Use of the MagArray Immunoassay System as a Platform for Pathogenic *Escherichia coli* Detection

"Enabling Better Outcomes through Better Diagnostics"

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Abstract

Background: In recent years, pathogenic *Escherichia coli* have been causing numerous foodborne outbreaks leading to mild to bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and even death of patients. Many foods with short shelf life are related to the public before a negative testing for *E. coli* is confirmed. Currently, the process by which regulatory agencies screen for pathogenic *E. coli* in foods takes over 3 days. The MagArray immunoassay system is a low-cost chip-based platform capable of simultaneously detecting up to 80 different analytes in as little as 10 min. The reduction in detection time of pathogenic *E. coli* can contribute to a faster recall of contaminated foods and can therefore limit the number of individuals ingesting the contaminated food and decrease the total cost of lost productivity and treatment. The objective of this study was to demonstrate on MagArray platform the simultaneous detection of two main types of pathogenic *E. coli* (i.e., O157 and O145) in ground beef with high sensitivity.

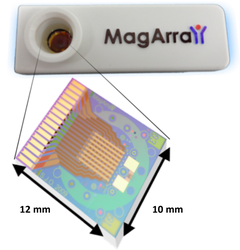
Methods: MagArray chips were first spotted with *E. coli* O145 and O157 antibodies. The chips were then blocked and ground beef samples were spiked with *E. coli* O145 and O157 for incubation. After incubating with detection antibodies, magnetic particles were then applied to generate signals. Different concentrations of E. coli were spiked to establish the standard curve and determine assay sensitivities.

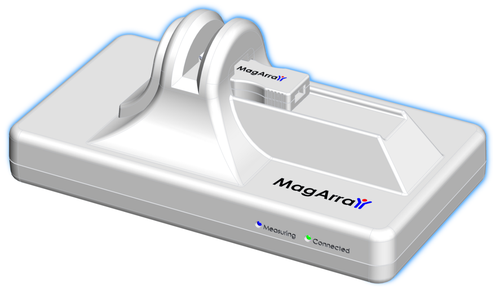
Results: In this 2-plex immunoassay in ground beef, detection of *E. coli* at a concentration as low as 2 cfu/µl was demonstrated. More specifically, for 2 cfu/µL of *E. coli* O145 and *E. coli* O157, the inter-run CVs were less than 10% for both types. And the results were compared and agree well with samples spiked to pure buffers. This sensitivity of detection was also achieved using a 30-min assay. And the results showed that assay sensitivity is minimally affected by changing assay media from pure buffer to ground beef.

Conclusions: The MagArray technology demonstrated that it can provide exceptional sensitivity with reasonable reproducibility for simultaneous detection of both *E. coli* O145 and O157 in ground beef. Thus we believe this technology provides a good fit for detecting multiple *E. coli* serogroups. This assay not only accelerates identification of pathogenic *E. coli*, but also holds the potential to help regulatory agencies to quickly issue a product recall for contaminated foods.

Introduction

- **Simple** - Small, portable system about the size of a tissue box. No moving parts. No microfluidics. Basic, easy to follow process.
- **Ultra-sensitive** - Has been able to distinguish concentrations as low as 50 attomolar. Depending on the target protein, typical detection limits are in the femtomolar / picomolar range.
- **Multiplex** - Multiplex assays with 80 individual sensors per chip. One chip per sample. Factoring in controls and replicates, researchers typically analyze 10-20 proteins per chip.





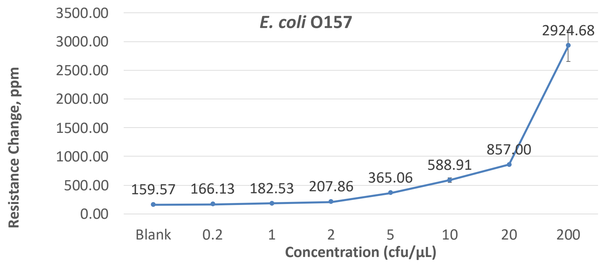
Methods

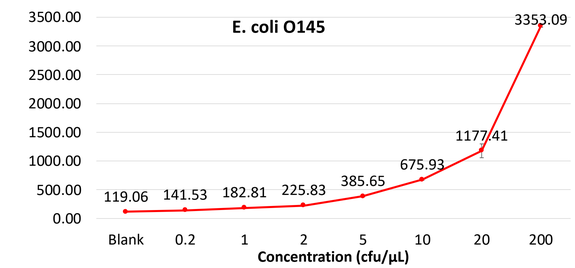
Assay condition:

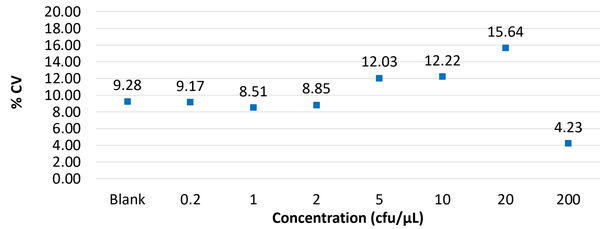
- Capture Antibodies - *E. coli* O145 and O157 (100 µg/mL diluted in PBS).
- Blocking - 5% BSA/PBS.
- Antigen - *E. coli* O145 and O157 (know cfu/µL diluted in enriched ground beef sample).
- Detector Antibodies (biotinylated) - *E. coli* O145+O157 (1 µg/mL diluted in 1% BSA/PBS).
- MNP - MACS, 1/20 dilution in PBS
- Rinsing - 0.1% BSA in TPBS.

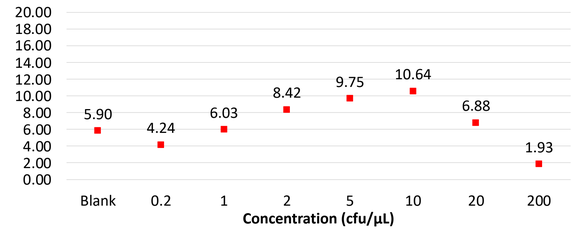
Results

2 h antigen → 1 h detection → 40 min reading

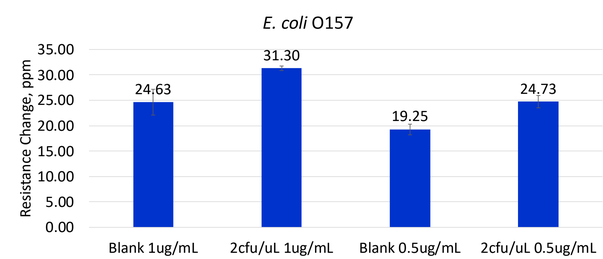


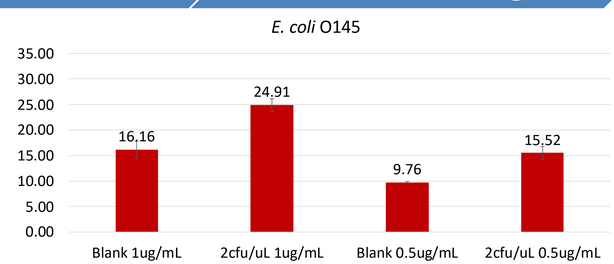


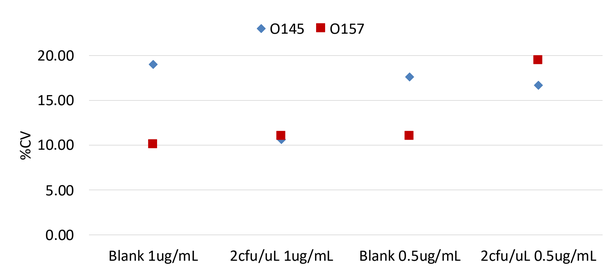




15 min antigen → 10 min detection → 5 min reading







Conclusion

Standard curve: For both *E. coli* O157 and O145, the LLOD was < 2 cfu/µL with CVs less than 10%.

30 min assay: Using 0.5µg/mL of detector Abs gave a lower noise level when compared to using 1µg/mL.

- O145: 9.76 vs 16.16 ppm
- O157: 19.25 vs 24.63 ppm

However the S/N was almost similar when 0.5µg/mL or 1µg/mL of detector Abs were used.

- O145: 1.59 vs 1.54
- O157: 1.28 vs 1.27