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.

E. coli O104:H4 is an entero-aggregative strain and is the agent of the 2011 European outbreak that caused 48 deaths and 3,785 cases. The "O" identifies the cell wall lipopolysaccharide antigen, and the "H" identifies the flagella antigen. This strain of *E. coli* is a novel strain that has acquired the Shiga toxin genes presumably by horizontal gene transfer. The strain is characterized by the following genetic markers: ● Shiga toxin stx2 positive ● terE positive (telluride resistance gene cluster) ● eae negative (intimin adherence gene) ● ß-lactamases ampC, ampD, ampE, ampG, ampH are present.

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 5B.02 "Detection and Isolation of non-O157 Shiga-Toxin Producing *Escherichia coli* Strains (STEC) from Meat Products".

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E. coli O104 IMS Beads

Immunomagnetic Separation Beads for E. coli O104

Product No. 543060 (2 mL)

1. General Description

The Abraxis E. coli O104 IMS beads are immunomagnetic separation beads designed for the rapid and selective concentration of *Escherichia coli* serogroup O104 to help with their confirmation on selective confirmation agars. The Abraxis E. coli O104 IMS beads should be used as part of the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5B.02 "Detection and Isolation of non-O157 Shiga-Toxin Producing *Escherichia coli* Strains (STEC) from Meat Products".

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 O104 IMS beads should be stored between 4–8°C (do not freeze) until the expiration date as shown 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 O104. The Abraxis *E. coli* O104 IMS beads should be used as part of the USDA-FSIS test protocol described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5B.02 "Detection and Isolation of non-O157 Shiga-Toxin Producing *Escherichia coli* Strains (STEC) from Meat Products". The MLG utilizes sample enrichment in modified tryptic soy broth, followed by multiplexed Real Time-PCR assays targeting stx_1, stx_2 and *eae* genes and the *wxz* gene in the O-antigen gene clusters of the six serogropus, 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 *in vitro* diagnostics use only.
-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* O104 in Food Samples Detection Scheme ((MLG) Chapter 5B.02)

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, O104, 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 (O104) 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 O104 latex agglutination kits (Abraxis PN 541060). 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* O104 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 uL)
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. Test tubes, glassware, loops, and pipettes
7. Wash Buffer Buffer: 0.15M NaCl, 0.01M Sodium Phopsphate buffer, pH 7.4, with 0.05% Tween-20. Autoclave at 121 °C for 15 minutes and store refrigerated. Also available in powder form, Sigma product # 740.02. Filter sterilize, pH 7.2 +/- 0.2.
8. Buffered peptone water (BPW). Filter sterilize, pH 7.2 +/- 0.2.
9. E Buffer: to 100 mL of BPW, add and mix 0.5 g of BSA, 50 µL of Tween-20. Filter sterilize, pH 7.2 +/- 0.2.
10. Separation Columns and Separation Magnets (MACS, Miltenyi Biotec) or Multi-6 Magnetic Separator (Abraxis PN 472260)
11. mRBA Plates
12. HCl, 1 N
13. Tube shaker or agitator; Incubator.
14. Vortexer
15. 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 Abraxis Magnetic Separator*)

Please refer to the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5B.02.

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 O104 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 a 3 minute separation.
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 Wash Buffer (E Buffer could also be used) 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 uL 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 Wash 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, please refer to procedure described in the USDA-FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5B.02.

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 O104 latex agglutination kits (Abraxis PN 541060). 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.

E. 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 5B.02 "Detection and Isolation of non-O157Shiga-Toxin Producing *Escherichia coli* Strains (STEC) from Meat Products".

H. 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 O104, 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 5B.02 "Detection and Isolation of non-O157Shiga-Toxin Producing *Escherichia coli* Strains (STEC) from Meat Products".

Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: *E. coli* O26, O45, O103, O104:H4, O111, O121, O145, O157:H7 Immunomagnetic Separation Beads
Product Code: 543000, 543010, 543020, 543060, 543030, 543040, 543050, 543070
1.2 Identified Use: Determination of *E. coli* O26, O45, O103, O104:H4, O111, O121, O145, O157:H7 in samples.
Restrictions on Use: For research use only.
1.3 Company: Abraxis, Inc., 124 Railroad Drive, Warminster, PA 18974 USA, info@abraxiskits.com +1(215) 357-3911, FAX +1(215) 357-5232
1.4 Emergency Telephone Number: +1(215) 357-3911

Section 2: Hazard(s) Identification

2.1 Classification of the mixture: Not a hazardous mixture.
2.2 GHS Label elements, including precautionary statements: Not applicable.
2.3 Hazards not otherwise classified (HNOC) or not covered by GHS: None known.
2.4 Unknown acute toxicity: None known.

Section 3: Composition / Information on Ingredients

3.2 Mixtures: *Contains no hazardous ingredients at levels requiring disclosure by the OSHA Hazard Communication Standard (29 CFR 1910.1200)*, however it contains minor amounts of materials considered hazardous. We recommend handling all substances with caution.

Section 4: First Aid Measures

4.1 Description of first aid measures: Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.
If inhaled: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact: Wash off with soap and plenty of water. Consult a physician.
In case of eye contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.
4.2 Most important symptoms and effects, both acute and delayed: No data available
4.3 Indication of any immediate medical attention and special treatment needed: No data available. Treat symptomatically.

Section 5: Fire-fighting Measures

5.1 Suitable extinguishing media: Use an extinguishing agent suitable for the surrounding fire.
5.2 Special hazards arising from the substance or mixture: None known
5.3 Advice for firefighters: Wear self-contained breathing apparatus for fire-fighting if necessary.
5.4 Further information: No data available

Section 6: Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures: Use personal protective equipment (see section 8). Avoid dust formation. Avoid breathing vapors, mist, dust, or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.
6.2 Environmental precautions: Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.
6.3 Methods and materials for containment and cleaning up: Solids (if applicable): Pick up and arrange disposal without creating dust. Sweep up and shovel. Liquids (if applicable): Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Keep in suitable, closed containers for disposal.
6.4 Reference to other sections: For information on safe handling see section 7.
For information on personal protection see section 8.
For information on disposal see section 13.

Section 7: Handling and Storage

7.1 Precautions for safe handling: See section 2. Avoid inhalation of vapors and contact with skin and eyes. Wear appropriate personal protective equipment. Do not eat, drink, or smoke in work area.

7.2 Precautions for safe storage: Keep container(s) tightly closed in a dry, well-ventilated place. Protect from physical damage. See label or product insert for appropriate storage temperature and additional specific information.
7.3 Specific end use(s): No data available

Section 8: Exposure Controls / Personal Protection

8.1 Control parameters: Not applicable.
8.2 Exposure controls:
Appropriate engineering controls: Provide adequate ventilation. Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday. Keep away from food and beverages.
Personal protective equipment: The usual precautionary measures, including eye/face/skin protection, should be taken when handling any chemical. Avoid contact with eyes, skin, and clothing.
Eye protection: As with handling of any chemical, wear approved safety goggles.
Skin protection: Handle with gloves. No specific information regarding glove material or thickness is available, but gloves must be impermeable and resistant to the substance being handled. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Respiratory protection: As with any chemical, where excessive vapor, mist, or dust may result, use a chemical fume hood or approved respiratory protection equipment.
Body protection: Lightweight, protective clothing.

Section 9: Physical and Chemical Properties

9.1 Information on basic physical and chemical properties of the mixture
Appearance: Multiple
Odor: Characteristic
Odor Threshold: No data available
pH: Multiple
Melting point/freezing point: No data available
Initial boiling point and boiling range: No data available
Flash point: No data available
Evaporation rate: No data available
Flammability (solid, gas): No data available
Upper/lower flammability or explosive limits No data available
Vapor pressure: No data available
Vapor density: No data available
Relative density: No data available
Water solubility: Various
Partition coefficient: n-octanol/water: No data available
Auto-ignition temperature: Not applicable
Decomposition temperature: No data available
Viscosity: No data available
Explosive properties: No data available
Oxidizing properties: No data available
9.2 Other information: No data available

Section 10: Stability and Reactivity

10.1 Reactivity: No data available
10.2 Chemical stability: Stable under recommended storage conditions.
10.3 Possibility of hazardous reactions: No data available
10.4 Conditions to avoid: No data available
10.5 Incompatible materials: No data available
10.6 Hazardous decomposition products: No data available. In the event of fire: see section 5.

Section 11: Toxicological Information

11.1 Information on toxicological effects
Acute toxicity: Not available. To the best of our knowledge, the chemical, physical, and toxicological properties of this product have not been thoroughly investigated.
Inhalation: No data available **Ingestion:** No data available

Skin contact: Irritant to skin and mucous membranes.
Eye contact: May cause eye irritation in susceptible persons.
Respiratory or skin sensitization: No data available
Aspiration hazard: No data available
Mutagenicity: No data available
Carcinogenicity
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.
Teratogenicity: No data available **Reproductive/fertility toxicity:** No data available
Specific target organ toxicity, single exposure: No data available
Specific target organ toxicity, repeated exposure: No data available

Section 12: Ecological Information

12.1 Toxicity: No data available
12.2 Persistence and degradability: No data available
12.3 Bioaccumulative potential: No data available
12.4 Mobility in soil: No data available
12.5 Results of PBT and vPvB assessment: No data available
12.6 Other adverse effects: An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Section 13: Disposal Considerations

13.1 Waste treatment methods
Product: All waste must be handled and disposed according to local, state, and federal regulations. Avoid disposing large volumes in sewer.
Contaminated packaging: All waste must be handled and disposed according to local, state, and federal regulations.
Refer to sections 7 and 8 for safe handling guidance.

Section 14: Transport Information

UN Number: Not regulated **UN Proper shipping name:** Not classified as dangerous in the meaning of transport regulations.
Transport hazard class(es): No data available **Packing group:** No data available **Environmental hazard:** No data available
Bulk transport: No data available **Special considerations:** No data available

Section 15: Regulatory Information

To the best of our knowledge, this product contains no substances which, at their given concentrations, are considered hazardous by other regulatory agencies. Refer to section 3.

Section 16: Other information

This information is based on our present knowledge. While Abraxis , Inc. believes that the data contained herein are factual and the opinions expressed represent a best effort to present accurate information, the data are not to be taken as a warranty or representation for which Abraxis , Inc. assumes legal responsibility. The information shall not be taken as being all-inclusive and is to be used only as a guide. The data are offered solely for the user's consideration, investigation, and verification. These suggestions should not be confused with either state, municipal, or insurance requirements, or with national safety codes and constitute no warranty. Any use of these data and information must be determined by the user to be in accordance with applicable federal, state, and local regulations.

All materials and mixtures may present unknown hazards and should be used with caution. Since Abraxis , Inc. cannot control the methods, volumes, or conditions of use of this product, Abraxis , Inc. shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material. This product is sold for research use only. It is not for any human or animal therapeutic or clinical diagnostic use.

Date this SDS was prepared: 5/24/2016
Version: 2
Changes from previous version: Abraxis, LLC changed to Abraxis, Inc.