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*A Research Note*

# Isolation of *Escherichia coli* 0157:H7 Using 0157 Specific Antibody Coated Magnetic Beads

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ABSTRACT

*Escherichia coli* 0157 specific antibody, coated on magnetic beads, was used to concentrate and remove the *E. coli* 0157:H7 from mixed cultures and meat samples. The problem of nontarget organism carryover was addressed by adding Protamine to the culture-bead sample, washing the beads three times in saline, and changing the test tubes with each wash. These modifications reduced the nontarget colony counts obtained from uninoculated meat samples. This procedure enabled consistent recovery of *E. coli* 0157:H7 from inoculated meat samples. The percentage of *E. coli* 0157:H7 cells captured, compared to the total number of cells captured, ranged from 48 to 100%. Two strains of *E. coli* 0157, :H7 and :non-H7, appeared to compete with one another and thus reduce or prevent isolation.

*Escherichia coli* 0157:H7 is an enteric pathogen known to cause bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. A few outbreaks of enteritis caused by this organism have been traced epidemiologically to beef (*8,10,17*); consequently, a number of methods have been developed to detect and/or isolate this organism from meat samples. These methods include ELISAs (*7,9,15,16*), toxin detection systems (*1,12*), cultural methods (*4-6,14*), and DNA probes (*11*). Some of these methods are detection systems only, while others detect and facilitate isolation and identification of this organism. With the exception of one ELISA kit (Petrifilm™ Test Kit-HEC-for Hemorrhagic Escherichia coli 0157:H7, 3M Health Care, St. Paul, MN), none of the methods that require monoclonal antibodies or DNA probes are commercially available at this time.

As *E. coli* 0157 non-H7 are common in meat, isolation is required for confirmation and serotyping (*4-6*). Isolation generally requires plating aliquots of the sample enrichment broth on selective and differential media to obtain isolated colonies. When the background growth is high and the number of *E. coli* 0157:H7 low, the target organism can be lost through overgrowth of contaminants and/or serial dilution of the enrichment culture necessary to obtain isolated colonies on plating media.

To solve this problem, a method was developed that would selectively capture the target organisms and remove them from the mixed culture. The method involves using magnetic beads coated with *E. coli* 0157 antibody. The coated beads, added to an enrichment culture of a meat product, captured the target organisms. The beads then could be removed from the culture with a commercial magnetic concentrator. This procedure facilitated concentration of the organisms for easy detection.

This type of system has been reported by Skjerve et al. (*13*) for *Listeria* and Morgan et al. (*3*) for *Pseudomonas*. The one problem experienced by these researchers was nontarget carryover. This paper describes a method that allows isolation of *E. coli* 0157:H7 while eliminating the nontarget carryover completely from many meat samples and greatly reducing it in others. The method can be performed using commercially available products.

## MATERIALS AND METHODS

### *Antibodies*

Affinity purified antibody to goat IgG, made in rabbit (Cat. No. 01-13-06) and BacTrace Affinity Purified Antibody to *E. coli* 0157:H7 made in goat (Cat. No. 01-95-90) were obtained commercially [Kirkegaard & Perry Laboratories, Inc. (KPL), Gaithersburg, MD]. KPL calls their product affinity purified antibody to *E. coli* 0157:H7; however, it was 0157 specific, but not H7 specific, in this study. Therefore, to eliminate confusion, this product is called *E. coli* 0157 antibody. The antibodies were diluted in 0.3 M sodium phosphate buffer to give a final concentration of 0.1 mg IgG/0.1 ml of buffer.

### *Preparation of beads*

The magnetic beads (Dynal™ Dynabeads™ M-280, Dynal, Inc., Great Neck, NY) were coated with sheep anti-rabbit IgG by the manufacturer. Following the manufacturers instructions, a

second antibody was bound to these beads, by rotating and tumbling them overnight at 5°C with 5 µg of KPL rabbit anti-goat IgG per mg of beads. Following this step, a coating of the KPL goat anti-0157 IgG was attached, using the same procedure.

*Preparation of samples*

Twenty-five-gm ground beef samples were inoculated with a dilution of washed *E. coli* 0157:H7 cells so that each sample received between 0.6 and 0.9 CFU *E. coli* 0157:H7/gm. The inoculated meat samples were placed in 225 ml modified EC + novobiocin (mEC + n) broth (*4*) and incubated on a shaker at 100 rpm for 6 h at 37°C. The cultures were then incubated statically at 35°C overnight.

*Isolation method*

Ten ml of mEC + n broth culture was transferred to a 16 x 150-mm screw cap test tube. One ml of a Protamine (P4005, Sigma Chemical Co., St. Louis, MO) solution (0.05 gm/ml distilled water) was added to the tube and mixed. In order to maintain the proportion of 1 µl of coated beads to 1 ml of broth culture used in preliminary trials without Protamine, 11 µl of coated beads was added to the sample. The tube containing the sample and beads was vortexed for 1 min and then allowed to stand at room temperature for 10 min. It was vortexed briefly again and then placed in the magnetic particle concentrator (MPC™-6, Dynal, Inc.) to immobilize the beads and captured cells. After 1 min, the culture fluid was removed by aspiration while the bead-cell complex was held by the magnetic concentrator. In order to maintain the same ratio of beads to liquid, 11 ml of sterile physiological saline was added, and the tube was removed from the magnetic concentrator. The bead-cell complex was washed by mixing for 10 min (Blood mixer, Drummond Scientific Company, Broomall, PA). The beads and wash solution were transferred to a clean sterile tube and again placed in the magnetic concentrator.

After 1 min the liquid was removed. The washing and tube transfer sequences were repeated twice, for a total of three separations, washes, and tube transfers. After the final wash, with the tube in the magnetic concentrator, the saline was removed and 1.1 ml of physiological saline was added to the bead-cell complex. The suspension again was mixed on a vortex mixer. One-tenth (0.1) ml of this bead-cell suspension was spread plated on MacConkey sorbitol agar (MSA, Difco 0079-17-7) plates and incubated at 35°C overnight. Colonies then were picked from the plates and tested biochemically and serologically to identify the *E. coli* 0157:H7 colonies.

## RESULTS AND DISCUSSION

The results of initial pure and mixed culture experiments showed that the 0157 antibody coated beads captured the target organism. The number of attached organisms could be varied by changing the time and temperature of incubation. However, with inoculated ground beef samples, the conditions for maximal attachment of *E. coli* 0157 to beads also allowed the most nontarget organism carryover.

The objective of this study was to combine the use of a commercially available ELISA kit, to screen out negative 0157:H7 samples, with antibody coated magnetic beads to recover the organism from ELISA presumptive positive samples. The purpose of using the bead recovery method was to separate the target organisms from contaminants (non-0157) before plating on the MSA plates.

Various wash solutions were tested with the bead-cell complex, and it appeared that physiological saline did as well as saline + 0.05% Tween 20 with or without 0.1% bovine serum albumin. Initially, the culture tube containing the bead-cell complex was placed in the magnetic concentrator to immobilize the beads, the culture fluid was aspirated, and wash solution was then added to the tube. The beads and cells were washed for 10 min by rotation and tumbling on the blood mixer. The tube was again placed in the magnetic concentrator and the wash solution aspirated. This procedure was repeated three times in the same tube before suspending the beads in 1.0 ml wash solution and making serial dilutions for plating on MSA. After incubation, total and differential (sorbitol positive vs. sorbitol negative) colony counts were done on the MSA plates. Twelve sorbitol negative colonies from each plate were tested to determine the percentage of 0157:H7 colonies among the 12 isolates. Using this percentage, the total number of 0157:H7 colonies in the sorbitol negative population and the percentage of 0157:H7 colonies in the total population were calculated.

In subsequent experiments, the wash solution with the beads and captured cells was transferred to a sterile test tube during each wash step prior to placement in the magnetic concentrator. This change markedly reduced the carryover of nontarget cells (Table 1). When meat with an aerobic plate count of 2.4 x $10^6$/gm was tested, there was an average decrease of 93.7% in the carryover of nontarget microorganisms when tubes were changed with each wash step. Although this was an impressive improvement, it still did not totally eliminate carryover from negative samples nor yield culture plates with only *E. coli* 0157 from positive samples.

TABLE 1. *Effect of changing test tubes between washes on the number of nontarget and target organisms per ml of washed/concentrated magnetic beads.*

| | Same tube[1] | | Change tubes[1] | | |
|---|---|---|---|---|---|
| No. of 0157:H7 in sample[2] | No. of cells/ml of beads | % 0157:H7 of total | No. of cells/ml of beads | % 0157:H7 of total | % Decrease in no. cells/ml of beads[3] |
| 0 | 3.2 x $10^5$ | 0 | 2.2 x $10^4$ | 0 | 93 |
| 74/gm | 1.2 x $10^6$ | 39 | 6.3 x $10^4$ | 69 | 95 |
| 0 | 3.7 x $10^5$ | 0 | 1.8 x $10^4$ | 0 | 95 |
| 0.82/gm | 8.3 x $10^5$ | 86 | 7.0 x $10^4$ | 79 | 92 |

[1] Three washes.
[2] Aerobic plate count of meat = 2.4 x $10^6$/gm.
[3] Decrease due to changing test tubes between washes.

The carryover of nontarget cells could be caused by the tendency of bacteria to adhere to solid surfaces. Meadows (*2*) reported that the polyamine, salmine (Protamine), reduced the number of *Aeromonas liguefaciens*, *E. coli*, and *Pseudomonas fluorescens* adhering to glass slides. This led to an investigation of compounds that could inhibit such adherence. One ml of aqueous Protamine solution (0.05 gm/ml) was added to 10 ml of the enrichment culture before adding the beads. The washing steps, with the tube

changes, were done as described previously. The beads were suspended in 1.1 ml of saline and spread plated on MSA plates. Preliminary experimental results on uninoculated meat samples revealed that Protamine decreased nontarget carryover by about 99%. The results of additional trials on both inoculated and uninoculated control meat samples indicated that adding Protamine, combined with changing tubes between washes, essentially eliminated carryover of nontarget bacteria (Table 2). In the first trial (meat sample #54), one colony (non-0157) grew on the uninoculated sample control plate and 340 colonies were found on the plate from the inoculated sample. All 340 colonies were *E. coli* 0157:H7. In the second trial (meat sample #55), there were no colonies on the uninoculated sample control plate and 19 colonies on the plate from the inoculated sample. Ninety percent of these colonies were 0157:H7. Sample #56 was prepared from a frozen ground beef sample that had been ELISA positive but cultural isolation negative for *E. coli* 0157 when first tested. After a month storage at -20°C, this sample was retested by the ELISA and found to be negative. Using beads, 76 colonies were obtained from the uninoculated sample plate (none were 0157), and 92 were obtained from the inoculated sample (48% were 0157:H7). Meat sample #57 was divided into 5 uninoculated and 5 inoculated subsamples. Serotype 0157:H7 was isolated from one out of five uninoculated subsamples. With one exception, 83% or more of the colonies from the five inoculated subsamples were 0157:H7 positive.

TABLE 2. *Effect of Protamine on the recovery of target and nontarget microorganisms from meat cultures using antibody coated magnetic beads.*

| | | Uninoculated control samples | | Inoculated samples (0.6-0.9 CFU/gm) | |
|---|---|---|---|---|---|
| Meat sample | APC[1] | Direct colony count from 0.1 ml of beads | % 0157:H7 colonies | Direct colony count from 0.1 ml of beads | % 0157:H7 colonies |
| 54 | 1.2 x $10^5$ | 1 | 0 | 340 | 100 |
| 55 | 2.0 x $10^6$ | 0 | 0 | 19 | 90 |
| 56 | 1.9 x $10^5$ | 76 | 0 | 92 | 48 |
| 57 | 5.3 x $10^5$ | 1 | 0 | 166 | 96 |
| " | " | 1 | 0 | 42 | 93 |
| " | " | 19 | 8 | 30 | 97 |
| " | " | 38 | 0 | 67 | 83 |
| " | " | 7 | 0 | 119 | 66 |
| 58 | 1.4 x $10^6$ | 24 | 0 | 155 | 0 |
| | | " | (19)[2] | " | (1.5)[2] |

[1] Aerobic plate count.
[2] Total % of sorbitol positive 0157 colonies.

Sample #58 provided some interesting information. Approximately 19% of the colonies recovered from the uninoculated sample were sorbitol positive *E. coli* 0157. When the sample was inoculated with *E. coli* 0157:H7 cells, the percentage of sorbitol positive *E. coli* 0157 colonies captured by the beads dropped to 1.5%, and no 0157:H7 were captured. This phenomenon will be investigated further. Generally, when nontarget organisms were captured and transferred to plates, they were sorbitol negative *Hafnia* or *Serratia*.

The nontarget organism carryover effect is possibly due to bacteria adhering to the walls of the glass test tubes. Meadows (*2*) suggested that the basic protein salmine (Protamine), which is positively charged at neutral pH, may reduce attachment because it adsorbs to the bacteria and to the glass and hence reduces their net negative charge. In our studies, Protamine reduced the adherence of nontarget bacteria to the glass tube, perhaps the beads, and facilitated their removal in the wash solution. In addition, changing tubes between washes physically removed bacteria that continued to adhere to the walls of the tube. Since the antigen-antibody reaction is a different form of binding mechanism, the target bacteria remained attached to the beads while the nontarget bacteria were removed.

Another attempt to reduce bacterial adherence and nontarget cell carryover involved coating the tubes with Prosil-28 (PCR Inc., Gainesville, FL), a concentrated organosilane surface-treating agent. This compound was as effective as Protamine in reducing nontarget organism carryover. As coating tubes with Prosil-28 is more laborious than adding Protamine, further work with this compound was discontinued.

Although this method needs further testing with a wider variety of meats, it provides a sensitive method for recovery of *E. coli* 0157:H7 by concentration and purification of the target organism. The net result is that even if the bead-cell complex contains some nontarget cells, the ratio of target organisms to contaminants (non-0157) transferred to selective plating media is improved significantly.



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