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Serogroup Variation with Use of Immunomagnetic Separation to Detect and Isolate Shiga Toxin-producing *Escherichia coli* O157 and the Big Six Non-O157

ABSTRACT

Immunomagnetic separation (IMS) is a technique used for detection and isolation of Shiga toxin-producing *E. coli* (STEC) O157 and the six major non-O157 serogroups. Official testing protocols for STEC used by the U.S. Department of Agriculture (USDA) include IMS to aid with the recovery of presumptive positive cells. This study assessed the magnetic beads capture efficiency for the detection of STEC O157 and non-O157. IMS was performed to separate cell cultures in enrichment broth and in inoculated ground beef, using different bacterial concentrations. For *E. coli* O111, IMS required at least 5.0 log CFU/ml of the microorganism to be present in the sample, which suggests that improvement of the anti-O111 magnetic beads is needed. Other serogroups required 3.0 log CFU/ml for accurate detection. After reducing the bead solution volume to 50%, IMS effectively ($P < 0.05$) recovered the target cells when the minimum detection limit of the microorganism was present in the sample. Cell recovery using IMS may be affected by the target STEC serogroup and not by the bead volume used, which could be reduced to half. Therefore, it is important to acknowledge that IMS should be used for cell isolation rather than microbial detection.

INTRODUCTION

STEC O157 was first recognized as a foodborne pathogen in the U.S. in 1982 after two major outbreaks associated with ground beef that caused several cases of hemorrhagic colitis *(13)*. The pathogen became a major concern to the public after a deadly multistate outbreak in 1993, which was associated with a national fast food chain in the U.S. *(4)*. During 2000 and 2010, FoodNet reported a total of 7,694 cases of *E. coli* O157 and non-O157 infections *(10)*. According to the Centers for Disease Control and Prevention (CDC) morbidity and mortality report, STEC infections, including O157 and non-O157 O groups, accounted for an estimated 1,082 cases, 275 hospitalizations, and 2 deaths in the United States during 2012 *(3)*. This situation led the USDA Food Safety and Inspection Service (FSIS) to declare STEC O157:H7 in ground beef and subsequently non-O157 serogroups (O26, O103,

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O45, O121, O111, and O145), commonly known as the "big six," as adulterants in non-intact beef products in 1994 and 2012, respectively *(27)*. Subsequently, FSIS implemented routine testing for the regulated STEC serogroups, particularly in ground beef and other non-intact beef products *(26, 27)*. Although use of molecular methods such as PCR is very common for STEC detection, isolation of viable colonies is frequently carried out using conventional techniques and is required to confirm positive results.

The ability to isolate presumptive STEC colonies is perhaps the primary challenge with STEC methodology, particularly when low numbers of microorganisms are present or when high concentrations of background microflora exist in the sample. One of the techniques most commonly used to facilitate STEC isolation is IMS, a method regarded as highly effective, even when used with samples having a high background microflora *(7)*. Hence, the technique is part of the FSIS STEC detection and isolation protocol once potential positive results are obtained via PCR *(28)*. IMS recovers target cells from the enrichment cultures using paramagnetic beads coated with polyclonal antibodies specific for STEC serogroups; the bead size ranges from 1 to 4 µm in diameter, with an average of 2.5 µm. Beads are mixed with the enrichment, incubated to allow binding to the cell-surface antigen, and subjected to repeated rinsing to remove non-specifically attached cells. An antibody-antigen complex is formed, and a magnetic field is used to capture and recover target cells *(21)*. Magnetic beads labeled with antibodies for O157 and the big six non-O157 STEC are commercially available *(1, 24)*. Because IMS efficiency may vary from serogroup to serogroup, the focus of this study is to evaluate differences in cell recovery for each serogroup.

The IMS method is efficient for bacterial cell recovery when the appropriate enrichment conditions are provided to the microorganisms *(8)*. Chapman et al. (1994) indicated that IMS is more sensitive than direct culture on selective agar and increases about 100-fold the ability to recover *E. coli* O157 from artificially inoculated bovine fecal samples *(6)*. Despite this, the technique works more efficiently when higher bacterial loads of the microorganisms are present in the sample *(11)*; hence, magnetic bead manufacturers recommend that a concentration greater than 100 cells per ml be present for STEC detection. This implies that lower bacterial concentrations in the sample may lead to false negative results.

The goals of this study were to evaluate the differences in capture efficiency of the magnetic beads for each of the regulated STEC serogroups (O157 and the big six non-O157), and to evaluate if the detection limits of IMS per serogroup is affected by modifying the bead volume used to perform the technique.

MATERIALS AND METHODS

Bacterial strains

Strains used for this study originated from human and animal sources *(Table 1)*. Strains were maintained frozen at -80°C in tryptic soy broth (TSB – EMD Millipore Chemicals; Darmstadt, Germany) with 10% glycerol. A 10 µl aliquot was transferred from the stock culture into a 9-ml TSB tube and incubated for 24 h at 37°C; a subsequent transfer of 10 µl into fresh TSB was performed.

Immunomagnetic separation

Antibody-coated magnetic microspheres were used to perform IMS. For STEC serotypes O157, O26, O103, O111 and O145, commercially available paramagnetic beads were obtained from one manufacturer (Dynabeads®, Invitrogen; Carlsbad, CA), while paramagnetic beads for serotypes O121 and O45 were obtained from a different supplier (Abraxis; Warminster, PA). IMS was conducted using an automated system (BeadRetriever™. Invitrogen; Carlsbad, CA) that captures the target microorganisms bound to antibody-coated magnetic beads by utilizing a pre-programmed magnetic bead processor. Instructions provided by the manufacturer of the bead retriever were followed, and the same procedure was applied to all serogroups as recommended by the bead suppliers. After the IMS process was completed, 50 µl of the bacteria-bead complex was plated onto tryptic soy agar (TSA. EMD Millipore Chemicals; Darmstadt, Germany) or mRainbow Agar (mRBA, Rainbow agar USDA recipe, MLG appendix 1.08), and incubated for 24 h at 37°C. Recovered colonies were always confirmed using latex agglutination tests specific to each O group, (Oxoid Ltd., Hants, UK) for the STEC O157, O26, O103, O111 and O145, and Abraxis (Warminster, PA) for O121 and O45). Total STEC concentration (CFU/ml) recovered after IMS from each sample was estimated by enumeration of colonies grown on the respective solid media used.

Sample preparation to conduct IMS

Cultures in enrichment broth

For each STEC serogroup (O157, O26, O103, O111, O145, O121, and O45), five-strain bacterial cocktails were prepared separately by combining 2-ml aliquots of each strain and homogenizing with a vortex mixer for about 5 sec. Serial dilutions were prepared in 9-ml buffered peptone water (BPW. EMD Millipore Chemicals; Darmstadt, Germany) to achieve the desired final culture concentrations of 1.0, 2.0, 3.0, 4.0, or 5.0 log CFU/ml. Bacterial concentrations in the sample subjected to IMS were confirmed during each repetition by plating on TSA and incubating for 24 h at 37°C. Colonies were enumerated and reported as CFU/ml. Log CFU/ml conversion was done as needed.

Ground beef

A five-strain cocktail of *E. coli* O157:H7 was prepared as previously described. The bacterial cocktail was centrifuged

TABLE 1. Strains used to prepare different bacterial cocktails

Serogroup/ serotype	Strains	Source	Confirmed genes
O157:H7	ATCC 43889	Human – HUS patient	*stx2*
O157:H7	ATCC 43894	Human – HC outbreak	*stx1 stx2*
O157:H7	A4 966	Not reported	Not reported
O157:H7	A5 528	Not reported	Not reported
O157:H7	I 040	Bovine feces	*stx2, eae, ehxA*
O26	7.1556	Ground beef	*stx* and *eae* negative
O26:H11	CSU FSL E1 022	Human	*hly, eae*
O26:H11	CSU FSL E1 126	Human	*hly, stx1, stx2, eae*
O26	CSU FSL E1 127	Human	*hly, stx1, eae*
O26	CSU FSL E1 128	Human	*hly, stx1*
O111	4.0005	Bovine feces	*stx1, stx2, eae*
O111:NM	CSU FSL E1 001	Not reported	*hly*
O111:H21	CSU FSL E1 151	Human	*hly, stx1*
O111	CSU FSL E1 152	Human	*hly, stx1, eae*
O111	CSU FSL E1 153	Human	*hly, stx1, eae*
O45:H3	CSU FSL E1 134	Human	*hly, stx1, eae*
O45:H25	CSU FSL E1 135	Human	*hly, stx1*
O45	CSU FSL E1 136	Human	*stx1, stx2*
O45	CSU FSL E1 139	Bovine feces	*hly, stx1*
O45:H2	CSU FSL E1 140	Bovine feces	*hly, stx1, eae*
O145	9.0538	Ground beef	*stx1, eae*
O145:H28	CSU FSL E1 165	Human	*hly, stx1, eae*
O145	CSU FSL E1 166	Human	*hly, stx1, stx2, eae*
O145	CSU FSL E1 167	Human	*hly, stx2, eae*
O145	CSU FSL E1 171	Bovine feces	*hly, stx2, eae*
O121	CSU FSL E1 155	Human	*hly, stx2*
O121	CSU FSL E1 156	Human	*hly, stx2*
O121	CSU FSL E1 157	Human	*hly, stx2*
O121	CSU FSL E1 158	Human	*hly, stx1, eae*
O121	CSU FSL E1 159	Human	*hly, stx1*
O103	97.1241	Bovine feces	*stx1, eae*
O103:H2	CSU FSL E1 142	Human	*hly, stx1*
O103	CSU FSL E1 143	Human	*hly, stx1, stx2*
O103	CSU FSL E1 144	Human	*hly, stx1, stx2, eae*
O103	CSU FSL E1 145	Human	*hly, stx1, eae*

(Centrifuge 5804R – Eppendorf AG; Germany) for 10 min at 5,000 rpm, RCF 4,500 × g, 4°C; the supernatant was discarded and cells re-suspended to the original volume with BPW. Ground beef was obtained from the Texas Tech University G.W. Davis Meat Laboratory at the Department of Animal and Food Sciences, an FSIS-inspected facility, and kept refrigerated at 4°C until used in the experiment. Five-gram aliquots of the meat were weighed, placed into

10 × 20 cm sterile bags, and inoculated to reach target bacterial concentrations of 2.0, 3.0, and 4.0 log CFU/g. The meat was thoroughly hand mixed for about 1 min and then refrigerated for 30 min to allow bacterial attachment to the ground beef. Bacterial concentrations in the inoculated beef samples were confirmed prior to IMS by plating on TSA and mRBA and incubating for 24 h at 37°C. Colonies were enumerated and reported as CFU/g. Log CFU/g conversion was done as needed.

IMS sensitivity

Assays were conducted using cultures in enrichment broth. IMS was performed individually for each of the seven target serogroups, using the five-strain cocktails previously mentioned. Bacterial concentrations of 1.0, 2.0, 3.0, 4.0, or 5.0 log CFU/ml were tested. Results were defined as positive when at least one colony was recovered after IMS, and as negative when no colonies were recovered after IMS. Recovered colonies were always confirmed via latex agglutination assays per serogroup. Sensitivity was estimated as: positive results/(positive results + negative results) × 100 *(20)*.

Testing magnetic bead volumes

Reduced volumes of magnetic beads were tested with different bacterial concentrations. For each serogroup, bead volumes of 20, 16, 14, 12, and 10 µl were evaluated at each bacterial concentration (1.0, 2.0, 3.0, 4.0, or 5.0 log CFU/ml), and IMS was performed to recover target cells in broth. For the inoculated ground beef, 20 and 10 µl of anti-O157 beads were evaluated at each bacterial concentration (2.0, 3.0, and 4.0 log CFU/g). Automated IMS was performed individually for each serogroup, following the bead retriever manufacturer's standardized protocol. As recommended in the standard procedure, 5-wells tube strips were used, and each volume of beads tested was divided in two and added to the corresponding wells (e.g., for 20 µl, aliquots of 10 µl were dispensed into wells 1 and 2, for 16 µl, aliquots of 8 µl were dispensed, etc.). After completion of IMS, 50 µl of the bacteria-beads complex were spread-plated onto TSA for cultures tested in enrichment broth and mRBA for ground beef, and incubated for 24 h at 37°C. Recovered colonies were confirmed using agglutination tests specific to each O group, ensuring the presence of the correct serogroup. Also, colonies were enumerated and the CFU/ml estimated.

Statistical analyses

For the evaluation of the magnetic beads capture efficiency and IMS detection limit, a full factorial experimental design was used. Experiments carried out with the pure culture had a 5 by 5 factorial design, with the factors of bacterial concentration (1.0 to 5.0 log CFU/ml) and bead volume (20, 16, 14, 12, and 10 µl). For the experiments carried out with inoculated ground beef samples, the factorial design was 3 by 5, with the factors of bacterial concentration (2.0, 3.0, and 4.0 log CFU/g) and bead volume (20, 16, 14, 12, and 10 µl). All plate counts obtained after IMS were log transformed prior to analysis. Duplicate samples were processed for each repetition, and five repetitions of the experiment were conducted. Correlations between factors were determined through analysis of variance (R Version 2.15.0, 2012. R Core Team). Means were separated by use of Tukey's test. Means were considered significantly different when $P \leq 0.05$.

RESULTS

IMS sensitivity and limits of detection

Sensitivity was evaluated at the detection limit indicated by the magnetic bead manufacturers, which is 100 cells/ml. Findings from this study indicate that the lowest bacterial concentration detected by IMS varied among serogroups. When the bacterial concentration in the sample was 2 log CFU/ml and 20 µl of beads (recommended by manufacturer) were used, sensitivity per serogroup was found to be 62.5, 87.5, 87.5, 50.0, 87.5, and 87.5, and 0.0% for O157, O103, O26, O145, O45, O121, and O111, respectively. Furthermore, other bacterial concentrations were tested to identify the detection limit at 100% sensitivity. *Table 2* shows the proportion of samples from which target cells were recovered after IMS. After conducting IMS on STEC cultures at 2 log CFU/ml, magnetic beads were not always effective in recovering target cells, indicating that 100% sensitivity was not achieved with the suggested 100 cells. For serogroups O157, O103, O26, O145, O45, and O121, the lowest bacterial concentration effective in recovering cells from 100% of the samples tested was 3.0 log CFU/ml. *E. coli* O111 was detected through IMS in all repetitions only when the concentration was at least 5.0 log CFU/ml.

Testing a reduced magnetic bead volume

To evaluate whether the bead volume was related to IMS sensitivity, the assays were performed individually for each STEC serogroup. Cell recovery was tested using the aforementioned cultures in enrichment broth. Samples contained bacterial concentrations of 1.0, 2.0, and 3.0 log CFU/ml (prior to IMS). Varying magnetic bead volumes of 20, 16, 14, 12, and 10 µl per culture concentration were evaluated to determine the optimum volume of beads required during IMS to separate target cells. The volume of beads recommended by the manufacturer for the standard automated IMS protocol is 20 µl, and lowering this volume to 10 µl did not affect IMS efficiency in recovering bacterial target cells from enrichment broth. No significant difference ($P > 0.05$) in cells recovered was found among the different bead volumes tested for any of the target STEC serogroups *(Table 3)*. While all tested serogroups were evaluated with reduced volumes of beads, using bacterial concentrations from 1 to 3 log CFU/ml, *E. coli* O111 was tested also, with additional concentrations of 4 and 5 log CFU/ml. With 4 log CFU/ml, the average CFU recovered was 2.13 and

TABLE 2. Proportion of O157 and non-O157 Shiga toxin-producing *E. coli* recovered after immunomagnetic separation, using 10 and 20 µl of magnetic beads

Bacterial concentration in sample[a]	Sensitivity (%) by serogroup[b,c]													
Log/ml	O157		O103		O26		O145		O45		O121		O111	
	10 µl	20 µl	10 µl	20 µl	10 µl	20 µl	10 µl	20 µl	10 µl	20 µl	10 µl	20 µl	10 µl	20 µl
1	12.5	50	0.0	0	0.0	25.0	12.5	25.0	75.0	62.5	60.0	50	0	0
2	87.5	62.5	100	87.5	62.5	87.5	37.5	50.0	75.0	87.5	80.0	87.5	12.5	0
3	100	100	100	100	100	100	87.5	100	100	100	100.0	100	37.5	62.5
4													100	75
5													100	100

[a]Bacterial culture concentration present in the sample prior to IMS, and verified by direct plating

[b]Sensitivity of recovery per serogroup, reported in percentage of samples in which at least one colony of the target microorganism was recovered after IMS

[c]No statistical differences ($P > 0.05$) between the two magnetic bead volumes, when the minimum detection limit of the microorganism was present in the sample, being 5 log CFU/ml for O111, and 3 log CFU/ml for the rest of the serogroups. For each serogroup and each magnetic bead volume, n = 50

TABLE 3. *E. coli* O157 and non-O157 Shiga toxin-producing *E. coli* recovered after immunomagnetic separation with reduced magnetic beads

		Initial Culture Concentration[a]					
		1 log CFU/ml[b]		2 log CFU/ml[b]		3 log CFU/ml[b]	
	Dynabead volume[c] →	10 µl	20 µl	10 µl	20 µl	10 µl	20 µl
(Log CFU/ml)	O45	4.63 (5.13)	6.75 (12.86)	6.63 (8.14)	12.0 (9.53)	9.88 (5.11)	3.25 (4.20)
	O121	10.40 (11.45)	8.80 (10.39)	9.60 (9.88)	9.40 (10.13)	10.70 (7.36)	12.10 (8.88)
	O145	0.56 (0.18)	0.56 (0.18)	0.81 (0.53)	1.25 (1.22)	5.19 (3.78)	8.69 (9.74)
STEC Serogroup recovery	O103	0.50 (0.0)	0.50 (0.0)	2.63 (1.06)	2.56 (1.29)	16.63 (6.93)	29.63 (10.74)
	O26	0.50 (0.0)	0.75 (0.53)	1.81 (1.28)	2.69 (1.58)	21.13 (12.19)	20.38 (9.96)
	O157	0.13 (0.35)	0.63 (0.74)	3.88 (4.42)	2.88 (3.87)	18.88 (11.21)	34.63 (16.03)
	O111	0 (0.0)	0 (0.0)	0.13 (0.35)	0 (0.0)	0.63 (1.02)	1.38 (1.19)

[a]Reported values correspond to the average of the colonies recovered after IMS. Values in parenthesis correspond to the standard deviation. For each serogroup and each magnetic bead volume, n = 50

[b]Concentration of each serogroup culture before conducting IMS

[c]Two different magnetic bead volumes tested per serogroup and per bacterial concentration

3.15, at 10 µl and 20 µl, respectively. With 5 log CFU/ml, the average CFU recovered was 23.63 and 36.25, at 10 µl and 20 µl, respectively. Similar to results obtained with the other serogroups, no significant difference ($P > 0.05$) was observed when the magnetic bead volume was varied.

Capture efficiency of O157 on ground beef

Because previous findings indicated that the volume of magnetic beads used to perform IMS did not affect the capture of the cells, only 20 (manufacturer's recommendation) and 10 µl were tested with ground beef.

IMS was performed on inoculated ground beef samples with an estimated bacterial concentration of ca. 2.0, 3.0, and 4.0 log CFU/g.

With regard to the capture efficiency of IMS, results were comparable to those in the previous experiment in broth using pure cultures. Using a smaller volume of beads, or 50% of the recommended amount, did not affect ($P > 0.05$) the recovery of bacterial cells in ground beef. Similar to results of assays with pure cultures, significant differences ($P < 0.05$) were seen among the different bacterial concentrations tested; however, cells were always captured during each repetition after performing IMS and recovered on solid media after incubation of plates. Given that samples were always positive for the target STEC serogroup after IMS, it could be said that the test showed 100% sensitivity under the conditions of this study with inoculated ground beef (2.0, 3.0, or 4.0 log CFU/g) *(Fig. 1)*.

DISCUSSION

Recognizing the value of using IMS, this study investigated the variations of the method when it is used to recover the major seven STEC serogroups. The IMS detection limit for STEC as estimated by the manufacturer of the paramagnetic beads is roughly100 cells/ml after enrichment for all serogroups; with a lower bacterial cell concentration, IMS might not capture target cells effectively, thus providing a false negative result. Therefore, confirmation of performance metrics is crucial to reliable implementation of IMS-based food screening protocols. As presented earlier, IMS sensitivity varied among serogroups. When IMS was performed to separate the target cells in enrichment broth, the limit of detection was found to be 3.0 log CFU/ml for serogroups O157, O103, O26, O145, O45, and O121 and 5.0 log CFU/ml for O111, when the sensitivity was 100%. Results suggest that anti-O111 beads do not capture the target cells as efficiently as the other O groups. Possible explanations are

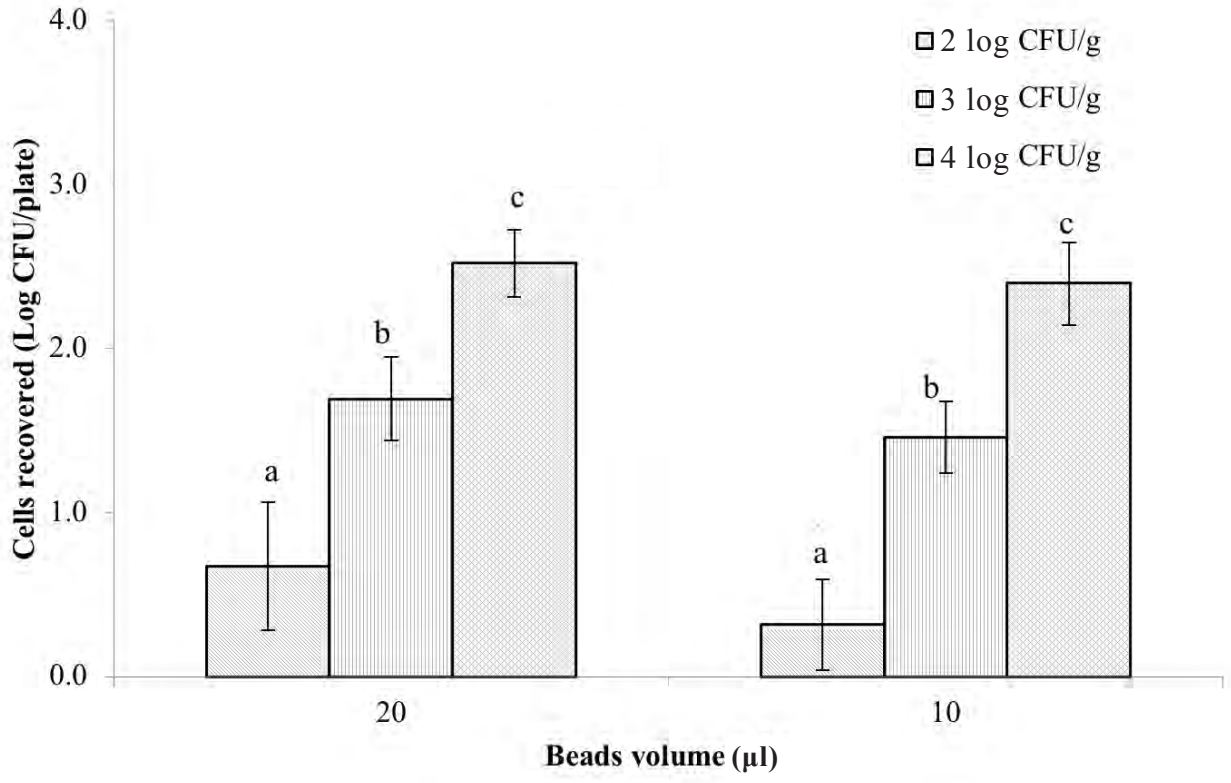

Figure 1. Colonies of *E. coli* O157 recovered after IMS conducted on ground beef with different bacterial concentrations, using two volumes of magnetic beads.

Different letters in the graph denote significant difference ($P < 0.05$) between bacterial concentrations tested. Initial culture concentrations are represented in the graphs as: 2 log CFU/ml diagonal stripes bars, 3 log CFU/ml horizontal stripes bars, and 4 log CFU/ml crossed lines bars.

that (1) a low antibody affinity for the target cell exists; (2) differences among strains provide different binding abilities; (3) a longer incubation time is required during the automated IMS to allow attachment of cells to magnetic beads; or (4) bead size and number of beads per ml do not allow for capture of a sufficient number of the target cells. Using a five-strain bacterial cocktail, and yet not recovering cells, could indicate that such low binding affinity is not strain dependent but serotype related. Other authors *(12)* have found that using IMS with samples containing competitive microflora or with complex matrices reduce the ability of the beads to bind to the target cells.

Experiments conducted using inoculated ground beef with serogroup O157 showed a detection limit of 2.0 log CFU/ml, in which 100% of the samples were identified as positive. We hypothesize that the difference between ground beef and pure culture could be associated with small variations during the repetitions, or perhaps the nature of the ground beef sample (fat and protein content) may have influenced the ability of the beads to bind to the target cells. Karch et al. (1996) evaluated IMS for the detection and isolation of *E. coli* O157 in stool samples from patients diagnosed with hemolytic uremic syndrome and compared the technique with direct plating and serological methods *(13)*. Using the IMS method, they were able to isolate O157 strains from 90% of the patients with a positive O157 serology, and the detection limit was found to be 2–3 log CFU/g. Chapman et al. (1994) evaluated the isolation of *E. coli* O157 using Dynabeads® anti-O157 and compared performing IMS on enrichment cultures to the direct plating of *E. coli* O157 on solid media for isolation of the microorganism *(6)*. Although specific detection limits were not reported, their results indicated that IMS was more sensitive, but >100 cells are needed to isolate colonies. Verstraete et al. (2010) found similar results regarding serogroup variations *(29)*. One of their findings demonstrates the importance of enrichment to increase bacterial concentration to enhance the recovery of target cells by IMS, particularly for serogroups O157, O103, O26, and O145. Samples containing up to 900 CFU/ml did not always provide positive results after IMS. They also found that magnetic beads for O157 have the highest recovery rate among the several STEC serogroups tested and that the lowest percentage of recovery was for O111, followed by O145, which suggests that the sensitivity of IMS varies depending on the target microorganism. Hall et al. (2006) found support for a similar theory after their studies with *E. coli* O26 in bovine feces *(11)*.

Immunomagnetic separation is considered to be an effective method for detection of STEC *(9, 18, 30)*. However, the detection limits and sensitivity of the test need to be taken into account during serogroup detection, since some serogroups seem to have a different affinity for magnetic beads. Because the infectious dose of STEC is proposed to be much lower than 100 cells *(14, 16, 23, 25)*, it is recommended that IMS be used only for isolation and not for detection. Adequate enrichment is important to ensure the growth of cells in the samples to detectable levels with use of IMS, as well as to avoid producing false negative results *(8)*.

It is believed that improvement of STEC detection assays has enhanced the ability to detect STEC infections. Mingle et al. (2102) has suggested that the introduction of new methods contributed to the changes observed in STEC epidemiological data, particularly between 1999 and 2011, that showed an increase in observed prevalence *(17)*. Some of the most frequently STEC non-O157 serogroups reported comprise O26, O103, and O111 *(2, 10, 17)*. A summary of the non-O157 STEC outbreaks in the USA shows that from 38 single-etiology outbreaks, O111 accounted for 66% *(15, 16)* confirmed through molecular methods, which could be indicative of a high O111 prevalence. On the other hand, studies in which IMS has been used as the method for detection and isolation of STEC consistently show a very low prevalence of O111 *(5, 22)*. This situation led us to question whether there is a true low O111 prevalence, whether inadequacy of anti-O111 magnetic beads is biasing results. Possible solutions could involve the improvement of these beads, an increase of the incubation time during IMS capture to allow a better cell binding to the antibody-coated magnetic beads, or use of an enrichment media that specifically targets O111, which does not necessarily existent.

On the other hand, the present study revealed that it is possible to lower by 50% the bead volume used to perform IMS, with respect to the manufacturer recommendation, without affecting the cell recovery efficiency. This means that IMS sensitivity is not affected by bead concentration used per test. Results were very consistent throughout all the experimental repetitions. When bacterial concentration in the samples was at least equal to that in which the sensitivity approached 100%, no false negative results were found with the lowest amount of beads tested (10 µl). Research laboratories frequently performing IMS and analyzing large numbers of samples could benefit from the associated cost reductions.

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DISCLOSURE STATEMENT

No competing financial interests exist. The authors are not associated with any of the manufacturers of IMS supplies used during the present study.

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