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Project Title: **Multiplex Immuno-magnetic separation (IMS) for detection of Shiga-toxin producing *Escherichia coli* (STEC)**

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Introduction: The current FSIS method for detection of STEC requires that to isolate the microorganisms it is necessary the use of the Immunomagnetic separation (IMS) technique. IMS can be a costly and time-consuming process to perform for each of the seven serogroups regulated by USDA. In addition, false negatives could potentially be diagnosed if a lower number of cells with respect to the detection limit are present in the enrichment. Given the importance of the STEC to the public health and to the meat industry, it is necessary to have reliable and efficient methods for the detection of these pathogens.

Objective: The objective was to evaluate the IMS detection limits as applied to STEC, and to propose a multiplex IMS process by conjugating magnetic beads available for the STEC serogroups.

Experimental treatments: Five-strain cocktails were prepared per serogroup by growing each strain in TSB, incubating overnight at 37°C, and combining equal aliquots of the strains into a sterile test tube. Cocktails were centrifuged at 5000 rpm for 10 min at 4°C, the supernatant discarded, and the cells resuspended to the original volume with BPW. Ground beef and bovine fecal samples were inoculated with different STEC serogroup combinations: i) O111, O145, and O103; ii) O111, O157, and O26; iii) O121, and O45; or iv) O157, O26, O145, and O103. Attachment of the microorganisms was allowed, and serial dilutions performed to achieve culture concentrations of 3 and 4 Log_{10} CFU/ml. IMS was performed using an automated system. Two levels of magnetic beads were tested: 20 and 10 μL. To perform the multiplex IMS, beads belonging to each serogroup were mixed according to the serogroup combinations. Recovered cells were plated on modified Rainbow agar and incubated overnight at 37°C. The resulting colonies were confirmed through latex agglutination kits and counted to evaluate the ability to simultaneously recover the O groups inoculated per serogroup combination.

Key results: Statistical analysis revealed no significant difference (P>0.05) between the two volumes of beads at any of the bacterial concentrations tested. However, significant differences (P<0.05) were found between the initial bacterial concentration previous to IMS and the cells recovered. In other words, lowering the volume of magnetic beads does not affect the cell capture, as long as the detection limit of 2 Log_{10} CFU/g for O157, O26, O145, O45, O103, and O121, and 4 Log_{10} CFU/g for O111 is present. With regards to the ability to capture cells through multiplex IMS with the different serogroups combinations, all serogroups were recovered as expected, except for O111, which could not be consistently recovered from any of the serogroups combinations.

How this information can be applied in the industry: This study revealed that multiplex IMS can be conducted to recover *E. coli* O157, O26, O145, O121, O45 and O103 using a smaller volume of magnetic beads and culturing on a selective media that allows for O group differentiation. Since the amount of beads can be reduced and less time will be required during multiplex IMS, the proposed method could be considered as a cost and time efficient alternative to isolation of STEC.

Table 1.
Serogroups recovered after multiplex IMS