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Immunomagnetic Capture of *Bacillus anthracis* Spores from Food

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ABSTRACT

Food is a vulnerable target for potential bioterrorist attacks; therefore, a critical mitigation strategy is needed for the rapid concentration and detection of biothreat agents from food matrices. Magnetic beads offer a unique advantage in that they have a large surface area for efficient capture of bacteria. We have demonstrated the efficient capture and concentration of *Bacillus anthracis* (Sterne) spores using immunomagnetic beads for a potential food application. Magnetic beads from three different sources, with varying sizes and surface chemistries, were functionalized with monoclonal antibodies and polyclonal antibodies from commercial sources and used to capture and concentrate anthrax spores from spiked food matrices, including milk, apple juice, bagged salad, processed meat, and bottled water. The results indicated that the Pathatrix beads were more effective in the binding and capture of anthrax spores than the other two bead types investigated. Furthermore, it was observed that the use of polyclonal antibodies resulted in a more efficient recovery of anthrax spores than the use of monoclonal antibodies. Three different magnetic capture methods, inversion, the Pathatrix Auto system, and the new *i*CropTheBug system, were investigated. The *i*CropTheBug system yielded a much higher recovery of spores than the Pathatrix Auto system. Spore recoveries ranged from 80 to 100% for the *i*CropTheBug system when using pure spore preparations, whereas the Pathatrix Auto system had recoveries from 20 to 30%. Spore capture from food samples inoculated at a level of 1 CFU/ml resulted in 80 to 100% capture for milk, bottled water, and juice samples and 60 to 80% for processed meat and bagged salad when using the *i*CropTheBug system. This efficient capture of anthrax spores at very low concentrations without enrichment has the potential to enhance the sensitivity of downstream detection technologies and will be a useful method in a foodborne bioterrorism response.

Food is vulnerable to intentional contamination, and the tainting of salad bars in the United States with *Salmonella* Typhimurium highlights this *(31)*. Recent accidental outbreaks of *Escherichia coli, Salmonella, Listeria,* and botulinum toxin in the North American food supply further demonstrate the vulnerability of our food supply *(29)*. While we have enjoyed a greater diversity in food choice, we have become even more susceptible to terrorist threats due to our global food sourcing *(7, 10)*. There is an increasing need for rapid sample preparation procedures in order to detect accidental and intentional contamination with foodborne pathogens and/or biothreat agents, such as *Bacillus anthracis*. The complexity of food matrices makes this a significant challenge, considering the presence of proteins, fats, and carbohydrates that can interfere with immunochemistry. The separation of biothreat agents, such as *B. anthracis* spores, from sample particulates, removal of inhibitory compounds associated with the food matrix, and provision of sample size reduction with recovery of virtually all of the spores need to be addressed.

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B. anthracis is a spore-forming bacterium and the causative agent of anthrax, which can infect grazing herbivores, such as cattle and bison *(36)*. However, the 2001 *Amerithrax* attacks (inhalation anthrax) in the United States, in which 22 people were infected and 5 died, highlighted the use of anthrax as a bioterrorist weapon *(15)*. In humans, the ingestion of anthrax spores can lead to gastrointestinal anthrax, which can be fatal. In Africa, gastrointestinal anthrax occurs occasionally through ingesting contaminated meat *(20, 26)*. Of particular concern is milk, as the typical pasteurization process has been shown to have little effect on the viability of anthrax spores *(11, 19, 23),* and studies have indicated that the pasteurization process actually enhances *Bacillus* spp. spore germination and growth in milk *(11, 23)*.

The current ''gold standard'' for pathogen isolation and detection is bacteriological culture, which is time-consuming, taking several days to obtain a positive identification *(3)*. Immunomagnetic beads (IMB) are playing an increasing role in the design of biosensing systems and have been commonly used for the isolation and concentration of target organisms in food sample preparation over the last 20 years *(1, 2, 28, 37)*. Significant improvements have been made by the introduction of immunomagnetic separation (IMS) after

TABLE 1. *Immunomagnetic bead properties*

Immunomagnetic particles	Size (μm)	Functional group
NRC-A beads	0.150	Carboxylic acid and poly(ethylene glycol)
NRC-B beads	0.164	Carboxylic acid
BcMag carboxyl-terminated magnetic beads	1	Carboxylic acid
Pathatrix beads with custom coating	~1	Carboxylic acid

the enrichment step, which facilitates downstream detection using methods such as PCR by isolating the pathogen of interest from background bacterial contamination. While enrichment and IMS have significantly improved detection time and sensitivity over culture, from several days to 10 to 24 h, there is still room for improvement *(5, 6, 33)*. Furthermore, IMS brings an advantage in terms of the design of novel foodborne pathogen detection strategies. The main impediment to achieving a high level of recovery has been interference from fats, proteins, and viscosity of food matrices that can impair recovery of IMB prior to enrichment steps and can carry over to have detrimental effects on downstream detection strategies *(14, 16)*.

In this study, several IMB with varying sizes and surface chemistries were functionalized with different polyclonal and monoclonal antibodies and were evaluated for the specific and efficient capture of *B. anthracis* spores from food. Several magnetic capture procedures, utilizing powerful rare earth magnets, were used to enhance the recovery of *B. anthracis* spores from bottled water, apple juice, whole milk, ham, and bagged salad. Further improvements have also been made by the added use of filtration systems to remove particulate material from food and dilution of highly viscous foods that aided in our ability to enhance recovery of pathogens from complex food matrices without an enrichment step. Here, we present a rapid and highly efficient method of sample preparation that enables specific separation of *B. anthracis* (Sterne) spores from food at concentrations as low as 1 CFU/ml.

MATERIALS AND METHODS

Bacterial spore and cell preparation. *B. anthracis* Sterne or *Bacillus cereus* ATCC 14579 strains were grown overnight on tryptic soy agar (Difco, BD, Sparks, MD) supplemented with 5% sheep blood (TSBA) at 37°C. Three colonies were selected, transferred into 5 ml of tryptic soy broth (Difco), and incubated overnight at 37°C. From the overnight culture, 100 μl was inoculated into a sterile polystyrene culture flask containing 50 ml TSBA. The inoculum was dispersed by the addition of 15 g of 4-mm glass beads. The flasks were incubated at 37°C for 1 to 2 weeks. Sporulation was verified by endospore staining with malachite green. Spores were harvested by adding 10 ml of phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBST) and rocking the glass beads over the agar surface to resuspend the spore culture. The suspension was transferred to a conical tube and centrifuged at 5,000 × *g* for 15 min at 4°C to pellet the spores. The supernatant was discarded, and the pellet was washed five times with PBST. To kill the residual vegetative cells, the pellet was resuspended in 40 ml of 50% ethanol in PBST and placed on a rocking shaker at 100 rpm for 60 min. The spores were centrifuged and resuspended in 40 ml of 0.01% PBS with 1% bovine serum albumin (BSA; pH 7.0) and centrifuged again. The spores were adjusted to a concentration of 10^9 to 10^{11} spores per ml in PBS with 1% BSA and stored at −20°C until use.

E. coli ATCC 25922 was grown overnight in tryptic soy broth at 37°C to a concentration of ~10^9 and serially diluted in PBS with 1% BSA to determine the actual CFU per milliliter.

Magnetic bead functionalization with anti–*B. anthracis* antibodies. Pathatrix magnetic beads, supplied with their custom coating kit (Matrix MicroScience Ltd., Newmarket, UK), were functionalized with anti–*B. anthracis* antibody as described in their custom coating kit protocol, with slight modifications. Briefly, 50 mg of magnetic beads with carboxyl functional groups were suspended in 1 ml of buffer A and mixed for 15 min at room temperature. The beads were pelleted using a magnetic particle concentrator, washed in buffer B, resuspended in 1 ml of buffer C containing anti–*B. anthracis* antibody at a concentration of 1 mg/ml, and placed on an orbital shaker at 300 rpm for 6 h at room temperature. The beads were washed three times with buffer C and resuspended in buffer D with 1% BSA (filter sterilized). The solution was placed on the orbital shaker at 300 rpm for 30 min and then washed three times in buffer D. The beads were resuspended to a final concentration of 20 mg/ml in buffer D and stored at 4°C until use.

Three other types of magnetic beads (NRC-A beads and NRC-B beads, fabricated by the National Research Council, Ottawa, Ontario, Canada) *(22)* and BcMag carboxyl magnetic beads purchased from Bioclone, Inc. (San Diego, CA), were tested after being functionalized using the same anti–*B. anthracis* antibody. The properties of these beads are shown in Table 1. The same functionalization method was used for all three types of magnetic beads. In a microcentrifuge tube, aliquots containing 50 mg of suspended beads were pelleted using a magnetic particle concentrator, and the storage buffer was removed. Magnetic beads were resuspended in 250 μl of freshly made ethyl(dimethylaminopropyl) carbodiimide (EDC)–*N*-hydroxysuccinimide (NHS) (Sigma-Aldrich, St. Louis, MO) solution (20 mg/ml EDC, 30 mg/ml NHS in PBS, pH 8.0) and placed on a shaker for 15 min at low speed. One milligram of rabbit anti–*B. anthracis* immunoglobulin G (IgG) antibody (Tetracore, Rockville, MD) was added to the magnetic beads to a final concentration of 1 mg/ml, and the solution was placed on a shaker for 6 h at room temperature. Ten microliters of 1 M hydroxylamine (Sigma-Aldrich) was added to the solution, and the solution was placed on the shaker for another 15 min. The magnetic beads were then pelleted and washed three times with 1 ml of PBS (100 mM $NaPO_4$, 140 mM NaCl, pH 8.0). One milliliter of blocking buffer (50 mM Tris, 140 mM NaCl, 1% BSA, pH 8.0) was added to the magnetic particles and mixed by inversion using a Revolver (Labnet International Ltd., Woodbridge, NJ) for 30 min at room temperature. The functionalized beads were washed four times with wash buffer (50 mM Tris, 140 mM NaCl, 0.05% Tween 20, pH 8.0), resuspended at a final concentration of 20 mg/ml in PBS, and stored at 4°C until use.

Comparison of immunomagnetic capture methods for *B. anthracis* spores. Three methods to capture *B. anthracis* spores

in food samples were tested. These included the use of inversion, the Pathatrix Auto system (Matrix MicroScience Ltd.), and the *i*CropTheBug system (FiltaFlex Ltd., Almonte, Ontario, Canada). All three methods used 1 mg of Pathatrix IMB functionalized with rabbit anti–*B. anthracis* IgG and 50 ml of spore suspension (buffered peptone water with 1% Tween 20 [pH 7.2; BPWT] containing either 2.5 or 25 CFU/ml of *B. anthracis* spores).

For the inversion method, the beads and spore suspension were placed in a 50-ml conical tube. The solution was mixed by inversion using the Revolver for 60 min at room temperature. The conical tube was placed on a magnetic separation stand (Novagen, EMD Chemicals, Gibbstown, NJ) for 10 min and the supernatant was removed. The pelleted beads were resuspended in 1 ml of wash buffer (50 mM Tris, 140 mM NaCl, 0.05% Tween 20, pH 8.0) and transferred to a microcentrifuge tube. The beads were washed three times in washing buffer, resuspended in PBS, plated on TSBA plates (TSBAP), and incubated overnight at 37°C for colony enumeration.

For the Pathatrix Auto system, the beads and spore suspension were added to the sample vessel and tubing and 35 ml of BPWT was used for washing within the system. The mixing cycle was run for a total of 60 min at room temperature, and the magnetic beads were transferred to a microcentrifuge tube. The beads were washed three times in washing buffer, resuspended in PBS, and enumerated as described above.

For the *i*CropTheBug system, the beads and spore suspension was placed in a 250-ml disposable Erlenmeyer flask and incubated for 60 min at room temperature on an orbital shaker. The beads were then pelleted from the solution by placing the flask on the *i*CropTheBug IMB collector, a powerful settling magnetic platform that pellets the magnetic beads. The magnetic beads were collected using the IMB pipette and transferred to a microcentrifuge tube. The magnetic beads were washed three times using washing buffer and resuspended in PBS, and colonies were enumerated as described above. All the above-described capture experiments were repeated three times in duplicate.

Comparison of different antibodies for the capture of *B. anthracis* spores. Four different antibodies (polyclonal rabbit anti–*B. anthracis* IgG [Tetracore], polyclonal goat anti–*B. anthracis* IgG [Tetracore], monoclonal antibody (MAb) anti-anthrax 8G4 [Tetracore], and monoclonal anti-anthrax, clone 2C3 MAb 8300 [Millipore, Billerica, MA]) were investigated for both specificity and sensitivity to *B. anthracis* spores. The antibodies were separately used to functionalize the NRC-A beads using the protocol described above. One milligram of functionalized beads was mixed with 1 ml of BPWT containing *B. anthracis* spores (10^2, 10^3, and 10^4 CFU/ml), *B. cereus* spores (10^4 CFU/ml), or *E. coli* cells (10^4 CFU/ml) and captured using the inversion method. Bacterial recovery was measured by plating captured beads on TSBAP and enumerating the colonies as described above. The experiment was run once in duplicate for each antibody.

Comparison of different IMB for the capture of *B. anthracis* spores. Three different beads (Pathatrix beads, NRC-B beads, and BcMag carboxyl magnetic beads) (Table 1) were functionalized with polyclonal rabbit anti–*B. anthracis* IgG antibody as described above and tested for both specificity and sensitivity to *B. anthracis* spores. Using the *i*CropTheBug bead mixing and capture method (described above), 1 mg of functionalized beads was mixed with 50 ml of BPWT containing *B. anthracis* spores (0.5, 2.5, 25, and 250 CFU/ml), *B. cereus* spores (250 CFU/ml), or *E. coli* cells (250 CFU/ml). Bacterial recovery was measured by plating captured beads on TSBAP and enumerating colonies as described above. Experiments involving each bead were repeated twice in duplicate.

Preparation of spiked food samples. Bottled water, apple juice, whole milk (3.25% milk fat), black forest ham processed meat, and prewashed (romaine lettuce) bagged salad were purchased from a local grocery store and used for the food spiking experiments. *B. anthracis* Sterne spores, *B. cereus* ATCC 14579 spores, and *E. coli* ATCC 25922 cultures were adjusted to a concentration of 10^9 and serially diluted to 10^3 to 10^5 CFU/ml in PBS containing 1% BSA. Spores or cells were added to 25 ml of bottled water, apple juice, or whole milk to achieve an inoculation of 1, 5, 50, or 500 CFU/ml *B. anthracis* alone or a combination of *B. anthracis* with *B. cereus* and *E. coli*. The bottled water and milk samples were diluted with 25 ml of BPWT, and the apple juice sample was diluted with 25 ml of BPW containing 0.2 M Na_2HPO_4 and 1% Tween 20 (pH 8.0). The apple juice acidity was adjusted to enhance the capture efficiency as previously reported *(30)*.

For spore capture in processed meat, 50 g of sliced black forest ham was cut into 1-cm^2 pieces and placed into a stomacher bag. The samples were inoculated with 1, 5, 50, or 500 CFU/g *B. anthracis* spores and hand massaged to evenly distribute the spores throughout the ham. Fifty milliliters of BPWT (pH 7.2) was added (1:1 dilution wt/vol), and the mixture was stomached with the Stomacher 400 Circulator (Seward Ltd., Worthing, UK) for 2 min at 230 rpm. The liquid portion of the mixture was transferred to a filter stomacher bag to remove any food debris. The liquid was further passed through a sponge filter and a 50-μm stainless steel mesh filter using a vacuum pump, and the filtrate was collected.

For bagged salad, 50 g of salad greens was weighed into a stomacher bag and inoculated with 1, 5, 50, or 500 CFU/g *B. anthracis* spores. The samples were hand massaged to evenly distribute the spores throughout the salad. Fifty milliliters of BPWT (pH 7.2) was added (1:1 dilution, wt/vol), and the mixture was hand massaged for another 1 min. The liquid portion of the mixture was transferred through a filter stomacher bag and placed into 50-ml Falcon tubes. The tubes were placed in a water bath at 80°C for 10 min to reduce bacterial contaminants. The liquid was then passed through a sponge filter and 50-μm stainless steel mesh filter using a vacuum pump, and the filtrate was collected.

Immunomagnetic capture of *B. anthracis* from spiked food samples. Following the preparation of spiked food samples, 50 ml of the prepared food sample was mixed with 1 mg (50 μl) of Pathatrix beads functionalized with rabbit anti–*B. anthracis* polyclonal antibody. The beads were mixed and captured according to the *i*CropTheBug method described above. Bacterial recovery was evaluated by plating captured beads on TSBAP and enumerating colonies. Experiments involving each food matrix and spore concentration were repeated three times in duplicate.

Data analysis. Data were analyzed by dividing the total number of spores captured by the total number of spores added to the food samples and expressing this as a percentage. The total number of spores added was determined by plate enumeration of prepared spore stock prior to each run. Standard deviations were calculated from the mean results of the replicate experiments.

RESULTS

Evaluation of different beads and antibodies for the capture of *B. anthracis* spores. The polyclonal antibodies showed very high sensitivity for *B. anthracis* (recovery efficiencies of 90 to 100%), with the rabbit anti–*B.*

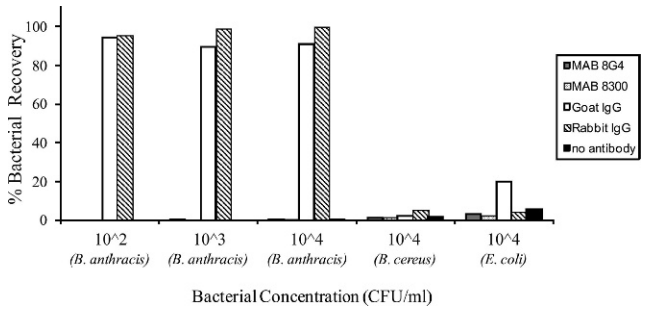

FIGURE 1. *Comparison of different antibodies for the capture of* B. anthracis *spores. NRC-A IMB were functionalized separately with polyclonal rabbit anti–*B. anthracis *IgG (rabbit IgG), goat anti–*B. anthracis *IgG (goat IgG), monoclonal anti-anthrax (MAb 8G4), and anti-anthrax clone 2C3 (MAb 8300) antibodies and used for the capture and recovery of* B. anthracis *Sterne spores using the* i*CropTheBug system. The percentage of bacterial recovery was determined by plating on TSBAP and incubating at 37°C overnight.*

anthracis antibody showing relatively higher levels of recovery than the goat anti–*B. anthracis* antibody (Fig. 1). The recovery of nonspecific organisms was low for both the rabbit anti–*B. anthracis* and goat anti–*B. anthracis* antibodies (5 and 2% for *B. cereus* spores and 4 and 20% for *E. coli,* respectively) (Fig. 1). The MAbs were ineffective in the capture of *B. anthracis* spores and also showed lower recoveries for *B. cereus* and *E. coli* than the polyclonal antibodies (Fig. 1). The Pathatrix beads functionalized with polyclonal rabbit anti–*B. anthracis* antibody and tested for recovery efficiency using the *i*CropTheBug method showed the most sensitive IMB-antibody combination while maintaining a low level of nonspecificity (Fig. 2).

Evaluation of spore capture methods. The Pathatrix Auto system showed low recoveries of 31.9 and 17% for 25 and 2.5 CFU/ml, respectively, whereas the inversion method and *i*CropTheBug system had recoveries of >80% for *B. anthracis* spores (Fig. 3). Samples containing a bead-to-liquid ratio of 1:50 (wt/vol) (1 mg of beads:50 ml of BPWT and 5 mg of beads:250 ml of BPWT) showed similar recoveries of >90%, whereas a ratio of 1:250 (1 mg of beads:250 ml of BPWT) decreased the recovery to ~50% (Fig. 3).

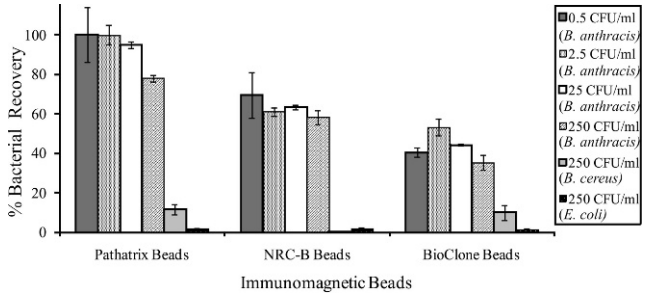

FIGURE 2. *Comparison of different IMB for the recovery of* B. anthracis *spores. Different IMB were functionalized with rabbit polyclonal anti–*B. anthracis *antibody and compared for the recovery of* B. anthracis *Sterne spores using the* i*CropTheBug system. The percentage of bacterial recovery was determined by plating on TSBAP and incubating at 37°C overnight. Error bars represent standard deviations based on the results of two different experiments.*

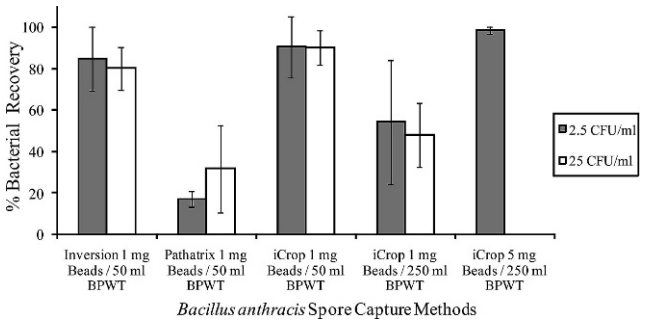

FIGURE 3. B. anthracis *spore recovery methods. Comparison of the inversion method, Pathatrix Auto system, and* i*CropTheBug system for capture of* B. anthracis *Sterne spores (2.5 or 25 CFU/ml) in BPWT with varying amounts of functionalized beads. The percentage of bacterial recovery was determined by plating on TSBAP and incubating at 37°C overnight. Error bars represent standard deviations based on the results of three different experiments. 25 CFU/ml in 250 ml of BPWT per 5 mg of beads was not tested with the* i*CropTheBug system.*

Food studies. Whole milk had recovery efficiencies ranging from 80 to 100%, bottled water had 93 to 98%, and apple juice was between 84 and 93% (Fig. 4). Processed meat (ham) and bagged salad spiked with *B. anthracis* spores had recovery efficiencies ranging from 65 to 78% (Fig. 4). An additional heating step was required for the bagged salad to reduce the contaminating bacteria as *B. anthracis* colonies were very difficult to enumerate due to high background interference, particularly at low inoculation levels.

B. anthracis spore recovery (1, 5, 50, and 500 CFU/ml) in apple juice in the presence of large numbers of both *B. cereus* spores (500 CFU/ml) and *E. coli* cells (500 CFU/ml) showed recoveries of 60 to 88% of the spores. Recovery of the non–*B. anthracis* spores was very low, approximately 5% for *B. cereus* spores and less than 1% for *E. coli* (data not shown).

DISCUSSION

Despite the recent advances in foodborne pathogen detection *(21, 34),* the concentration of organisms of interest

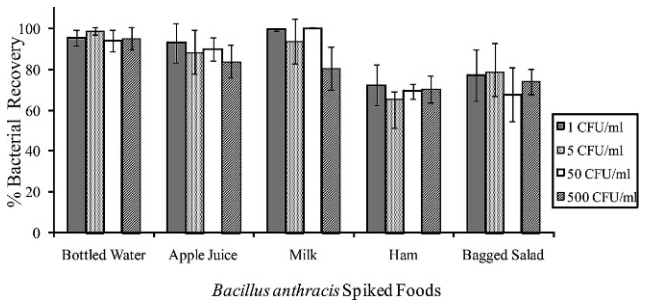

FIGURE 4. *Recovery of* B. anthracis *spores from spiked food. Food was spiked with* B. anthracis *Sterne spores and mixed with IMB. The IMB were recovered using the* i*CropTheBug system. The percentage of bacterial recovery was determined by plating on TSBAP and incubating at 37°C overnight. Error bars represent standard deviations based on the results of three different experiments.*

from complex food matrices still poses a significant challenge, and the sensitivity of downstream detection technologies hinges on this. The use of several rapid detection platforms may be expanded if the bacteria can be efficiently separated, concentrated, and purified from the food matrix prior to detection. The advent of IMS applications is becoming a key focus for foodborne pathogen detection, and the potential benefits of this technology in terms of enhancing the sensitivity and specificity of downstream detection are receiving growing attention *(13, 27, 37)*.

The comparison of polyclonal antibodies and MAbs in this study clearly showed that the polyclonal antibodies outperformed the MAbs for *B. anthracis* spore capture (Fig. 1). Polyclonal antibodies generally provide the ability to recognize multiple antigen epitopes, thus allowing for a higher sensitivity for the target organism *(12)*. The polyclonal rabbit anti–*B. anthracis* IgG antibody used has been tested by Tetracore against 145 *B. anthracis* isolates from around the world with 143 testing positive, 49 nonhemolytic non–*B. anthracis Bacillus* species with all testing negative, and 52 hemolytic non–*B. anthracis Bacillus* species genetically closer to anthrax, wherein 12 were positive and 40 negative (http://tetracore.com/pdfs/Tetracore_RedLine_Alert_Test.pdf).

The testing of the three different magnetic beads (Table 1) functionalized with rabbit anti–*B. anthracis* IgG polyclonal antibody using the *i*CropTheBug method indicated that the Pathatrix beads outperformed the other bead types in terms of spore recovery (Fig. 2). The size of the IMB and its surface chemistry appear to be the most important characteristics that enhance recovery and specificity for the *B. anthracis* spores *(32)*. The size of the bead is important because a critical aspect of any magnetic confinement assay is the magnetophoretic mobility of the IMB itself. Simply confining the beads used in this study suggests that the beads with diameters at 1 μm or more can be magnetically isolated faster than the smaller NRC-B beads (0.164 μm) (Table 1). This suggests that larger beads should perform better; however, it has been shown that smaller magnetic beads (0.5 to 1 μm) have larger surface-to-volume ratios and bind more antibody and hence are able to capture more bacteria *(24)*. Conversely, smaller beads also contain less magnetic material in their cores than larger particles *(25),* as was demonstrated by the comparatively low recovery of the NRC-B magnetic particles. Additionally, and perhaps more importantly, smaller particles present in large numbers are capable of better surrounding the bacteria (i.e., more small nanoparticles can bind to the bacteria surface), potentially enhancing the magnetic capture *(9, 17, 24)*. Despite the fact that the surface area–to–volume ratio of the smaller NRC-B beads (0.164 μm) is significantly greater than that of the Pathatrix beads (~1 μm), the Pathatrix beads performed better. It is not clear why the performance of the BcMag beads was not comparable to that of the Pathatrix beads. This may be due to other unknown properties associated with the BcMag beads. In this case, the Pathatrix beads appear to provide the best combination of size and magnetic content which results in the effective capture of spores.

The *i*CropTheBug system, which has been previously used for the concentration of norovirus in ready-to-eat foods *(18),* was shown to perform better than the Pathatrix Auto system *(1, 35)* in our current study. This could be due in part to the nature of the instruments, where with the Pathatrix Auto system, the IMB are trapped by the magnets prior to coming into contact with the whole sample solution, which decreases the chance of contact between IMB and bacteria (http://www.matrixmsci.com/). The inversion method and *i*CropTheBug system allow for free movement of the IMB during the mixing, thus increasing the chance of IMB coming into contact with the target bacteria. The *i*CropTheBug system has an added advantage in being flexible and allowing scaling up and processing of greater sample volumes without compromising recovery (Fig. 3).

Recent publications using molecular methods such as real-time PCR in combination with enrichment culture and IMS have been shown to have detection limits for foodborne pathogens within the range of 10^0 to 10^3 CFU/ml or g *(4, 8, 9)*. More recently, advances have been made in sample preparation methods that do not require culture enrichment for sensitive detection. Previously, we demonstrated detection of *Yersinia pestis* at levels of 10^1 to 10^3 CFU/ml in milk and 10^2 to 10^5 CFU/g in ground beef using a combination of IMS and a simple boiling lysis protocol *(1)*. Previous reports also indicate a limit of detection of 10^3 CFU/ml for the immunomagnetic detection of *Bacillus stearothermophilus* spores from food and environmental samples *(2)*. These studies indicate that the use of IMS procedures that leave samples relatively free of PCR inhibitors and a well-validated method of DNA extraction can match the detection limits observed when using IMS and culture enrichment. We found that we could effectively recover as little as 1 CFU/ml in liquid food samples or 1 CFU/g in solid food samples in 1.5 h without enrichment, which would enable us to go from sample to identification in an 8-h shift depending on the detection system being used, such as real-time PCR. Eliminating the need for enrichment would allow food testing laboratories to truly harness the benefits of many emerging detection technologies.

In conclusion, the efficient capture and recovery of *B. anthracis* spores (1 CFU/ml) from food without enrichment and the high specificity of the assay demonstrated a significant improvement and should potentially enhance the sensitivity and specificity of downstream detection platforms, such as the use of biosensors and nucleic acid-based technologies. Furthermore, the use of the *i*CropTheBug system in this study is novel for *B. anthracis* spore capture in food and offers a new tool for sample preparation in biodefense applications.

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