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Monitoring Pathogenic Viable *E. coli* O157:H7 in Food Matrices Based on the Detection of RNA Using Isothermal Amplification and a Paper-Based Platform

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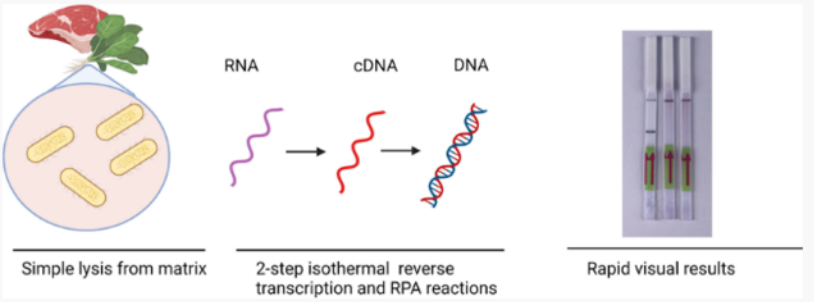

ABSTRACT: In recent years, the number of product recalls and contamination incidents involving pathogenic bacteria has significantly increased, and the ensuing infections continue to be an ongoing problem for public health and agriculture. Due to the widespread impact of these pathogens, there is a critical need for rapid, on-site assays that can provide rapid results. In this work, we demonstrate the development of a rapid and simple test based on the combination of reverse transcription with recombinase polymerase amplification followed by lateral flow strip detection of viable *Escherichia coli* O157:H7 cells by detecting the RNA of the pathogen. The optimized method can be performed for approximately 2 h with a detection limit of 10 CFU/mL of *E. coli* O157:H7 in buffer, spinach, and ground beef samples. Our assay is sensitive, detecting only *E. coli* O157:H7 and not nonpathogenic *E. coli* or other similar pathogens. This strategy was able to distinguish viable from nonviable bacteria and more significantly was able to detect viable but nonculturable bacteria, which is a major issue when using culture-based methods for monitoring pathogenic bacteria. An important advantage of this test is that it can provide timely identification and removal of contaminated consumables prior to distribution without an extensive sample preparation.

1. INTRODUCTION

Food contamination via pathogenic bacteria has become a topic of increasing importance in recent years due to the frequency of cases and its impact on public health. Pathogenic bacteria cause thousands of emergency room visits each year, many of which result in hospitalization and even fatalities.[1] These outbreaks do not only burden the healthcare system but they also create a cascade of economic effects ranging from individual loss of productivity to major financial losses in the restaurant and agricultural industries.[2] *Escherichia coli* (*E. coli*) is a bacterium found natively in the human gastrointestinal flora.[3] However, there are four different subsets of *E. coli* that can also cause illness in the human body: enterotoxigenic, enteroinvasive, enteropathogenic, and enterohemorrhagic.[4] One example of these pathogens that is at the forefront of foodborne outbreaks is *E. coli* O157:H7, a enterohemorrhagic strain producing a virulent protein called Shiga toxin (Stx) that is known to disrupt protein synthesis.[5] This organism is a Gram-negative coliform bacterium that is found without ill effects in the intestines of ruminant animals such as cows. However, upon ingestion and colonization of the human intestinal tract, the bacterium can cause gastroenteritis and hemorrhagic colitis.[6] As per the CDC, *E. coli* O157:H7 accounts for around 95,000 cases of infection per year alone, which equates to 36% of the total Stx-related *E. coli* infections.[7] Stx is an AB5 ribosomal toxin that removes a specific adenine from 28S rRNA, blocking protein synthesis and promoting hemolytic uremic syndrome/kidney failure via cellular apoptosis.[8] As such, it is critical to discover the presence of such pathogens before public health is placed at risk.

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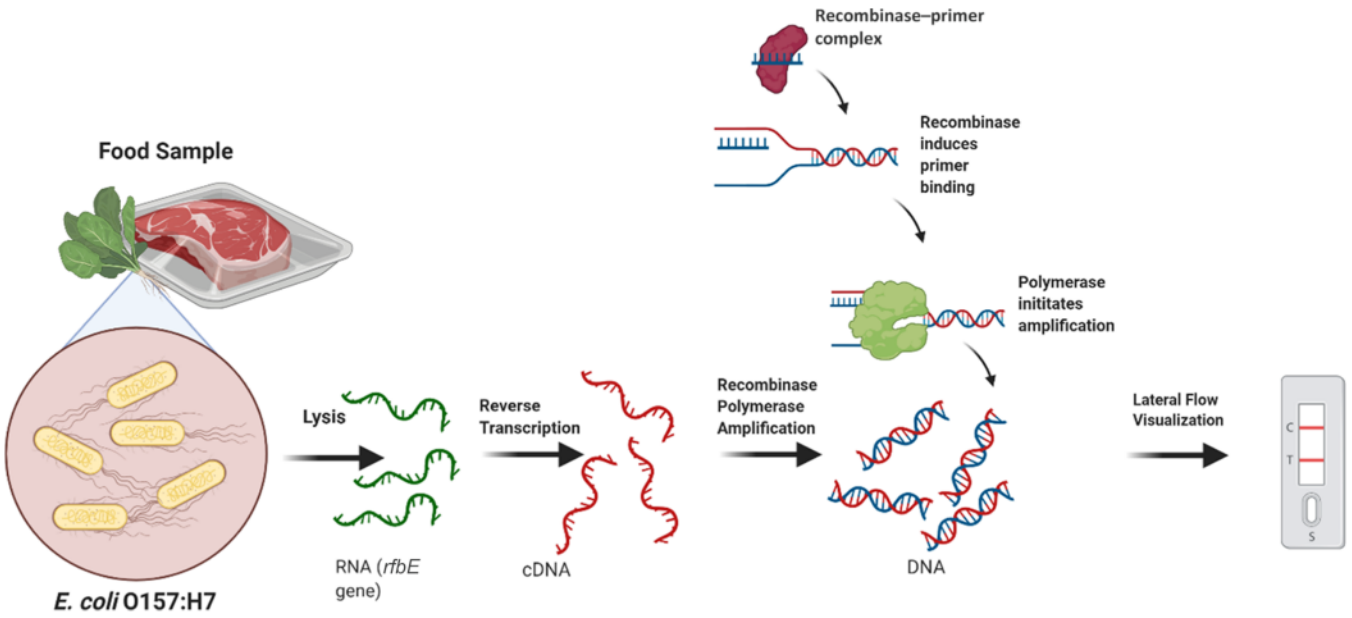

Figure 1. Schematic of the assay protocol from sample to results. Briefly, the pathogenic bacteria in the sample are lysed, and genomic DNA is removed via DNase I digestion. The RNA of the *rfbE* gene of the pathogenic bacteria is reverse transcribed and the ensuing cDNA is amplified utilizing RPA. The reaction products are then visualized on a lateral flow paper strip assay. Created using BioRender.com.

Current methods for pathogenic bacteria detection tend to be time-consuming, such as selective culture, or require costly supplies and skilled personnel to run them, as in PCR-based amplification methods.[9] With the necessity of off-site analysis, results can take days or weeks, leaving significant opportunity for the contaminated product to reach the market. As such, it is of great importance to develop detection techniques that are not only rapid and simple but also capable of detecting viable cells, as these are a better indication of a current health threat. DNA has proven to persist in the environment much longer than RNA; therefore, detection of RNA will lead to a more accurate representation of viable pathogenic *E. coli*. In fact, *E. coli* RNA has a demonstrated half-life of no longer than 20 min in the environment.[10] More significantly, it is important to develop technologies that are able to detect pathogens at relevant concentrations and also capable of rapid and on-site analysis. Recent advances in amplification technology have bolstered the potential for development of on-site nucleic acid testing.

As an alternative to traditional PCR, recombinase polymerase amplification (RPA) has shown promise for on-site and point-of-care applications due to isothermal operation at a single lower temperature that can be completed in around 20 min. Briefly, RPA is able to amplify DNA isothermally by means of a recombinase.[11] The recombinase forms a complex with the primers, which then searches the dsDNA for the sequence homologous to the primers. Strain invasion occurs, and the open DNA is stabilized by single-stranded binding proteins. Once the DNA is open, the DNA polymerase can elongate the sequence. This process cycles and is exponential. When paired with a reverse transcriptase, single-pot amplification of bacterial RNA can be realized.[12−16] Although this technique is most commonly applied to viral RNA amplification, the isolation and recovery procedures are similar, and the amount of RNA recovered from equivalent concentrations of bacteria is typically significantly higher than those for viruses. This comparatively high yield allows techniques such as direct amplification following lysis to be applied avoiding problems related to high nonspecific background.

Despite the many recent advances in on-site detection of pathogenic *E. coli* (please see Table S1 in the Supporting Information),[17] some issues such as assay complexity, costs, and detection limits still exist. For example, several countries in Africa have reported outbreaks, but total infections can be underestimated due to the use of outdated detection methods.[18] In addition, few assays focus on detecting viable bacteria, a concern because current methods such as culturing do not account for viable but nonculturable bacteria (VBNCs). VBNCs are a major problem because they remain virulent and infectious without being detectable using the "gold standard" culturing technique,[19] a dangerous occurrence as this can potentially lead to false negatives via traditional testing means. The assay described herein aims to circumvent current limitations in detection by its ability to distinguish viable bacteria and provide a broader indication of a health threat. To achieve this, we combined reverse transcription with an isothermal amplification technique to rapidly detect pathogenic *E. coli* with minimal extraction procedures and simple visualization on a lateral flow paper strip platform. This is advantageous as there is not a major requirement for expensive equipment, only a simple battery-operated centrifuge and a method of heating. Furthermore, this strategy does not require an enrichment step. A full depiction of the assay from sample to visualization with lateral flow paper strips can be seen in Figure 1.

2. EXPERIMENTAL SECTION

2.1. Materials. All bacterial strains were purchased from ATCC (Manassas, VA). Oligos were purchased from Sigma (St. Louis, MO). Milenia HybriDetect Lateral flow paper strips and TwistDx RPA Basic kits were obtained from TwistDx (Cambridge, UK). M-MLV reverse transcriptase was from Promega (Madison, WI). dNTPs and herring sperm DNA were obtained from New England Biolabs (Ipswich, MA).

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Anal. Chem. XXXX, XXX, XXX−XXX

RNaseOUT, TURBO DNA-free Kit, and RT-PCR grade water were purchased from Thermo Fisher (Waltham, MA). Sodium hydroxide was purchased from VWR (Radnor, VA). Triton-X 100 was from Alfa Aesar (Tewksbury, MA). Bacterial viability assay was purchased from Abcam (Cambridge, UK).

2.2. Bacterial Culturing. All bacterial strains (*E. coli* O157:H7, *E. coli* O6, and *Pseudomonas aeruginosa*) were inoculated in 5 mL of nutrient broth and incubated overnight with shaking at 250 rpm/37 °C. The following day, cultures were diluted to a starting concentration of 10^6 CFU/mL using sterile water before being used or further diluted from 10^6 CFU/mL to concentrations as low as ∼1 CFU/mL via 10-fold dilutions in our assay. All cultures and waste products were disposed of and handled according to proper BSL-2 safety handling guidelines.

2.3. Isolation of RNA from Bacterial Cells. 1.0 mL of bacterial culture was removed and centrifuged for 5 min at 4000*g*. The supernatant was aspirated, and the pellet was resuspended in 100 μL of lysis buffer (50 mM NaOH and 5% Triton-X 100). This bacterial suspension was vortexed and then incubated at room temperature for 10 min. Lysed cells were then treated with 2.0 μL DNase I (Thermo Fisher Scientific, Waltham, MA) with 10.0 μL of DNase I reaction buffer as per the manufacturer's protocol for 30 min and then treated with 20.0 μL of inactivation reagent for 5 min. During this incubation, samples were periodically vortexed to prevent the resin from settling out of the solution. Samples were then centrifuged for 2 min at 1500*g*, and the supernatant containing the RNA was distributed into 15−20 μL aliquots prior to immediate use or storage at −80 °C.

2.4. Optimization of Bacterial Lysis Conditions. 1 mL of 10^6 CFU/mL *E. coli* O157:H7 was prepared for each condition. Samples were centrifuged for 4000*g* for 5 min and the media was aspirated. 100 μL of lysis buffer (50 mM NaOH and 5% Triton-X 100) was added, and the samples were briefly vortexed before being put on either ice, heated at 37 °C, or left in room temperature. After either 10, 20, or 40 min, samples were treated with 2 μL of DNase I and 10.0 μL of DNase I reaction buffer. DNase I is removed using 20.0 μL of inactivation reagent before samples are reverse transcribed using M-MLV reverse transcriptase (see Section 2.5) and then amplified with RPA (see Section 2.6). 2.0 μL of each product was visualized on a lateral flow paper test strip (see Section 2.7).

2.5. Reverse Transcription of RNA. RNA was reverse transcribed using the M-MLV reverse transcription kit from Promega. Briefly, 2.5 μL of the reverse primer (final concentration 962 nM), 2.0 μL of RNA (The concentration of RNA was found to range approximately from 300 to 1000 ng/μL. Due to the variable nature of RNA concentrations we always added the same amount each time to standardize the assay protocol. Our concentrations were predominantly derived from the amount of bacteria rather than the RNA concentration as this is more relevant to sample analysis.), and water up to 15.0 μL were incubated at 70 °C for 5 min before adding the remaining reagents in their respective order (5.0 μL of RT 5× buffer, 5.0 μL of dNTPs, 1.0 μL of RNaseOUT, and 1.0 μL of M-MLV RT). Samples are then incubated for 1 h as per the manufacturer's protocol at 37 °C.

2.6. Amplification of cDNA. A 2.0 μL aliquot of cDNA per sample was transferred to a PCR tube containing the RPA reaction components. These components included 29.5 μL of rehydration buffer, 2.4 μL of the forward and 2.0 μL of the reverse primers, 1.0 μL of 100 ng herring sperm DNA, and 11.2 μL of water, similar to the manufacturer's protocol (TwistDx, Cambridge, UK). Primers and DNA were all added to separate sides of the wall rather than into the mixture to prevent early interactions. Tubes were then vortexed and briefly centrifuged prior to transferring the entire contents to the manufacturer-supplied lyophilized enzyme pellets. A 2.5 μL aliquot of the reaction initiator, 280 mM magnesium acetate (MgOAc), was added to the PCR tube caps, and the reaction tubes were then briefly centrifuged, vortexed, and centrifuged again. Tubes were immediately incubated for 20 min at 37 °C prior to visualization of the product.

2.7. Lateral Flow Assay. Milenia HybriDetect 1 lateral flow paper strips were purchased from TwistDx (Cambridge, UK). Lateral flow paper strips and buffers were first allowed to acclimate to room temperature before use. An aliquot of 2.0 μL of sample was pipetted onto the sample pad prior to dipping the strips into 1.5 mL microcentrifuge tubes containing 100 μL of manufacturer-supplied running buffer. Strips were allowed to run for 3 min before removing from the buffer for visualization.

2.8. Detection of Viable *E. coli* O157:H7. *E. coli* O157:H7 was grown overnight in a 5 mL culture in nutrient broth. Cultures were then diluted to 10^6 CFU/mL. A one mL portion of the culture was lysed, RNA was isolated and reverse transcribed-RPA amplified as per our previous methods above. Another one mL portion was also taken and heated at 85 °C for 35 min before being lysed and used in our assay as a nonviable sample. Both samples were then visualized on a lateral flow paper assay as described above.

2.9. Induction of a Viable but Nonculturable State. *E. coli* O157:H7 was grown overnight in a 5 mL culture in nutrient broth. Cultures were then diluted to 10^6 CFU/mL in nutrient broth. Cells were then incubated for 4 h at 50 °C with periodic manual shaking to allow for consistent heat application. Samples were then verified to be nonculturable by being plated on MacConkey agar (MAC) plates and assessed for viability using a viability assay from Abcam (Cambridge, UK).

2.10. Food Sample Handling and Spiked Sample Studies. Ground beef was purchased from a local chain supermarket and aliquoted into 1.00 g amounts before being stored at −20 °C until use. Just prior to use, samples were thawed and homogenized into a 10% w/v suspension utilizing a mortar and pestle. Into this suspension, 100 μL of bacterial cultures per mL of liquid volume was added for total concentrations ranging from 10^6 to 10^1 CFU/mL. These samples were then incubated for 10 min with 1.00 mL of 50 mM NaOH and 5% TritonX-100 (Tx-100) before an aliquot of 1.00 mL was centrifuged at 4000*g* for 5 min 100 μL of supernatant was removed and treated with DNase I as described above prior to reverse transcription and amplification as per the above protocols. Results were then visualized on a lateral flow paper strip just as previously described.

3. RESULTS AND DISCUSSION

3.1. RPA Optimization and Primer Selection. Primers were designed against the open reading frame of the strain-selective *rfbE* region of *E. coli* O157:H7,[20,21] responsible for coding a synthetase involved in O-antigen production. The O-antigen is the portion of the lipopolysaccharide on the outside of the bacterial membrane consisting of repeats of oligosaccharides.[22] The O-antigen conveys most of the

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Anal. Chem. XXXX, XXX, XXX−XXX

variability between the different pathogenic *E. coli* strains. This region was chosen as it is more specific to this particular strain and because the genes associated with the O-antigen are highly conserved.[20] Primers were designed by scanning the open reading frame based on ideal product size and generating multiple 30 base pair sequences. These primers were verified as unique via Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (see Figure S1) and compared for optimal product formation in standard RPA assays using genomic DNA purchased from ATCC (Manassas, VA). The finalized primer set was then used to generate a 255 bp product via reverse transcription of bacterial RNA and isothermal amplification of the cDNA product utilizing RPA (Figure 2). Primer sequences used in this manuscript are displayed in Table 1 under the Experimental Section. The product obtained was verified by DNA sequencing. The primers chosen resulted in the amplification of a strain-selective region of the gene as expected, and hence, the chosen primer sequence was employed in the next studies.

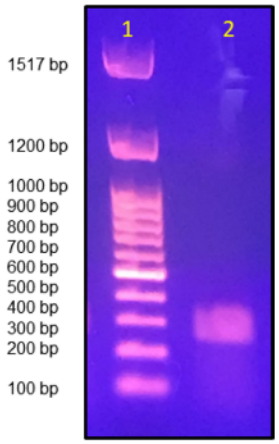

Figure 2. RPA amplification of *E. coli* O157:H7 cDNA visualized in a 2% agarose gel. Lane 1 is a 100 bp ladder and lane 2 is the amplified product. The product size is 255 bp. Results were repeated at least three times and a representative image is shown.

Table 1. Oligonucleotide Sequences Utilized in This Work

primer	sequence
forward FAM primer	5′-(FAM)-TTGTTCCAACACTGACATATATAGCATCAG-3′
reverse biotin primer	5′-(TEG Biotin)-TAGAACCAAAGGCTTCAGCGCAATCTTCAA-3′

3.2. Cross-Reactivity Analysis. In order to evaluate the performance and specificity of our assay, we assessed our optimized primer pair against a nonpathogenic *E. coli* strain (O6) and a dissimilar pathogen (*P. aeruginosa*). This is important because the food samples could be contaminated with nonpathogenic strains that are ubiquitously present. For that purpose, samples containing 10^6 CFU/mL of either *E. coli* O157:H7, *P. aeruginosa*, or *E. coli* O6 were lysed and then treated with DNase I. The corresponding RNA was then reverse transcribed, and the cDNA was amplified before being visualized on a lateral flow paper strip and a 2% agarose gel. These lateral flow assays are able to be visualized and interpreted by the naked eye without any use of equipment. The test bands appear due to the gold nanoparticles that are attached to an antibody against FAM. This antibody recognizes the labeled primer of the amplicon that becomes immobilized due to the interaction of the biotin on the other labeled primer and the biotin ligand present in the test lines. A positive sample is indicated by two colorimetric lines—the bottom line is the captured amplicon, and the top line is a control line that just captures the gold and antibody conjugate. This control line acts as a quality control line to ensure the assay is functioning properly and is required for the validity of the lateral flow assay. With that, a negative sample would only have one top line present. In both the gel and in the lateral flow paper strips, the *E. coli* O6 or *Pseudomonas* strains provided no observable activity (Figure 3A,B) as expected because the primers designed are highly specific and should not amplify the genes of other organisms. Only the sample containing *E. coli* O157:H7 resulted in a positive response. This means that there is negligible cross-reactivity with similar nonpathogenic strains or other pathogens. Additionally, cross-reactivity against other major foodborne bacteria were assessed via Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for sequence homology and confirmation of the primer specificity. Blast results can be seen in Supporting Information, Figure S2. No significant homology was seen in the commonly found foodborne pathogens. This type of sequence homology-based screening is an accepted method for cross-reactivity study by the FDA because this allows for screening against a large number of pathogens, which is not feasible to study through a laboratory-based screening.[23] Additionally, this method is indicated in a protocol set forth by Park *et al.* for the optimization of primers.[24]

3.3. Optimization of Direct Bacterial Lysis. Direct lysis has better potential for incorporation into an on-site test due to its simplicity compared to other conventional lysis methods that may need use of extra equipment, steps, and/or conditions that are not favorable for on-site handling. For that, we utilized a chemical lysis method consisting of a buffer containing 50 mM NaOH and 5% Triton-X 100. To determine the optimal temperature and time for lysis, bacteria were cultured, and 1 mL aliquots of 10^6 CFU/mL bacteria were spun down and then media was aspirated. A volume of 100 μL of lysis buffer was added to each sample and was incubated for either 10, 20, or 40 min either on ice, at room temperature, or at 37 °C. Samples were then treated with DNase I to remove any interference from DNA that could be present in the sample. This DNase I was then removed with an inactivation resin, and then, cDNA was synthesized, amplified, and finally visualized on a lateral flow paper strip (Figure 4). While all conditions yielded good lysis results, in the interest of simplification and reduced assay time of the on-site assay, we decided to use room temperature for only 10 min as our lysis conditions. This lysis and direct use of sample also simplified the sample preparation step, which can be performed on-site.

3.4. Evaluation of the Assay for Detection of O157:H7. Because the infectious dose of *E. coli* O157:H7 is fairly low (<700 cells), the need for high sensitivity is imperative to prevent potential false negatives.[25] To test the detection range of our assay, bacterial RNA was isolated and then diluted to varying concentrations (from 10^6 to ~1 CFU/mL). This range was designed to mimic an infectious range of typical contaminated food consumption. Results were visualized on a lateral flow paper strip (Figure 5). From these results, it was apparent that the detection limit was

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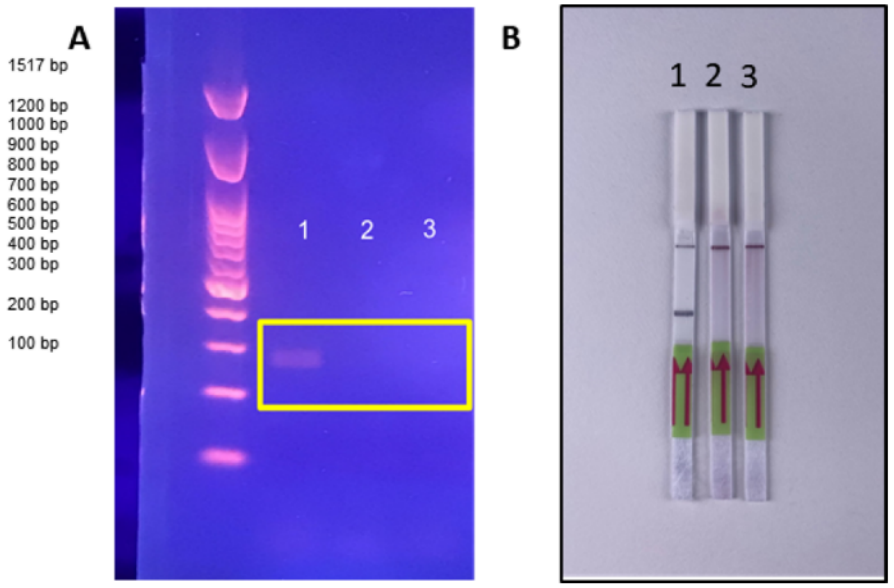

Figure 3. Cross-reactivity assessment. (A) 2% agarose gel visualization of amplified cDNA of (1) *E. coli* O157:H7, (2) *E. coli* O6 (nonpathogenic variant), and (3) *P. aeruginosa*. (B) Paper strip visualization of amplified products from (A). Paper strips are of, respectively, the following: *E. coli* O157:H7 (1), *E. coli* O6 (2), and *P. aeruginosa* (3). While only one representative replicate is shown in the figure, results were repeated at least three times in separate isolation experiments.

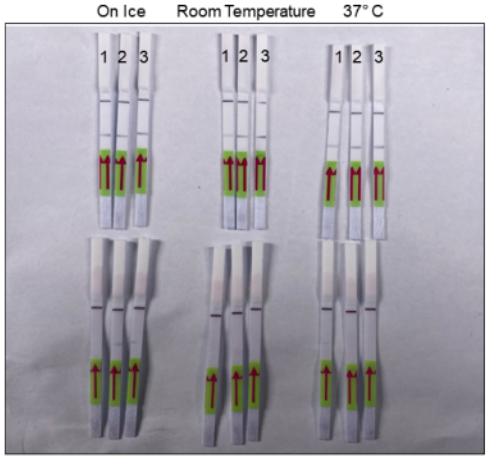

Figure 4. Optimization of lysis time and temperature. As shown, samples of isolated *E. coli* bacterial cells were incubated either for (1) 40, (2) 20, or (3) 10 min with the lysis buffer either on ice, at room temperature, or at 37 °C. The second row of strips represents the negative controls of each condition displayed in the first row. Results were repeated in at least three separate experiments, with one representative set being shown.

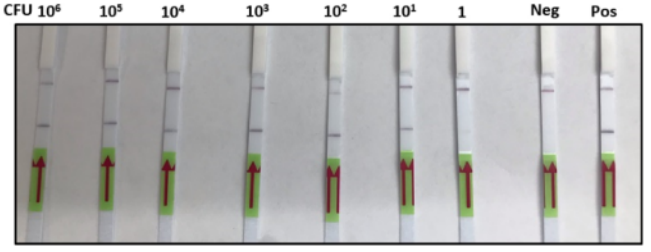

Figure 5. Assessment of assay range in media. Bacterial RNA was isolated from varying concentrations of bacteria and then synthesized into cDNA before being amplified and visualized on a lateral flow paper strip assay. As seen here, we can visually detect as low as 10 CFU/mL of bacteria. The negative control is human genomic DNA and positive control is *E. coli* O157:H7 genomic DNA. Results were repeated in at least three separate experiments, with one representative set being shown.

approximately 10 CFU/mL, which is relevant in terms of the infective range for *E. coli* O157:H7. This detection limit is comparable or better than other literature-reported methods (Table S1, Supporting Information). Additionally, this detection limit was achieved using isothermal amplification reaction in a total assay time of 2 h from sample to result with a good sensitivity and without any need for an enrichment step. These experiments were performed in biological triplicate (separate RNA isolations) to ensure reproducibility and rigor.

3.5. Detection of Viable *E. coli* O157:H7. The detection of viable bacteria is important when considering pathogen identification, as viable cells indicate a potential public health threat. In that regard, RNA can be used as a viability marker because it is produced only when bacteria are actively multiplying. In addition, the shorter half-life and faster degradation of RNA, as compared to DNA that can stay stable in the sample even if the bacteria is no longer viable, make it more suitable for viability studies than DNA.[26] In particular, the mRNA of *E. coli* has been found to be rather unstable and have a half-life of at most 20 min.[27] If fragments of the RNA were to remain, these remaining fragments may not be amplifiable with our primers. Even if some RNA fragments remained and were able to be detected, this would imply that the bacteria were viable in a recent enough time to indicate a potential health threat. Additionally, other methods commonly used for viability determination such as propidium monoazide dye can have limitations and false positives.[28] In order to validate the use of RNA as a means to distinguish viable cells from nonviable cells, 10^6 CFU/mL of *E. coli* O157:H7 was incubated at 85 °C for 35 min prior to performing a 10 min chemical lysis followed by immediate reverse transcription and amplification. This temperature was chosen based on methods used for heat inactivation in the literature,[29] which avoided the risk of inducing a VBNC state.

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Anal. Chem. XXXX, XXX, XXX−XXX

These samples were treated under these conditions to induce a nonviable state. Heat-killed samples were compared to identical samples that were only chemically lysed. As seen in Figure 6, heat-killed samples could not be detected on the

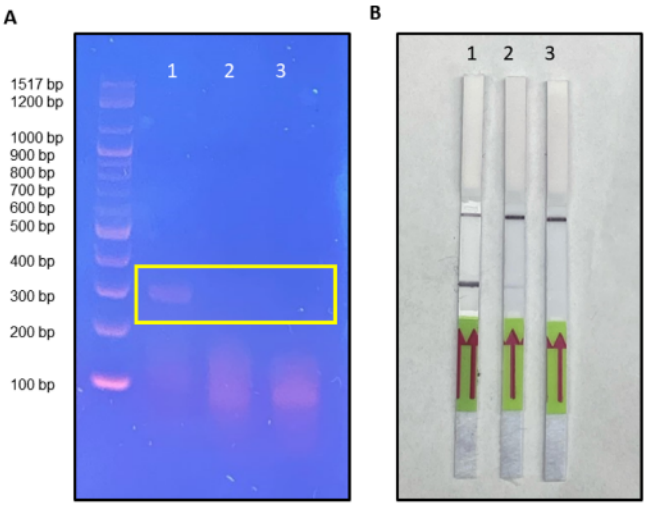

Figure 6. Comparison of heated nonviable bacteria to viable bacteria. (A) 2% agarose gel visualization of (1) 10^6 CFU/mL *E. coli* O157:H7, (2) 10^6 CFU/mL heat-treated *E. coli* O157:H7, and (3) negative control without bacteria. (B) Lateral flow assay visualization of products. 1 represents 10^6 CFU/mL of live cells, while 2 represents 10^6 CFU/mL of heat-treated cells, and 3 represents a negative control with no bacteria present. Results were repeated in at least three separate experiments, with one representative set being shown.

lateral flow paper strip assay, indicating that using RNA as a marker allows for discrimination of viability. While we use a strain-specific gene for the determination of the presence of a particular pathogen, it is not inherently determining viability. Being able to detect mRNA in a sample allows the determination of a viable sample. By utilizing RNA and a strain-specific gene, we are able to achieve two goals—strain detection and viability determination.

3.6. Detection of Viable but Nonculturable Bacteria. Several pathogens have been found to enter the VBNC state when exposed to different stressors.[30] This is hallmarked by a loss of culturability on agar, meaning that standard culturing techniques are unable to detect them. These cells, while not culturable, are still found to undergo respiration and other metabolic processes such as transcription.[31] This state creates a public health risk and, as such, requires additional means to detect these cells outside of standard culturing. *E. coli* O157:H7 is known to enter this state in response to a variety of stressors and allow for its survival in a variety of conditions.[32] As a result, we sought to be able to detect bacteria that have entered this state. For that, we adapted a protocol from Fu *et al.* that utilized heat treatment to achieve a VBNC state and treated cells at a concentration of 10^6 CFU/mL.[33] We placed our cells in a dry incubator at 50 °C for 4 h with occasional shaking. Upon treatment, we verified the culturability of our cells by plating the cells on MAC for 24 h at 37 °C. We observed that the cells were unable to grow, indicating that they reached a VBNC state. We also assessed the viability with a bacterial viability assay and detection of the RNA of bacteria in the VBNC state using our method. We found that we were able to detect these cells utilizing our detection strategy, and bacteria were found to be viable using the Abcam cell viability kit. The results of these experiments are depicted in Figure 7. The significance of these findings is that our test can provide a deeper insight into a potential public health threat that could be misevaluated by culturing methods alone.

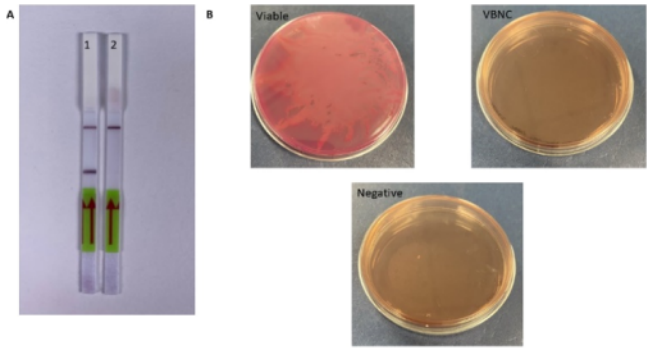

Figure 7. (A) VBNC-induced bacteria were compared to a negative control. Results were visualized on a lateral flow paper assay. (1) is the VBNC induced cells and (2) is a negative control where no bacteria are present. (B) Cells plated on MAC plates. The first plate is a viable positive control followed by the VBNC-induced cells and a negative control plate.

3.7. Detection of *E. coli* O157 in Two Real Sample Matrices. To determine the applicability of our assay in real samples, we decided to use ground beef and spinach as two examples of a sample matrix that are known to be a common cause of *E. coli* contamination leading to food poisoning in humans.[34,35] Our experiments in nutrient broth had established a detection limit of 10 CFU/mL; thus, we decided to use a range of 10^6 to 10 CFU/mL to obtain an LOD within the beef and spinach sample matrices. *E. coli* O157:H7 was spiked into the 10% w/v solutions of either ground beef or spinach in water. Samples were prepared via crude homogenization with a mortar and pestle before the bacteria were spiked. These samples upon lysis and centrifugation were treated with DNase I and used in our assay as previously described. Our data as shown in Figure 8 demonstrate that we can reliably detect as low as 10 CFU/mL in both food matrices, indicating no or a negligible effect of either of the food matrices in the assay. As in all prior experiments, this result was performed in biological

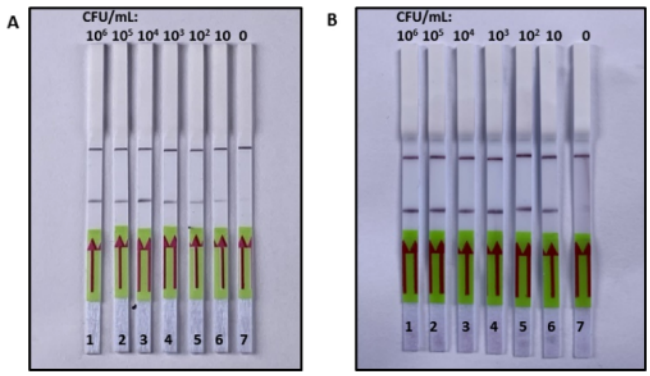

Figure 8. LOD determination (A) in ground beef and (B) in spinach. Strips 1−6 show varying ranges of bacteria in the matrix, from 10^6 to 10 CFU/mL, and a negative control in 7. Results were repeated in at least three separate experiments, with one representative set being shown.

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Anal. Chem. XXXX, XXX, XXX−XXX

triplicate (from separate RNA isolation points) to ensure reproducibility and rigor of the assay.

4. CONCLUSIONS

In this study, we present a novel application of reverse transcription and RPA for the rapid and sensitive on-site detection of the viable RNA of Stx-producing *E. coli* O157 pathogen. The assay developed demonstrated a LOD of 10 CFU/mL of bacteria in samples of *E. coli* O157:H7 in ground beef and spinach matrices. This detection limit is significant given that less than 700 cells present in contaminated food can initiate infection in humans upon ingestion. Furthermore, our obtained limit of detection is comparable to the most sensitive on-site assays described in the literature. A comparison of these methods is highlighted in Table S1. Furthermore, no significant interference was observed from nonpathogenic *E. coli* or other pathogenic organisms. Moreover, our assay could be completed within 2 h with minimal instrumentation (only a centrifuge and a heating method, which is reasonable for on-site applications and significantly shorter than conventional methods such as culturing). These types of instrumentation are easily amenable to portable versions allowing for on-site applications. There was also no need for any sample enrichment step—which allowed for more rapid results. We also showed that our method is able to detect bacteria in a viable but nonculturable state, which overcomes the limitation of culture-based methods as described in the prior sections. Ultimately, with the addition of portable machinery, this assay could potentially see use in the food industry as an initial on-site screening tool for quality control. The inexpensive nature of lateral flow assays combined with the short assay time will allow the end user to run multiple tests, allowing for a good representative sample to be taken. Ideally, this assay would be used to determine if a product is safe for the consumer from the agriculture location all the way to restaurants and grocery stores. Finally, the method developed for our assay could be easily adapted for the detection of other foodborne or waterborne pathogens by redesigning the primers to recognize a different target, making it amenable for use as a general platform for an initial pathogen screening tool in potentially contaminated food or agricultural samples as well as in clinics and environmental monitoring.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c04305.

Blast searches against common foodborne pathogens and detailed comparison of similar methods (PDF)

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Conceptualization by S.K.D. and S.D.; Investigation, S.P. and C.C.; Methodology, S.P. and D.B.; Software, E.D.; Validation, S.P. and C.C.; Formal Analyses, S.P.; Data Curation, S.P. and C.C.; Resources, S.K.D. and S.D.; Writing—original draft preparation, S.P. and C.C.; writing—review and editing, S.K.D., S.D., D.B., and E.D.; Supervision, S.K.D., S.D., D.B., A.K., and E.D.; Project Administration, S.K.D. and S.D.; Funding Acquisition, S.K.D. and S.D. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Mead, P. S.; Slutsker, L.; Dietz, V.; McCaig, L. F.; Bresee, J. S.; Shapiro, C.; Griffin, P. M.; Tauxe, R. V. *Emerging Infect. Dis.* **1999**, *5*, 607−625.
(2) Hussain, M.; Dawson, C. *Foods* **2013**, *2*, 585−589.
(3) Richter, T. K. S.; Michalski, J. M.; Zanetti, L.; Tennant, S. M.; Chen, W. H.; Rasko, D. A. *mSystems* **2018**, *3*, No. e00047.
(4) Padhye, N. V.; Doyle, M. P. *J. Food Prot.* **1992**, *55*, 555−565.
(5) Donohue-Rolfe, A.; Acheson, D. W.; Keusch, G. T. *Rev. Infect. Dis.* **1991**, *13*, S293−S297.
(6) Hunt, J. M. *Clin. Lab. Med.* **2010**, *30*, 21−45.
(7) Su, C.; Brandt, L. J. *Ann. Intern. Med.* **1995**, *123*, 698−714.
(8) Melton-Celsa, A. R. *Microbiol. Spectrum* **2014**, *2*, No. EHEC-0024-2013.
(9) Deisingh, A. K.; Thompson, M. *J. Appl. Microbiol.* **2004**, *96*, 419−429.
(10) Selinger, D. W.; Saxena, R. M.; Cheung, K. J.; Church, G. M.; Rosenow, C. *Genome Res.* **2003**, *13*, 216−223.
(11) Lobato, I. M.; O'Sullivan, C. K. *TrAC, Trends Anal. Chem.* **2018**, *98*, 19−35.
(12) Li, Y.; Li, L.; Fan, X.; Zou, Y.; Zhang, Y.; Wang, Q.; Sun, C.; Pan, S.; Wu, X.; Wang, Z. *Sci. Rep.* **2018**, *8*, 17760.
(13) Zhao, C.; Sun, F.; Li, X.; Lan, Y.; Du, L.; Zhou, T.; Zhou, Y. *J. Virol. Methods* **2019**, *263*, 96−100.
(14) Sun, N.; Wang, W.; Wang, J.; Yao, X.; Chen, F.; Li, X.; Yinglei, Y.; Chen, B. *Mol. Cell. Probes* **2018**, *42*, 25−31.
(15) Liu, L.; Wang, J.; Zhang, R.; Lin, M.; Shi, R.; Han, Q.; Wang, J.; Yuan, W. *BMC Vet. Res.* **2018**, *14*, 263.
(16) Ma, L.; Cong, F.; Zhu, Y.; Wu, M.; Xu, F.; Huang, R.; Moore, R. J.; Guo, P. *Mol. Cell. Probes* **2018**, *41*, 27−31.
(17) Law, J. W.; Ab Mutalib, N. S.; Chan, K. G.; Lee, L. H. *Front. Microbiol.* **2014**, *5*, 770.
(18) Lupindu, A. M. *South. Afr. J Infect. Dis.* **2018**, *33*, 24.
(19) Liu, J.; Zhou, R.; Li, L.; Peters, B. M.; Li, B.; Lin, C.-w.; Chuang, T.-L.; Chen, D.; Zhao, X.; Xiong, Z.; Xu, Z.; Shirtliff, M. E. *Res. Microbiol.* **2017**, *168*, 188−193.
(20) Iguchi, A.; Shirai, H.; Seto, K.; Ooka, T.; Ogura, Y.; Hayashi, T.; Osawa, K.; Osawa, R. *PLoS One* **2011**, *6*, No. e23250.
(21) Franz, E.; Klerks, M. M.; De Vos, O. J.; Termorshuizen, A. J.; van Bruggen, A. H. C. *Appl. Environ. Microbiol.* **2007**, *73*, 2180−2190.
(22) Wang, L.; Reeves, P. R. *Infect. Immun.* **1998**, *66*, 3545−3551.
(23) Barra, G. B.; Santa Rita, T. H.; Mesquita, P. G.; Jácomo, R. H.; Nery, L. F. A. *Genes* **2020**, *11*, 1183.
(24) Park, M.; Won, J.; Choi, B. Y.; Lee, C. J. *Exp. Mol. Med.* **2020**, *52*, 963−977.
(25) Tuttle, J.; Gomez, T.; Doyle, M. P.; Wells, J. G.; Zhao, T.; Tauxe, R. V.; Griffin, P. M. *Epidemiol. Infect.* **1999**, *122*, 185−192.
(26) Liu, Y.; Gilchrist, A.; Zhang, J.; Li, X.-F. *Appl. Environ. Microbiol.* **2008**, *74*, 1502−1507.
(27) Pedersen, S.; Reeh, S.; Friesen, J. D. *Mol. Gen. Genet.* **1978**, *166*, 329−336.
(28) Magalhães, A. P.; França, Â.; Pereira, M. O.; Cerca, N. *Sci. Rep.* **2019**, *9*, 13639.
(29) Liu, Y.; Mustapha, A. *Int. J. Food Microbiol.* **2014**, *170*, 48−54.
(30) Li, L.; Mendis, N.; Trigui, H.; Oliver, J. D.; Faucher, S. P. *Front. Microbiol.* **2014**, *5*, 258.
(31) Lleò, M.; Pierobon, S.; Tafi, M. C.; Signoretto, C.; Canepari, P. *Appl. Environ. Microbiol.* **2000**, *66*, 4564−4567.
(32) Dinu, L.-D.; Bach, S. *Appl. Environ. Microbiol.* **2011**, *77*, 8295−8302.
(33) Fu, Y.; Jia, Y.; Fan, J.; Yu, C.; Yu, C.; Shen, C. *Environ. Microbiol. Rep.* **2020**, *12*, 568−577.
(34) In *Escherichia coli O157:H7 in Ground Beef: Review of a Draft Risk Assessment*, Washington (DC), 2002.
(35) Heiman, K. E.; Mody, R. K.; Johnson, S. D.; Griffin, P. M.; Gould, L. H. *Emerging Infect. Dis.* **2015**, *21*, 1293−1301.

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