Enhanced Automated Immunomagnetic Separation (eAIMS) for Escherichia coli O157

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Introduction

Dynabeads® anti-E.coli O157 are designed for rapid, selective concentration of E.coli O157 directly from a pre-enriched sample aliquot using immunomagnetic separation (IMS). Dynabeads anti-E.coli O157 reacts with all E.coli O157 strains including pathogenic and non-pathogenic, sorbitol fermenting and non-sorbitol fermenting isolates. Dynabeads anti-E.coli O157 are simply incubated with an aliquot of the pre-enriched sample and the antibodies coated onto the beads will specifically bind the target bacteria. The bead-bacteria complexes are subsequently separated by applying a magnetic field. The whole IMS process can be automated using a BeadRetriever™ instrument or performed manually. Automated IMS (AIMS) using the BeadRetriever™ allows the user to selectively concentrate E.coli O157 from 15 samples at once. Utlilizing a new custom coated MyOne™anti-E.coli Dynabead® with a greater surface area than the M280 Dynabead® and improvement of the BeadRetriever™ protocol with optimized buffers and a higher starting volume of sample, a 60% higher capture rate has been realized over the standard product & protocol. Detection down to 10 CFU/25g of ground meat beef was detectable after only a 4 hour pre-enrichment Inclusion of ChargeSwitch® Technology (CST) for the pre-extraction of DNA prior to qPCR increased the detection of E.coli O157 to 1CFU/25g of sample with 100% detection of samples at 10CFU/25g of sample. These advances allow testing of up to 15 separate food samples per instrument, in one work day with the high sensitivity required for today's food market.

Table 1 – The capture efficiency of the standard EPEC/VTEC protocol and the VTEC enhanced protocol

No.	CFU/assay	Detection by new E.coli Dynabead (%)	Detection by EPEC/VTEC (%)
Set 1	10	80	20
Set 2	100	95	55
Set 3	1000	97	80
Set 4	10000	100	100

Table 1. The sensitivity of the VTEC enhanced protocol and the standard AIMS protocol were compared by measuring the efficiency of detection at 10, 100, 1000, and 10,000 CFU, from pure bacterial cultures. The Results (Table 1) indicate a 60% difference in the performance of the two methods when 10 CFU of pure bacterial culture are placed in the system.

Table 2 – Comparison of AIMS procedures

Level of contamination	Dynal EPEC/VTEC (%)	Dynal eAIMS (%)	Competitor M (%)
Low	40 (10CFU/25mg)	100 (10CFU/25g)	65 (~5CFU/25g)

Table 2. Comparison of the original EPEC/VTEC protocol with the enhanced protocol using qPCR as the final detection method. A 6 hour pre-enrichment was used for these samples. Competitor M results are for culture only with a 3 hour pre-enrichment. Reported values for this competitor state a detection level of between 1-10 CFU/25g sample using culture or PCR (AOAC Validation Report).

Figure 1 – Effect of prior DNA extraction on the level of detection by qPCR

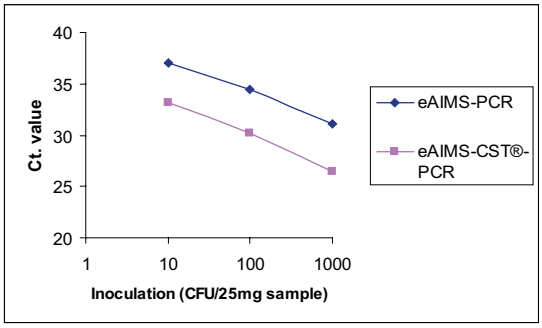

Figure 1. The effect of extracting DNA from cultures, prior to qPCR, was determined by processing two samples of the same bacterial culture through the enhanced AIMS protocol. After AIMS, DNA was extracted from the samples using the CST® nucleic acid extraction kit. Both the DNA extracted and non-extracted samples were then used in the qPCR assay. The results demonstrate that the DNA extracted samples produced lower Ct values, indicating higher target concentrations resulting in enhanced detection at all levels of inocula.

Figure 2 – Detection of E.coli O157 in beef by eAIMS-CST®-qPCR

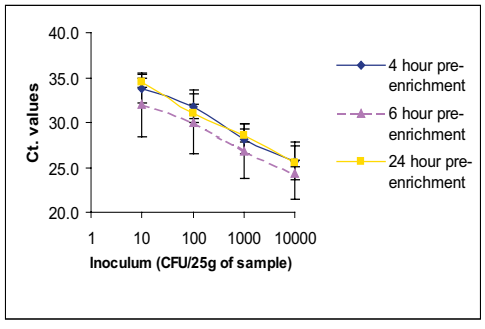

Figure 2. Spiked ground beef exhibited the lowest Ct values at 6 hour pre-enrichment. At 10 CFU/25g of sample all pre-enrichment samples produced a positive result which was below our cutoff of 37 (statistical analysis of previous data showed that Ct values of 37 or higher were not confirmable with culture). Other food types tested with similar results (data not shown).

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Results & Conclusions

→ IMS has been demonstrated to improve detection of Escherichia coli O157 by 10-100 fold over conventional pre-enrichment and culture.

→ Improvement of the IMS method and inclusion of an enhanced custom coated Dynabead has lead to an increase of 60% for the recovery of Escherichia coli O157 using the culture method.

→ Molecular techniques such as PCR are rapid, specific, and sensitive for detecting Escherichia coli O157.

→ Combination of enhanced AIMS plus ChargeSwitch® Technology for DNA extraction results in significantly lower Ct. values for enhanced detection in multiple food types.

→ CST® 96 well PCR plates used in this method provide easy to use nucleic acid extraction that fits seamlessly into detection work flow.

→ 4 hour and 6 hour pre-enrichment of samples were found to adequately accelerate the detection process. Highest sensitivity found to be with 6 hour pre-enrichment.

→ The enhanced protocol along with qPCR has reduced the time necessary for detection from 48 hours to less then 10 hours.

→ Enhanced AIMS for detection of Escherichia coli O157 reduces labour cost and time required plus gives the added benefit of greater sensitivity when compared to other methods.

References:

1. Khan, A. (2006) University of Central Lancashire, UK
2. Dynabeads anti-E.coli O157, Rev. No. 007, Dynal Biotech, Oslo, Norway