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Sensitive and Rapid Detection of *Escherichia coli* O157:H7 by Coupling of Immunomagnetic Separation with Fluorescence Immunoassay

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Abstract:

Background: Our previous study demonstrated the feasibility of immunomagnetic separation fluorescence immunoassay (IMS-FIA), for the rapid detection of *E. coli* O157:H7. The objective of this study was to further optimize the reaction conditions to improve the detection sensitivity and reproducibility.

Methods: Antibody-coated magnetic beads were added to the test samples to specifically capture *E. coli* O157:H7 cells. The bead-bound cells were recognized by an anti-O157:H7 polyclonal antibody labeled with Cy5 dye to form an immuno-sandwich. In this study the assay is modified by first washing away any unbound dye, then dissociating the antibody/dye/cell complex from the immuno-sandwich by pH adjustment and magnetic separation, and finally, measuring the fluorescence signal of the supernatant with Creatv's Signalyte™-II spectrofluorometer. Various blocking and dissociation reagents were evaluated.

Results: Eight blocking buffers were tested for IMS-FIA detection of *E. coli* O157:H7 cells. Blocking with PBST containing 2% BSA showed the best results in reducing the background noise. Several dissociation buffers were compared for efficiency in releasing the antibody/dye/cell complex from the magnetic beads. The highest dissociation efficiency (>98%) was obtained by incubating the beads with 0.1 M glycine (pH 2.0) for 5 min, followed by brief vortexing. Finally, 10-fold serial dilutions of *E. coli* O157:H7 were tested using the modified IMS-FIA protocol. The results showed that the Cy5 fluorescence intensity from the supernatant was highly correlated with the original *E. coli* O157:H7 cell concentrations. A threshold detection limit was established based on the average fluorescence intensity, plus three times the standard deviation of the negative controls. The limit of detection for *E. coli* O157:H7 was 10 cfu/ml using this IMS-FIA approach.

Conclusions: Reagents and protocol were significantly improved to enhance the sensitivity of the IMS-FIA for detection of *E. coli* O157:H7. A detection limit of 10 cfu/ml *E. coli* O157:H7 was consistently achieved. The assay is also rapid; it can be completed in less than 3 hours.

Topic: P04 Isolation and Detection ; P01 Foodborne Pathogens

Keyword: *E. coli* O157 ; immunoassay ; food safety

