Importance of STEC Determination

Shiga toxin-producing *Escherichia coli* strains (non-O157 STEC) have become an increasing public health concern. Some of the non-O157 STEC possess the same range of virulence factors as *E. coli* O157:H7, including the locus of enterocyte effacement and production of Shiga toxin. STEC has been implicated in numerous outbreaks, causing serious illness (hemolytic uremic syndrome), or death.

A study from the CDC showed that from 1982 to 2002 approximately 70% of non-O157 STEC infections in the USA were caused by strains from one of six major serogroups: O26, O45, O103, O111, O121, and O145. Non O-157 STEC has been found in ground beef and in cattle hides, and in feces at levels comparable to those of *E. coli* O157. Bovine feces can be a source of environmental contamination (soil, water) which can lead to secondary contamination of produce growing in fields.

It is difficult to distinguish pathogenic non-O157 STEC strains from non-pathogenic *E. coli* strains because the former rarely possess any distinguishing phenotypic or biochemical characteristics from the latter. Therefore, methods such as PCR and latex agglutination tests have been developed by the USDA-Agricultural Research Service Eastern Regional Research Center (USDA- ARS-ERRC) to help on the identification of these STEC strains. In order to conduct the confirmation methods, enrichment of the sample followed by immunomagnetic (IMS) separation is performed. The IMS method described in this user's guide is part of the testing protocol utilized and mandated by the FSIS for testing ground beef and beef trim, and described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges".

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E. coli O103 IMS Beads

Immunomagnetic Separation Beads for E. coli O103

Product No. 543020 (2 mL)

1. General Description

The Gold Standard Diagnostics' E. coli O103 IMS beads are immunomagnetic separation beads designed for the rapid and selective concentration of *Escherichia coli* serogroup O103 to help with their confirmation on selective confirmation agars. The Gold Standard Diagnostics E. coli O103 IMS beads should be used as part of the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges".

2. Safety Instructions

Biological waste should be decontaminated by autoclaving or by using another effective method. Discard samples according to local, state and federal regulations.

3. Storage and Stability

The E. coli O103 IMS beads should be stored between 2-8°C (**do not freeze)** and may be used until the last day of the month as indicated by the expiration date on the label. All reagents and samples to be analyzed should be at room temperature before use.

4. Test Principle

The IMS beads provided in the kit are coupled to antibodies against *E. coli* serotype O103. The Gold Standard Diagnostics' *E. coli* O103 IMS beads should be used as part of the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identificationof Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges". The MLG utilizes sample enrichment in modified tryptic soy broth, followed by multiplexed Real Time-PCR assay s targeting *stx1, stx2* and *eae* genes and the *wxz* gene in the O-antigen gene clusters of the six serogroups, and then immunomagnetic separation (IMS) followed by plating onto Rainbow Agar (mRBA) (Fratamico et al). Colonies are then tested for specific O antigens using latex agglutination and positive colonies are purified on Sheep Blood Agar (SBA) and confirmed using PCR and biochemical identification.

5. Limitations of the Test

If a positive result is obtained on an unknown organism, further test such as PCR should be carried out for confirmation. Apply good judgment to any test result, particularly when preliminary positive results are observed.

6. Warning and Precautions

-This product is for research purposes only;not for diagnostic or *in vivo* use.
-**Do not freeze reagents**.
-Do not allow reagents to become contaminated by using dirty transfer pipettes.
-Use reasonable judgment when interpreting the test results.
-Prior to use, ensure that the product has not expired by verifying that the date of use is prior to the expiration date on the label.
- Avoid cross-contamination of samples by using a new sample stick for each sample.
-Specimens may contain pathogenic organisms, handle with appropriate precautions.
-Ensure that reagent bottle caps are tight after each use to prevent drying of reagents.
-Reagents contain 0.05-0.1% sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to produce metal azides which might cause explosion. To prevent azide accumulation in plumbing, flush with copious amounts of water immediately after disposal.
-Dispose according to local regulations.

7. Summary of E. *Coli* O103 in Food Samples Detection Scheme ((MLG) Chapter 5)

7.1 Enrichment

325 +/- 32.5 gm sample is combined with 975 +/- 19.5 mL of modified TSB + casamino acids and 8 mg/L novobiocin (mTSB), stomached and incubated static at 43 +/- 1 °C for 15-22 hours. Positive and negative controls need to be included.

7.2 Screening Using Real-Time PCR

Enriched samples are screened for the presence of *stx* and *eae* genes. Samples with positive results (both gene targets) on the initial *stx/eae* PCR screen will be tested by three additional Real-time PCR assays to determine if a top six serogroup (O26, O45, O103, O111, O121 or O145) is present.

7.3 Immunomagnetic Separation (IMS)

Samples positive by the screening test are potential positives. Isolation of non-O157 STEC is carried out using immunomagnetic separation with specific anti-serotype beads (see protocol below, section D). A post-IMS acid treatment is performed to reduce background flora on the mRBA plate. Following the 1 hour acid treatment, immunomagnetic beads with adhering bacteria (O103) are diluted 1:1 and 1:10. Then, 0.1 mL is spread (plated) onto mRBA. Plates are incubated at 35 +/- 2 °C for 20-24 hours.

7.4 Identification

Colonies are picked from RBA plates and tested for agglutination with antisera specific for the serogroup of interest (at this stage the target serogroup is known). At least one colony of each morphological type on each plate is tested using O103 latex agglutination kits (Gold Standard Diagnostics PN 541020). A minimum of five latex positive colonies from each plate are streaked onto SBA plates and incubated at 35 +/- 2 °C for 18-24 hours. Colonies are then confirmed by specific latex agglutination test.

7.5 Confirmation

Latex positive colonies are confirmed by PCR assays followed by biochemical identification.

A. Materials Provided

1. Superparamagnetic beads attached with purified antibodies against *E. coli* O103 covalently bound to the surface. The beads are supplied in a suspension of PBS, pH 7.4 with 0.05% Sodium Azide, 2 mL vial.
2. User's guide.

B. Additional Materials (not provided with the test)

1. Pipettes (10-100 µL) with disposable filter tips
2. 1 mL dispenser pipette
3. mTSB (available from many media manufacturers)
4. Stomacher bags with or without mesh
5. Microcentrifuge tubes (1.5 mL) and 50 mL centrifuge tubes.
6. Sterile test tubes
7. Sterile glassware
8. Sterile inoculating loops, "hockey sticks" or spreaders
9. E Buffer: add 0.5 g of BSA, and 50 µL of Tween-20 to 100 mL of Buffered peptone water (BPW). Mix well. Filter sterilize, pH 7.2 +/- 0.2. (Sigma catalog # A7906-500G)
10. Separation Columns and Separation Magnets (MACS, Miltenyi Biotec) or Multi-6 Magnetic Separator (Gold Standard Diagnostics PN 472260)
11. mRBA Plates
12. HCl, 1 N
13. Tube shaker or agitator
14. Incubator
15. Vortexer
16. Disinfectant Solution, e.g. Sodium hypochlorite solution >1.3% w/w

C. Test Preparation

1. All reagents and samples to be analyzed should be at room temperature before use.
2. Thoroughly suspend the IMS beads by repeated gentle inversion of the vial.

D. Assay Procedure (Using Gold Standard Diagnostics Magnetic Separator*)

Please refer to the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.

1. For each sample positive for one of the top six serogroups, transfer 2-5 mL from the overnight enrichment through a 40 µm cell strainer into a 50 mL conical centrifuge tube.
2. Transfer 1.0 mL aliquot of the enrichment filtrate sample to a sterile 1.5 mL centrifuge tube.
3. Add 20 µL of the IMS O45 beads to the sample and vortex for 10-15 seconds to mix.
4. Incubate the sample(s) at room temperature (18-30 °C) with rocking for 15 minutes.
5. Place the sample tube(s) onto the magnetic separation rack. Allow to separate for 3 minutes.
6. Using a pipette, carefully remove the liquid from the sample tube (**NOTE:** be careful so as not to remove or touch the IMS beads on the side of the tube closest to the magnet).
7. Remove the sample tube(s) from the magnet. Add 1.0 mL of **E Buffer** and vortex for 10-15 seconds to re-suspend the IMS beads.
8. Repeat steps 5 to 7, 2 more times (a total of 3 washes).
9. After the final wash step, reconstitute the sample with 1.0 mL of **E Buffer** and vortex 10-15 seconds to mix.
10. Plate 0.1 mL of the bead suspension into mRBA plates. In addition, plate 1:10 and 1:100 dilutions of the IMS beads (sample) diluted in **E Buffer**. Use a hockey stick, swab or spreader to spread plate the beads. Incubate for 20-24 hours at 35 +/- 2 °C.
11. **Acid Treatment:** for each sample, transfer 450 µL of the undiluted bead suspension (step 9) to a microcentrifuge tube. Add 25 µL of 1N HCl and vortex for 10-15 seconds (pH should be 2.0-2.5).
12. Place tube(s) in tube rotator and rotate for 1 hour at 18-30 °C.
13. Dilute the IMS bead suspension by adding 475 µL of **E Buffer**.
14. Vortex for 10-15 seconds to re-suspend beads and plate 0.1 mL of the bead suspension into mRBA plates. In addition, plate a 1:10 dilution of the IMS beads (sample) diluted in **E Buffer**. Use a hockey stick, swab or spreader to spread plate the beads. Incubate for 20-24 hours at 35 +/- 2 °C.

NOTE: All mixing sticks, tubes, etc. should be sterile.

*Separation Columns and Separation Magnets (MACS, Miltenyi Biotec) can also be used, pleas e refer to procedure described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5.

E. Identification

Colonies are picked from RBA plates and tested for agglutination with antisera specific for the serogroup of interest (at this stage the target serogroup is known). At least one colony of each morphological type on each plate is tested using O103 latex agglutination kits (Gold Standard Diagnostics PN 541020). A minimum of five latex positive colonies from each plate are streaked onto SBA plates and incubated at 35 +/- 2 °C for 18-24 hours. Colonies are then confirmed by specific latex agglutination test.

F. Interpretation of Results/Additional Analysis

Positive samples must be confirmed as described in the USDA-FSIS test protocol described in the USDA- FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges".

G. References

(1) Fratamico, P., Bagi, L., Cray, W. Jr., Narang, N., Yan, X., Medina, M., Liu, Y. Detection by Multiplex Real-Time Polymerase Chain Reaction and Isolation of Shiga Toxin-Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121 and O145 in Ground Beef. Foodborne Pathogens and Disease 8(5):601-607.

(2) USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5 "Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges".