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Checkpoint enrichment for sensitive detection of target bacteria from large volume of food matrices

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Abstract

A gap in biosensor development is the ability to enrich and detect targets in large sample volumes in a complex matrix. To bridge this gap, our goal in this work is to propose a practical strategy, termed as checkpoint-style enrichment, for rapid enrichment of the target bacteria from large volume of food samples with particulates and evaluate its enrichment and improvement in detection. The checkpoint-style enrichment was conducted with antibody modified polyethylene terephthalate (PET) pads as capture substrates. In our approach, blended lettuce sample cocktail was circulated through antibody modified PET pads such as a checkpoint in the sample solution pathway, where target pathogens were selectively captured with immobilized antibodies. The obtained PET pads with the captured target pathogens were then used for enhanced detection by colorimetry. To render the checkpoint-style enrichment approach practical and applicable for on-site rapid screening tests, only a simple syringe-based setup with antibody modified PET pad was required. The developed method could process up to 50 ml of lettuce cocktail blended from 5g samples and purposefully inoculated with *E. coli* O157:H7. Overall, the enrichment method developed required only 40 min of sample processing time. After enrichment, as low as 100 CFU/ml of *E. coli* O157:H7 could be detected by a simple colorimetric procedure due to the enhancement from the proposed checkpoint-style enrichment in the presence of ~3000 CFU/ml of non-target bacteria. A linear response was obtained from blank to 100000 CFU/ml of E. coli O157:H7 in blended lettuce samples. The conceptualized approach demonstrates a promising means to improve the detection of target bacteria with a high degree of sensitivity and specificity and could be used in low resourse settings.

Keywords: enrichment, large volume, food matrix, *E. coli* O157:H7, colorimetric recognition

Introduction

Contamination of pathogenic bacteria is one of the major threats to the public health sector. The presence of pathogens in food could potentially lead to serious illness, and ultimately to death. For instance, *E. coli* O157:H7 and the corresponding Shiga toxin could induce mild diarrhea to deadly hemorrhagic colitis along with symptoms comprising of thrombotic thrombocytopenic purpurea and bloody diarrhea [1, 2] resulting in mortality rates as high as 40% [3]. The presence of ultra-low levels of live bacteria in food samples can render the food hazardous. Hence, regulatory requirements call for zero tolerance on pathogens, requiring detection techniques that are sensitive. Most sensing technologies have limitations when testing real food samples. For instance, PCR-based pathogen detection could offer extremely high sensitivity and good specificity but requires sample preparation and trained personnel. [4-6] In contrast, colorimetric strategies are usually rapid, simple, cost-effective and practical, however the unsatisfactory sensitivity makes them less desirable for monitoring pathogens in low numbers.[7-9] Although several detection strategies have been reported [10-18], the challenge to balance detection performance, simplicity of the method, detection time, sensitivity and specificity of the methods.

In food safety monitoring the target pathogens are often present in low numbers in large volume of food samples, necessitating an enrichment step. Basic steps involved in food matrix pretreatment include culturing, filtration, and concentration with nano/micro beads. Culturing is often used to increase the number of bacteria in food samples and usually takes a

long time, lasting from 8 to 48 hours [19-22], limiting its usage in rapid food safety monitoring. However, since the number of bacteria, including the non-target, could increase during culturing to potentially influence subsequent detection steps, additional steps were necessary to specifically enrich the target bacteria. Magnetic nanostructures have been used for selective concentration of target bacteria after modification with the corresponding antibody.[23-26] An external magnet could concentrate the target bacteria in food samples, making it fit for on-site screening. It should be noted that the magnetic force required to extract the target greatly reduces with distance from the capture magnetic particles to the external magnet, thus only a limited volume of samples could be processed by this method. It should be noted that nonspecific binding of food particulates and food constituents in the sample could also influence the magnetic nanoparticle-based enrichment. Filtration is another commonly used method[27-30], which could non-selectively concentrate bacteria from large volume of food matrices in a short operation period [31, 32]. However, it is clear that the particulates in food matrices which are larger than the size of bacteria would also be trapped to interfere with the isolation of target pathogens. A selective pretreatment strategy suitable for processing large volume of food matrices is still elusive.

Utilizing antibodies, selective enrichment of specific pathogens could be achieved. But for ideal enrichment, the key factor is to increase the rate of collision between antibody and target pathogens. Based on this principle, a strategy termed as net fishing utilizing antibody modified porous nitrocellulose (NC) membranes with large surface area-to-volume ratio as capture substrates for selective pathogen enrichment was adopted.[33] During the enrichment, the modified NC membrane was immersed in the sample solution like a net for the capture of

target *E. coli* O157:H7. However, the NC membrane net could only capture the bacteria proximal to it as determined by the diffusion of target bacteria, thus the enrichment required a capture time of 2 hours to process 10 ml of liquid samples. Limited by the size and corresponding surface area of the nitrocellulose membranes, the net fishing approach is not ideal for rapid processing large sample volumes, but still considered an improvement over the existing methods.

To achieve better enrichment, the key is to bring the target bacteria to the antibody conjugated on capture substrates. In this work, a rapid and selective capture of *E. coli* O157:H7 on porous polyethylene terephthalate (PET) pads modified with corresponding antibodies was achieved in large volume of samples. The antibody-modified PET pad was rolled up and loaded in a tube through which samples solution was driven by a syringe. During enrichment, the sample solution was circulated with the syringe and the modified PET pad in the solution pathway acted as a checkpoint: all the components in the sample will pass through the modified PET pad and the target bacteria in the sample would be captured by the antibodies on the pad. The increase in the number of circulation through the PET pads could increase the probability of contact between target bacteria and antibody and hence better enrichment of the targets. With a high flow rate of 8-10 ml/min, the proposed enrichment strategy could process large volume liquid sample in a short time; while the large interspaces of the PET pad would reduce the adherence of non-target materials in the samples, making this method more suitable for handling complex food samples. Compared to our previous diffusion-based net fishing method which required 2 hours for enrichment in diluted pineapple juice which is not as complex as blended lettuce, the current design could enrich

bacteria within 40 min from complex particulate samples such as blended lettuce. Furthermore, our checkpoint-style method could process liquid samples up to 50 ml compared to the previous net fishing method (handles only ~10 ml), which is a significant improvement over existing enrichment methods that can be integrated with the sensing module. The developed checkpoint-style enrichment module has the ability to process volumes as large as 50 ml of particulate samples in less than 40 minutes with a simple syringe-driven approach assisted by colorimetry to detect as low as 100 CFU/ml of *E. coli* O157:H7 in lettuce samples. In contrast, the previous net fishing enrichment methodology takes 2 hours to process a 10 ml of pineapple juice to detect 400 CFU/ml of target bacteria with colorimetric reporting. The advancement in enrichment time, ability to process matrices in larger volume with particulates, and enhancement on the colorimetric signal demonstrates the novelty and significant advances of the checkpoint-style enrichment which considers sample complexity, sample volume, detection sensitivity, and detection time. Furthermore, the proposed method requires no extra instrument or power supply, and is small enough to be portable, indicating the potential for not only on-site use but also its utility in low-resourse settings. Its capability to collect a low number of target bacteria from large volume complex samples in a rapid and simple procedure paves the way for practical implementation not only for food safety assessment, but also in environmental health, and medical diagnostics.

Experimental section

Chemicals

Sodium citrate, casein (sodium salt type) and $HAuCl_4 \cdot xH_2O$ were obtained from Sigma (St. Louis, MO). Polyclonal antibody from goat against *E. coli* O157:H7 (catalog number:

01-05-90) and positive control *E. coli* O157:H7 (catalog number: 50-95-90) were purchased from KPL (Gaithersburg, MD). NaOH was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Sulfo-NHS-LC-Biotin and streptavidin Poly-horseradish peroxidase (HRP) were purchased from Pierce (Rockford, IL). H_2O_2 was obtained from Macron Fine Chemicals (Center Valley, PA). Ethanol (200 proof) was bought from Decon Labs, Inc. (King of Prussia, PA). Tetramethyl benzidine (TMB) was purchased from Moss, Inc. (Pasadena, MD). PET pads (6613) were obtained from Ahlstrom (Holly Springs, PA). LB agar media was obtained from TEKNOVA (Hollister, CA). Biotin-labeled antibody from mouse against goat (catalog number: 31730) and and LB broth was obtained from Fisher Scientific (Waltha, MA). The reagents were used as received. Glasswares used were immersed in fresh aqua regia and washed with DI water.

PET pad modification

The modification of PET pad was performed based on the reported method with slight modification.[34, 35] Briefly, PET pads at 1.1 cm×6 cm were immersed in 50 ml of 1:1 DI water/ethanol mixture with 0.24 g NaOH and the solution was kept in 60 ^{o}C water bath overnight. From the solution, 10 ml of solvent was removed and 10 ml of 30% H_2O_2 was added, then the solution with PET pads was gently shaken and kept in 60 ^{o}C water bath for 5 hours. The obtained PET pads were washed with DI water 6 times and immersed in 5.7 ml of 10 mM MES buffer (pH=4.7). Then 0.3 ml of 400 mM EDC in MES buffer was added and the solution was gently shaken for 1 hour at room temperature, and 6 μl of 1 mg/ml anti-*E. coli* O157:H7 antibody was then added. The chemical reaction process of the modification was discussed in the works from Boxus et al. and Chollet et al..[34-36] Briefly, the PET

polymer chain was broken by hydrolysis with the addition of NaOH to generate hydroxymethyl groups. The obtained hydroxymethyl groups were oxidized by H_2O_2 to carboxyl groups, to which antibodies could be conjugated by EDC reaction. The PET pads were kept in the solution of antibody in MES buffer with EDC under shaking conditions for 24 hours and then washed with 10 mM PBS buffer (pH=7.4) 6 times. The obtained PET pads were immersed in 0.5% casein in 10 mM PBS buffer at 4 ^{o}C until further use.

Gold nanoparticle (GNP) probes preparation

GNPs used as probes were synthesized based on the work by Frens.[37] Briefly, 1 ml of 1% $HAuCl_4$ •xH_2O was added to 100 ml of DI water and heated to boiling. With strong stirring, 0.5 ml of 1% sodium citrate was quickly injected. The color of the solution changed from colorless to red in a few minutes. After boiling for 15 more minutes, the obtained GNPs were cooled down to room temperature and stored at 4^{o}C for subsequent experiments.

Modification of the GNP probes was performed based on our previous works.[12, 13, 33] To 1 ml of GNP solution, 100 µl of 10 mM PB buffer (pH=7.4) was added, followed by injection of 2 µl 0.5 M Na2CO3. After mixing well, 10 µl of 1 mg/ml anti-*E. coli* O157:H7 antibody was added. The obtained solution was gently shaken for 4 hours at room temperature. Then 110 µl of 5% casein in 10 mM PB was added and the mixture was shaken for two hours. The resulting GNPs were centrifuged and washed with 1 ml of 10 mM PBS two times. Then GNPs were redispersed in 1 ml of 10 mM PBS and 10 µg of Sulfo-NHS-LC-Biotin was added. The mixture was gently shaken for 1 hour and mixed with 100 µl of 5% casein in 10 mM PB. After 1 hour of shaking, the modified GNPs were centrifuged and washed with 10 mM PBS two times. The obtained GNP probes were

redispersed in 100 μl of 5% casein in 10 mM PBS and kept at 4^{o}C until use. Characterization of the GNP probes is provided in the Supporting Information and Reference 13.

Pathogen enrichment from lettuce sample

Briefly, 25 g of lettuce purchased from a local store was blended with 225 ml of 10 mM PBS for 1 min. The obtained mixture was filtered to remove the large solid lettuce particulates to prevent blockage of modified PET pads. Serial concentrations of *E. coli* O157:H7 were then purposefully added and the samples were processed by the proposed checkpoint-style enrichment.

To collect *E. coli* O157:H7, the modified PET pad was rolled up and loaded into a rubber tube, which was linked to a 60 ml syringe (as in Scheme 1A). The tube was immersed in 50 ml of the sample. The sample solution was circulated with the syringe through the pad roll. The pad roll was then removed from the tube and rinsed with DI water.

Evaluation of capture efficiency of the proposed enrichment

Following a classic procedure, plate count was performed to evaluate the capture efficiency of the proposed enrichment. As shown in Figure S1, results indicated that in the processed lettuce sample, approximately 3280±230 CFU/ml of non-target pathogens were present before inoculation of target pathogen, which influenced the overall plate count determination of the target *E. coli* O157:H7. Capture efficiency was assessed with autoclaved lettuce samples, which were then inoculated with target *E. coli* O157:H7. The checkpoint-style enrichment was performed with the inoculated lettuce samples and the concentration of target bacteria left in the samples was determined by plate count. For plate count, 100 μl of the inoculated lettuce samples after the enrichment with the optimized

circulation times was spread on agar surface and incubated at 37 ^{o}C for 24 hours. The concentration of target bacteria in the samples without the proposed enrichment was also tested (control). The difference in concentration of the target *E. coli* O157:H7 between the enriched samples and control were the captured bacteria.

Colorimetric detection of *E. coli* O157:H7 with PET pads

After processing samples by the syringe-driven approach, the modified PET pad was taken out from the rubber tube and unrolled. Then the excess moisture in the pad was drained off with a tissue. The pad was next immersed in 1.5 ml of 10 mM PBS, followed by a 10 min sonication step to remove the non-target materials from lettuce. After rinsing, the pad was immersed in 475 µl of GNP probe solution (GNP probe stock solution mixed with 1% tween 20 in 0.5% casein in 10 mM PBS, well mixed with a pipette before use) and incubated for 10 min. The interaction between GNP probes and modified PET pads was checked based on the absorbance at 450 nm in the UV-vis spectra of the GNP probe solution before and after incubation. The results were included in Supporting Information. After the rinse and dry steps, 475 µl of HRP solution (10 µg/ml mixed with 1% tween 20 in 0.5% casein in 10 mM PBS, well mixed with a pipette before use) was added and incubated for 10 min. The pads were then rinsed and dried 3 times. To the pads, 1 ml of TMB dilution (200 µl of TMB mixed with 800 µl 10 mM PBS) was added. After 30 min of incubation, the UV-vis spectra were recorded with a Thermo Scientific Nanodrop One spectrometer and the absorbance at 650 nm was used to determine the concentration of *E. coli* O157:H7 in the samples.

Results and discussion

The basic concept of the proposed checkpoint-style enrichment is provided in Scheme 1.

The NaOH and H_2O_2 treated PET pads were conjugated with antibodies. The modified pads were then rolled and loaded into a rubber tube linked to a 50 ml syringe as shown in the inset photo in Scheme 1A. The syringe was then used to drive all the sample solution through the antibody modified PET pad with large interspaces in opposite directions. The operation of the proposed enrichment and corresponding circulation of solution is shown in Scheme 1B. It should be noted that the modified PET pad was loaded at the far end of the rubber tube which was connected to the syringe to occupy the entire cross-section of the solution pathway in the rubber tube. Since all the sample solution passes through the rubber tube driven by the syringe, the antibody modified PET pad roll in the rubber tube acts as a checkpoint because all the sample solution has to pass through the PET pad. In this approach, the target bacteria will be captured on the pad due to antibody specificity, while other non-target materials will pass due to the large interspaces of the PET pad. The circulation of sample solution through the modified PET pad would increase the probability of contact between target bacteria and the conjugated antibody for better enrichment; meanwhile, the large interspaces and the flow of sample solution in opposite direction would result in minimal influence of other components and particulates in the food matrix.

To confirm antibody modification of the PET pads, the modified pad was reacted with HRP conjugated anti-goat antibody shown as the inset in Figure 1. After washing, the pads were immersed in the substrate for TMB reaction. Blue color was generated in the solution and on the pads which were modified with anti-*E. coli* O157:H7 antibody. Figure 1 shows photos of the pads with and without antibody modification and UV-vis spectra from the corresponding solution. It can be seen that the pads modified with antibodies exhibited a clear

blue color, while the pads without antibodies retained the original color of the pad. The UV-vis spectra showed a distinct peak from the enzymatic reaction with the antibody modified pads. In contrast, no distinct peak was observed from the pads without antibodies. Results in Figure 1 clearly establishes the modification of antibodies on the PET pad.

To investigate the capture efficiency of the proposed checkpoint-style enrichment in complex food matrices, blended lettuce cocktail, was purposefully inoculated with target *E. coli* O157:H7. Lettuce was used as a model food system to demonstrate the methodology in complex matrices, where the blocking from non-target materials, the viscosity of the blended cocktail and the influence of the activity of capture antibody will challenge the enrichment as one might expect when processing real samples. It can be inferred that the capture efficiency would relate to the flow speed through the antibody modified PET pads which determines the reaction time of capture. The proposed checkpoint-style strategy was operated with a hand-driven syringe, where the solid non-target particulate materials in food samples could block the interspaces of the antibody modified PET pads, potentially affecting the flow as determined by the nature of food samples. Thus, it is hard to precisely control the flow speed of the blended lettuce during the proposed enrichment. The checkpoint-style enrichment in this work was performed at the highest possible speed (8-10 ml/min). To determine the number of captured target *E. coli* O157:H7, plate count was performed with samples after enrichment for different circulation times (the output after the first pass from the tube is used as input). Capture efficiency was calculated in relation to the concentration of the control samples. It should be noted that in the un-inoculated lettuce samples, around 3280±230 CFU/ml of non-target colonies were found as shown in Figure S1. Therefore, during plate

count the liquid lettuce samples were autoclaved to exclude influence from these non-target colonies present in the original lettuce. Then the samples were inoculated with live *E. coli* O157:H7 at an average final concentration of 81±52 CFU/ml in 50 ml lettuce solution samples. Figure 2 shows the estimated capture efficiency with respect to circulation times during enrichment. The concentrations of target in the treated and control samples was determined by plate count and normalized with respect to the initial concentration. The capture efficiency (CE) was calculated as:

$$CE = (C_{control} - C_{checkpoint\text{-}style\ enrichment})/C_{control},$$

where $C_{checkpoint\text{-}style\ enrichment}$ is the concentration of target bacteria in the samples after different circulation times, while the $C_{control}$ is the concentration of control samples tested at the same time after the corresponding circulation. It can be seen that after the very first circulation, on an average a 36.4% capture efficiency was achieved, suggesting that fresh PET pads with antibody modified surface unblocked by non-target materials could offer a significant enrichment from 50 ml of lettuce samples. With more number of circulations, the capture efficiency increased though not as much as that from the first circulation, which could be attributed to the accumulation of non-target materials from food samples on antibody modified PET pads. It should be noted that when the samples were circulated 4 - 6 times the increase in capture efficiency was not as significant, suggesting an interference from non-target particulates trapped on PET pads. A corresponding change in the color of antibody modified PET pads, from white to light green was observed as an indicator of the accumulation of solid particulates from lettuce samples onto the PET pads.

The proposed checkpoint-style enrichment approach was used for enrichment as a

pre-detection step integrated with the detection unit. First, the target bacteria at low concentration in the original food matrix were concentrated to achieve higher detection sensitivity. Second, our approach separated the target bacteria from most of the non-target materials in the food sample with minimal interference from these materials at the detection step. However, some accumulation of non-target materials on the PET pads after enrichment was noted. To investigate the detection performance with the target-enriched PET pads, an enhanced colorimetric detection method was carried out. As shown in Figure 3A, antibody modified GNPs were labeled on the captured target bacteria and then linked to HRP to provide a colorimetric signal from an enzyme-amplified reaction. In colorimetric detection, the attached particulates would also affect the GNP probe labeling and HRP conjugation. Compared to our former net fishing enrichment work[33], the proposed checkpoint-style enrichment is fundamentally different because the capture mechanism is different. Further, a significant decrease in sample processing time, improvement in detection sensitivity and increased sample throughput with significant complexity was demonstrated. Different from the plate count, the lettuce samples used in the combined checkpoint-style enrichment-colorimetric detection procedure were not autoclaved, thus the influence of the non-target bacteria in the original lettuce samples was also included.

To study the effect of circulation on the colorimetric detection of target bacteria, results from non-autoclaved blank lettuce samples and samples intentionally inoculated with 100 CFU/ml of target bacteria were collected at different circulation times. The adsorption at 650 nm in the UV-vis spectra from the generated colorimetric product from TMB reaction was used as the colorimetric signal. From Figure 3B, it can be seen that with different circulation

times a stronger absorbance at 650 nm was noted from the processed samples with 100 CFU/ml of the target, compared to that from blank. Moreover, when the number of circulations increased from 1 to 3 times, a significant increase in absorbance at 650 nm was noted in the samples with 100 CFU/ml of target bacteria, which is consistent with the capture efficiency results shown in Figure 2. The non-zero signals from blank with serial circulation times could be assigned to the non-specific binding of GNP probes and HRP on the modified PET pads. When circulated six times, the signal from blank and 100 CFU/ml samples decrease significantly compared to the results with 4-time circulation, which could be attributed to the influence of the accumulation of non-target components on the PET pads.

To demonstrate the improvement due to enrichment in colorimetric detection, signals from the pads when the sample was circulated 4 times and those simply immersed in sample solution were compared, and the results are as shown in Figure 3C. The incubation time of 40 minutes for the pads immersed in the sample solution was similar to the 4-time circulation process which also took ~40 minutes. It can be seen that by simply immersing the antibody modified pads in the sample solution no significant difference in the signal was observed between the signals from the blank and 100 CFU/ml samples. While the proposed enrichment resulted in a marked increase in signals from 100 CFU/ml samples, establishing an improved detection sensitivity. Furthermore, considering the presence of 3280±230 CFU/ml of non-target pathogens in the lettuce sample, the achieved detection of 100 CFU/ml *E. coli* O157:H7 in the food samples suggested that the enrichment combined with colorimetric detection could be used to enrich and specifically detect targets in a large volume of food matrix.

After enrichment, the materials adhered on the PET pads could affect the colorimetric signal generation at the detection step. To understand this effect, different durations of sonication to remove the attached non-target materials from PET pads were performed after capture and the corresponding results are shown in Figure 4A. It can be seen that 10 minutes of sonication yielded the best sensitivity to detect 100 CFU/ml of the target. This shows that with sonication the interference from other components in the food matrix could be reduced to detect 100 CFU/ml of *E. coli* O157:H7. It is noted that longer sonication could also result in the detachment of the captured target bacteria.

Non-specific binding of GNP probes and HRP could influence detection sensitivity. To further query our enrichment approach, the effect of accumulated non-target components on the PET pad was examined for non-specific binding. The comparison of results from with and without blocking of the PET pads with casein and tween 20 are shown in Figure 4B. It can be seen that the signals from detection without blocking are stronger than that with tween 20 and casein. Meanwhile, without tween 20 and casein, the blank samples provided a slightly stronger signal than that from 100 CFU/ml, indicating the influence from non-specific binding of GNP probes and HRP on the PET pads. Our results suggested that the attachment of non-target materials present in food samples may not reduce non-specific binding of probes and HRP and further blocking with casein and tween 20 is necessary for detection.

With the optimized checkpoint-style enrichment and colorimetric signal generation conditions, including circulation times of sample solution in the enrichment system, sonication time, blocking agents and other detection conditions, the proposed enrichment could assist in the differentiation of 100 CFU/ml of *E. coli* O157:H7 in lettuce from blank

samples based on the absorbance in UV-vis spectra with a *p*-value of 0.048. With these optimized conditions, lettuce samples inoculated with serial concentrations of bacteria were processed and tested. The colorimetric signal change 'S' with respect to concentration is given by,

$$S = (I-I_0)/I_0,$$

where I is the absorbance at 650 nm from the positive samples and I_0 is the absorbance at 650 nm from blank samples. The detection results obtained are shown in Figure 5. Results show that as low as 100 CFU/ml of *E. coli* O157:H7 could be recognized in lettuce samples compared to blank. The S value increased with the concentration of target bacteria from blank to 10000 CFU/ml, suggesting the potential for quantification. Meanwhile, it should be noted that the increase in S from 10,000 CFU/ml to 100,000 CFU/ml is not as apparent (from 1.16±0.14 at 10,000 CFU/ml to 1.33±0.24 at 100,000 CFU/ml) as that in the concentration of pathogen less than 10,000 CFU/ml (from 0 of blank to 1.16±0.14 at 10,000 CFU/ml), which could be attributed to the saturated status of the PET pads.

The specificity of the proposed checkpoint-style enrichment with colorimetric detection was evaluated and the results were shown in Figure 6. Utilizing the same enrichment and detection procedure, 1000 CFU/ml of *Salmonella typhimurium*, *Listeria monocytogenes* and non-pathogenic *E. coli DH5-alpha* in 50 ml lettuce samples did not give a significant colorimetric response compared with 100 CFU/ml of target *E. coli* O157:H7. The obvious difference in colorimetric response from target bacteria and non-target bacteria indicated excellent specificity of our enrichment approach along with the colorimetric detection.

Conclusion

A simple enrichment strategy was proposed to selectively and rapidly concentrate target pathogens from a large volume of food samples. The sample solution was passed through antibody modified PET pads by repeated circulations, and the target bacteria were captured by antibodies immobilized onto the pads. The PET pads have larger size interspaces and the sample solution was circulated through the pads in opposite direction. Thus, the amount of non-target components adhering to the pads is minimized, facilitating efficient enrichment. The proposed strategy does not require complex instrumention, and can be utilized for on-site monitoring of pathogens in food samples. An excellent capture efficiency was demonstrated based on plate count with serial circulations of sample solution through the antibody modified PET padsWith a 40-minute enrichment, as low as 100 CFU/ml of *E. coli* O157:H7 could be detected in 50 ml of blended lettuce samples containing ~3000 CFU/ml of non-target bacteria. A possible influence from the accumulated non-target materials in the checkpoint-style enrichment from food samples to the detection protocol was also investigated. The proposed checkpoint-style method was found to be effective, selective and rapid to process large sample volumes (>50 ml). Its capability to process complex food samples exhibited the potential for routine screening of pathogens in food matrices. More importantly, the proposed enrichment module has the versatility to be combined with other analytical tools for enhanced diagnostics.

Conflicts of interest

There are no conflicts of interest to declare.

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Captions

Scheme 1. Concept of the proposed checkpoint-style pre-concentration method (A), images of circulation driven by a 50 ml syringe (B).

Figure 1. Characterization of anti-*E. coli* O157:H7 antibody modification on PET pads by HRP-conjugated secondary antibody with images and UV-vis spectra. The inset illustrates the label of anti-*E. coli* O157:H7 antibody with HRP-conjugated secondary antibody.

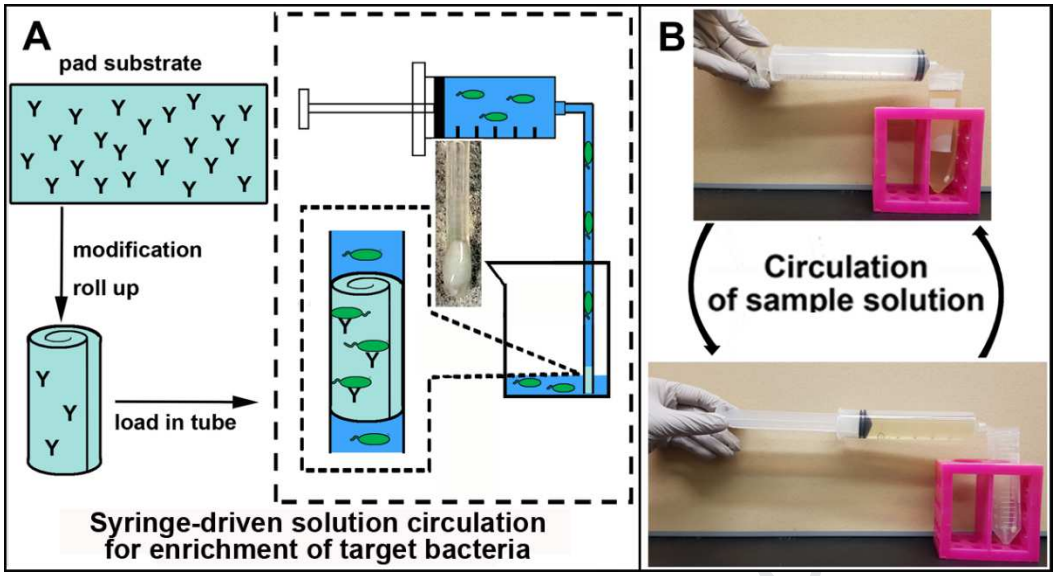
Figure 2. Capture efficiency of *E. coli* O157:H7 in lettuce samples with respect to circulation times during checkpoint-style enrichment.

Figure 3. Schematic of colorimetric determination based on pads with captured target *E. coli* O157:H7 after enrichment (A). Colorimetric signals from blank and 100 CFU/ml of lettuce samples at different circulation times (B), and comparison between 4-time circulation and simple incubation of modified PET pads in sample solution (C).

Figure 4. Absorbance at 650 nm from blank and 100 CFU/ml of lettuce samples with different sonication time (A) and labeling solution (B).

Figure 5. Colorimetric response of the proportional concentration of *E. coli* O157:H7 in 50 ml of lettuce samples.

Figure 6. Colorimetric response assisted with checkpoint-style enrichment from 100 CFU/ml of *E. coli* O157:H7 (A), 1000 CFU/ml of *Salmonella typhimurium* (B), 1000 CFU/ml of *Listeria monocytogenes*, and 1000 CFU/ml of non-pathogenic *E. coli* DH5-alpha in 50 ml of lettuce samples.



Scheme 1.

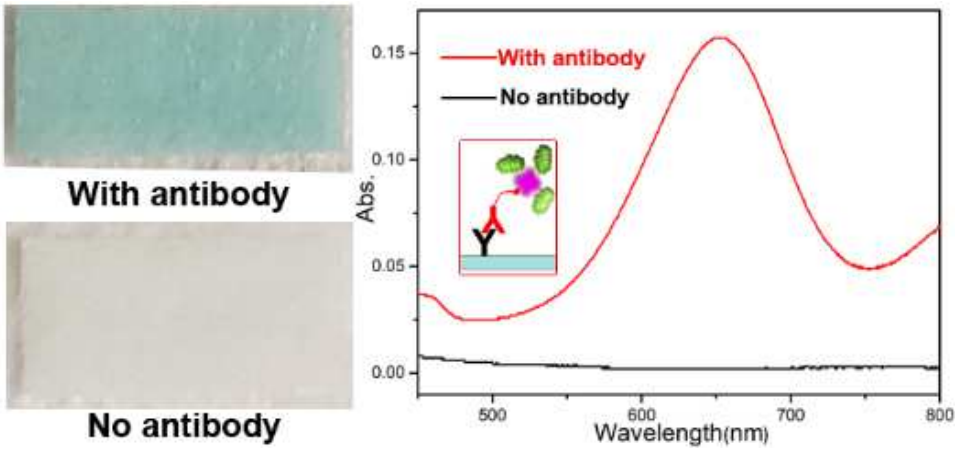


Figure 1.

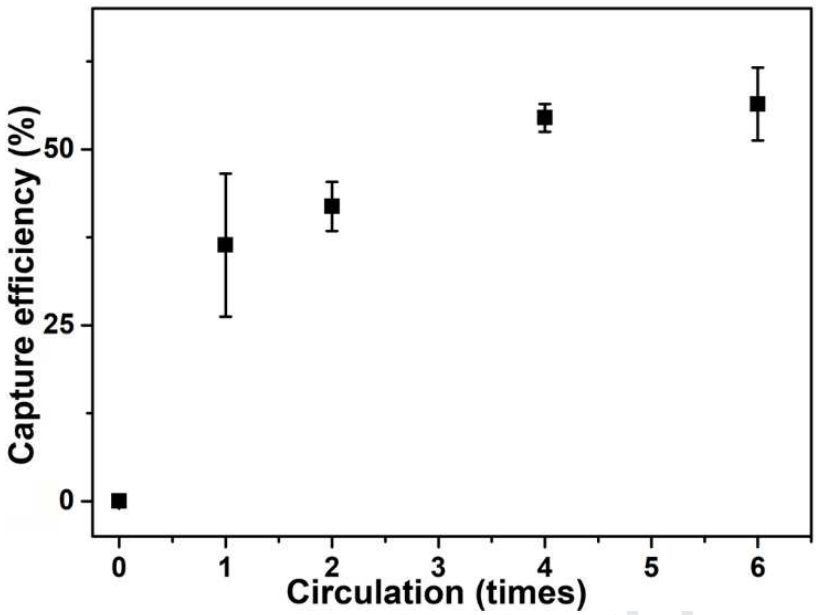


Figure 2.

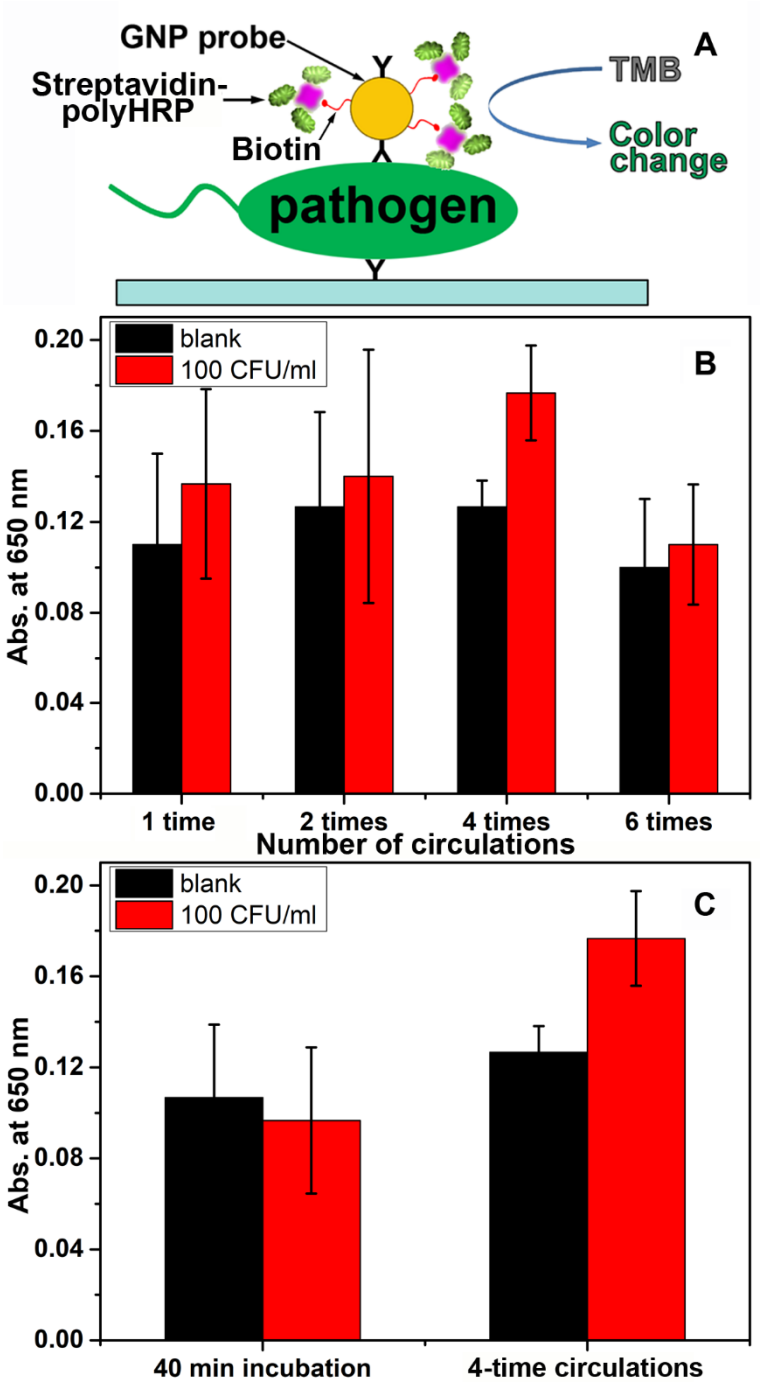

GNP probe
Streptavidin-polyHRP
Biotin
pathogen
TMB
Color change
A
B
blank
100 CFU/ml
Abs. at 650 nm
0.00
0.04
0.08
0.12
0.16
0.20
1 time
2 times
4 times
6 times
Number of circulations
C
40 min incubation
4-time circulations

Figure 3.

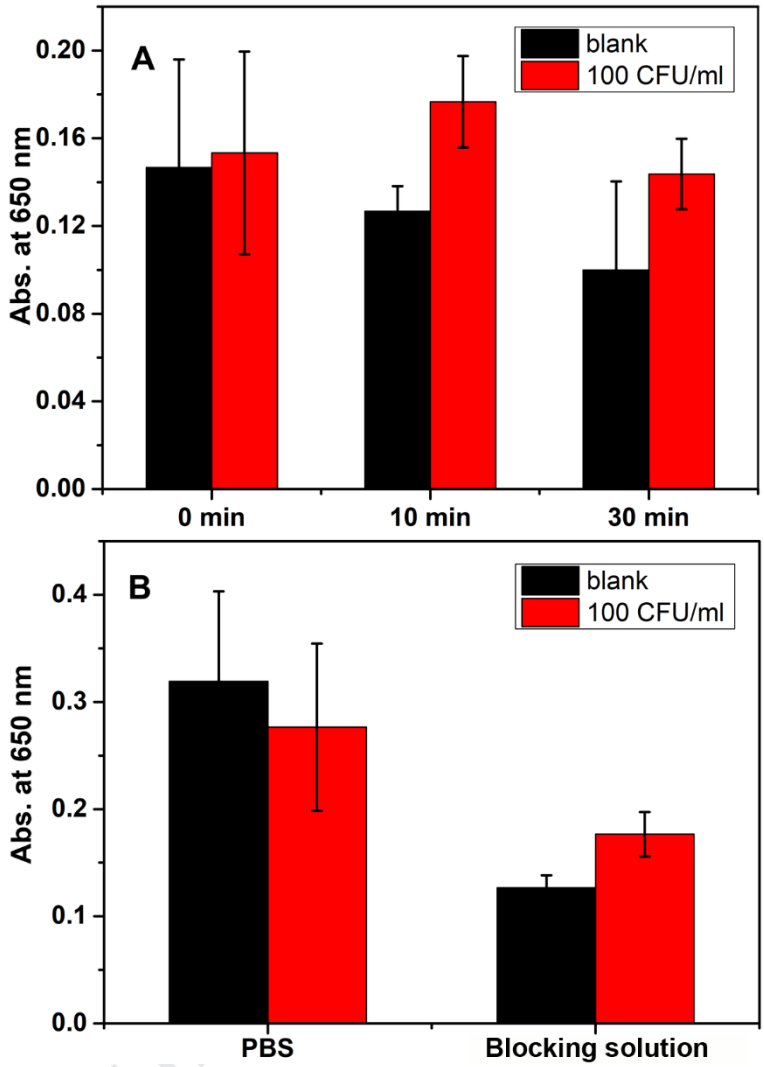
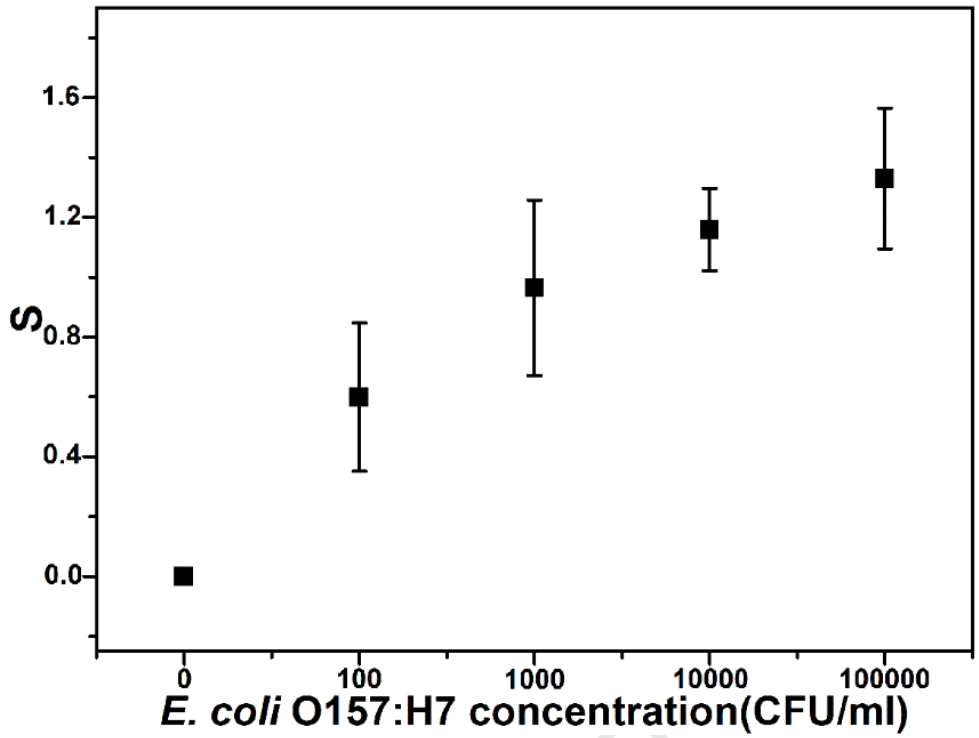

A
blank
100 CFU/ml
Abs. at 650 nm
0.00
0.04
0.08
0.12
0.16
0.20
0 min
10 min
30 min
B
blank
100 CFU/ml
Abs. at 650 nm
0.0
0.1
0.2
0.3
0.4
PBS
Blocking solution

Figure 4.



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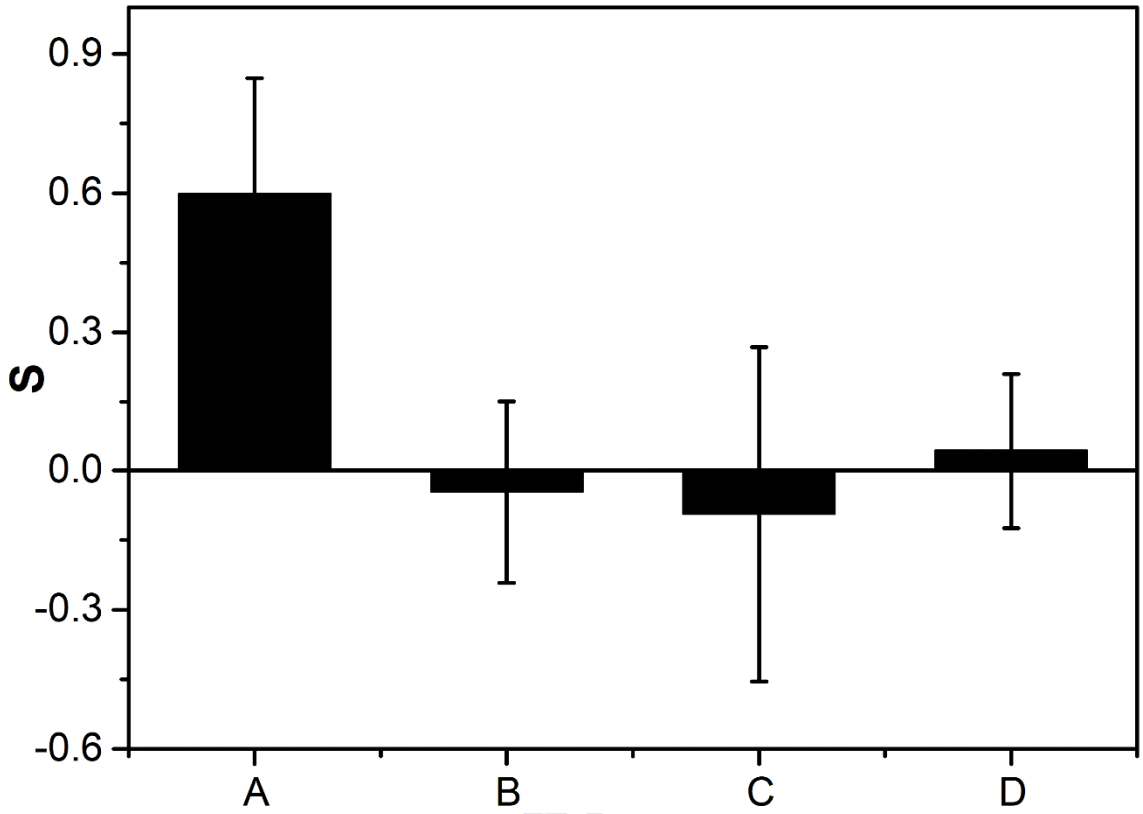
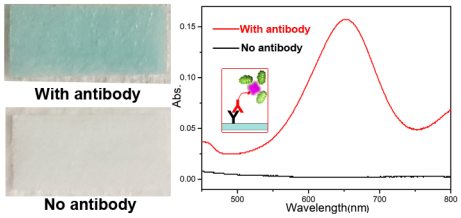
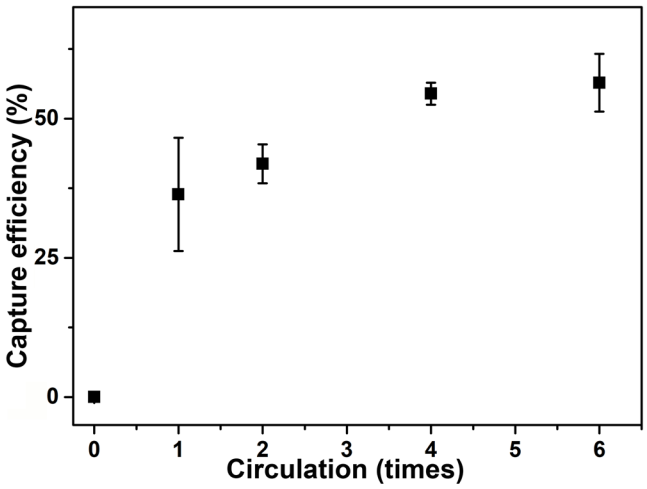
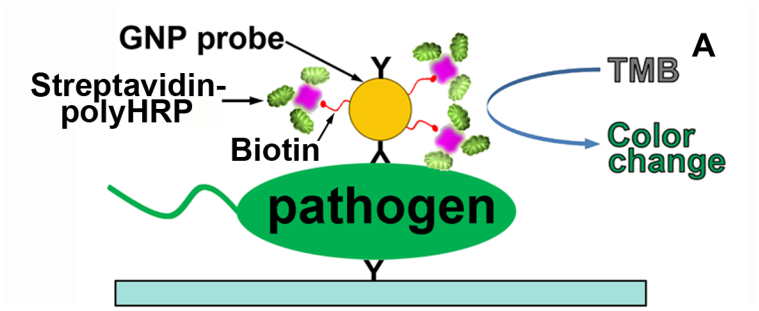
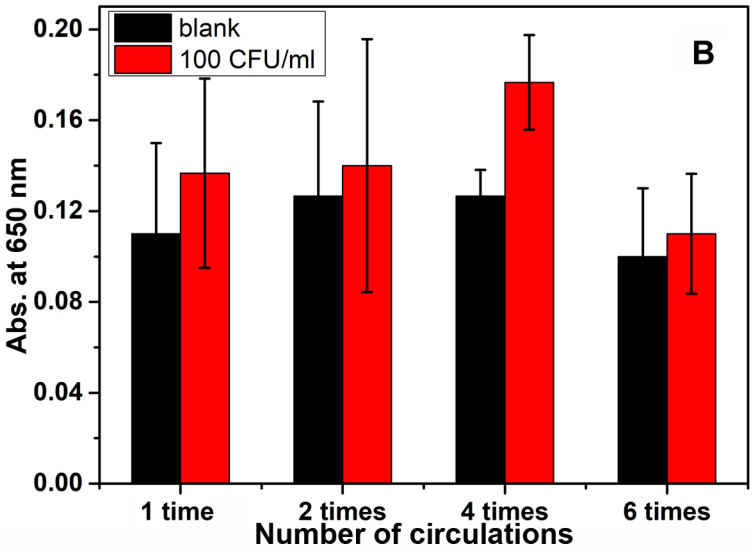


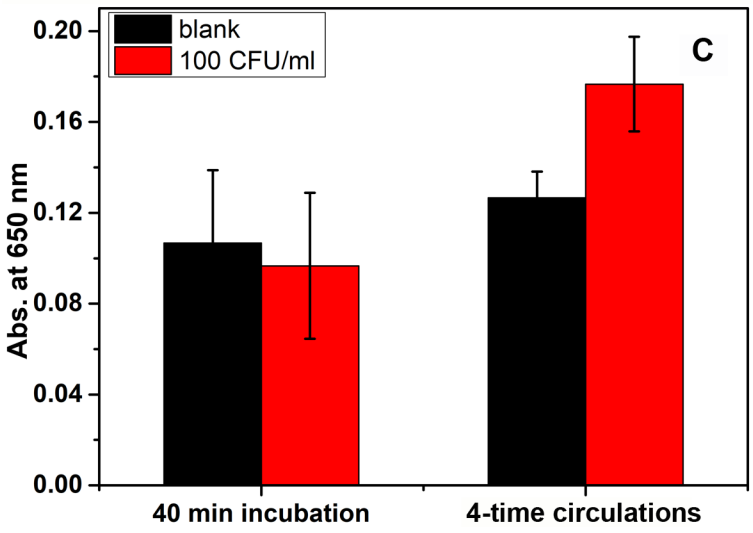
Figure 6.

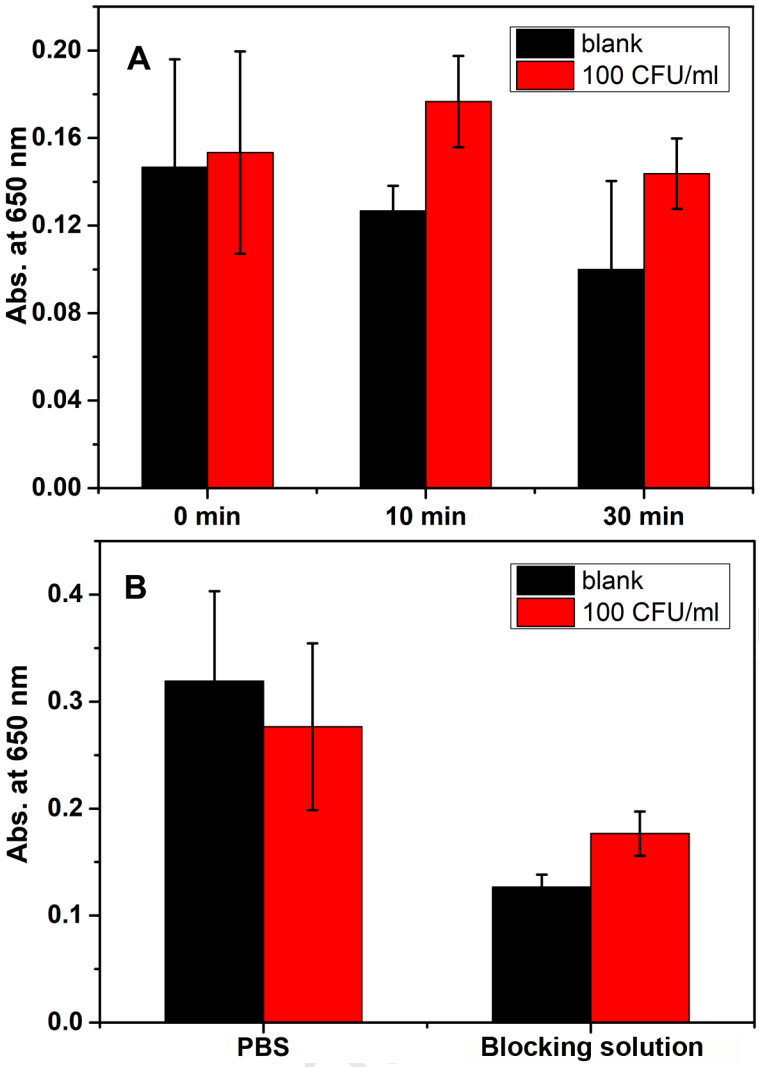


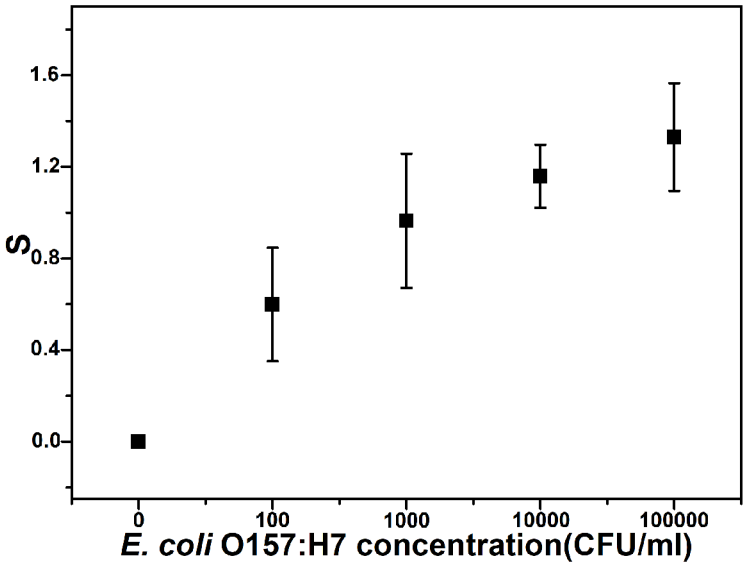


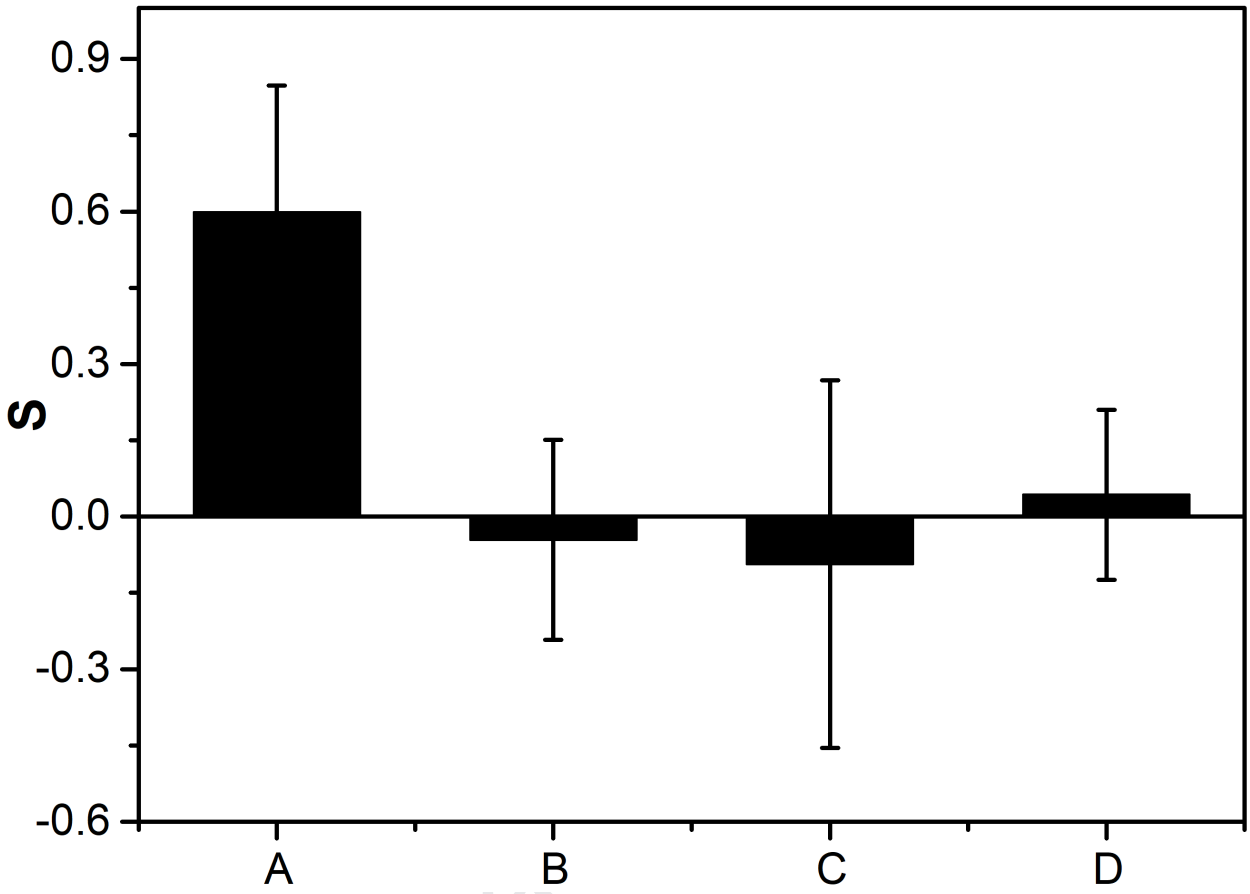

GNP probe
Streptavidin-
polyHRP
Biotin
pathogen
TMB
Color
change
A

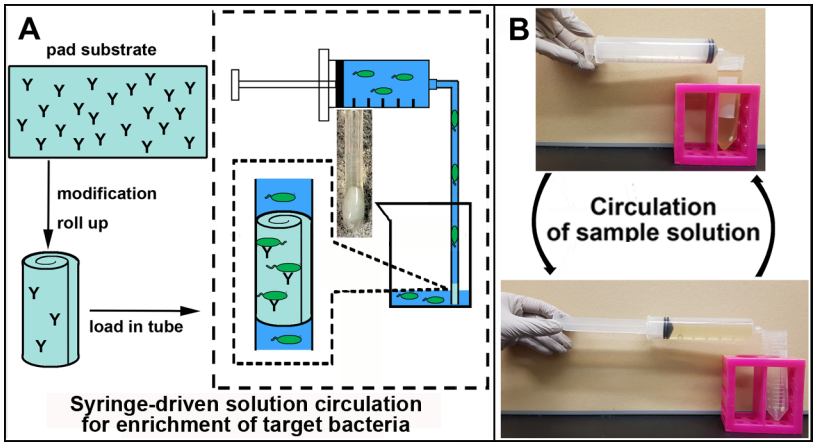

blank
100 CFU/ml
B
Abs. at 650 nm
0.20
0.16
0.12
0.08
0.04
0.00
1 time
2 times
4 times
6 times
Number of circulations


blank
100 CFU/ml
C
Abs. at 650 nm
0.20
0.16
0.12
0.08
0.04
0.00
40 min incubation
4-time circulations


A
blank
100 CFU/ml
Abs. at 650 nm
0.00
0.04
0.08
0.12
0.16
0.20
0 min
10 min
30 min
B
blank
100 CFU/ml
Abs. at 650 nm
0.0
0.1
0.2
0.3
0.4
PBS
Blocking solution







- Technology for enrichment and detection of *E. coli* O157:H7 in complex food samples
- Enrichment of pathogens in a large volume of up to 50 ml of food samples in 40 min
- Detection of *E. coli* O157:H7 in blended lettuce cocktail at 100 CFU/ml based on absorbance from UV-vis spectra
- On-site monitoring of infectious pathogens for food safety, disease monitoring and environmental health

Wen Ren: Conceptualization, Methodology, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing

Abigail Cabush: Investigation

Joseph Irudayaraj: Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: