. *Food Control*, *26*, 369–377. https://doi.org/10.1016/j.foodcont.2012.01.043

Tu, S. I., Uknalis, J., Gore, M., Irwin, P., & Feder, I. (2003). Factors affecting the bacterial capture efficiency of immuno beads: A comparison between beads with different size and density. *Journal of Rapid Methods & Automation in Microbiology*, *11*(1), 35–46. https://doi.org/10.1111/j.1745-4581.2003.tb00406.x

Vidic, J., Vizzini, P., Manzano, M., Kavanaugh, D., Ramarao, N., Zivkovic, M., & Gadjanski, I. (2019). Point-of-need DNA testing for detection of foodborne pathogenic bacteria. *Sensors*, 19. https://doi.org/10.3390/s19051100

Wang, Y., Ye, Z., Si, C., & Ying, Y. (2013). Monitoring of *Escherichia coli* O157:H7 in food samples using lectin based surface plasmon resonance biosensor. *Food Chemistry*, *136*(3–4), 1303–1308. https://doi.org/10.1016/j.foodchem.2012.09.069

Wang, Y., & Duncan, T. V. (2017). Nanoscale sensors for assuring the safety of food products. *Current Opinion in Biotechnology*, *44*, 74–86. https://doi.org/10.1016/j.copbio.2016.10.005

Wang, Y., & Salazar, J. K. (2016). Culture-independent rapid detection methods for bacterial pathogens and toxins in food matrices. *Comprehensive Reviews in Food Science and Food Safety*, *15*(1), 183–205. https://doi.org/10.1111/1541-4337.12175

Wu, S., Duan, N., Shi, Z., Fang, C., & Wang, Z. (2014). Simultaneous aptasensor for multiplex pathogenic bacteria detection based on multicolor upconversion nanoparticles labels. *Analytical Chemistry*, *86*(6), 3100–3107. https://doi.org/10.1021/ac404205c

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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