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 the latex agglutination test described in this User's Guide 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. This latex agglutination method 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 O111 Latex Test Kit

Latex Agglutination Test for the Detection of E. coli O111

Product No. 541030 (50 Test)

1. General Description

The Abraxis E. coli O111 Test is a rapid latex agglutination test, designed solely for the presumptive identification of *Escherichia coli* serogroup O111 cultured on TSA agar plate. The Abraxis E. coli O111 Latex Test Kits 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 O111 Latex Test Kit 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 polystyrene latex particles provided in the kit are coupled to antibodies against *E. coli* serotype O111 (according to Medina et al). When the latex particles are mixed on a test card with fresh colonies of *E. coli* O111, the bacteria will bind to the antibody causing the latex particles to agglutinate (positive reaction). Bacteria that are not *E. coli* O111 will not bind to the antibody and will not agglutinate the latex particles (negative reaction).

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.
-Use only the *E. coli* test reagents from one kit lot (do not mix with other lots), as they have been adjusted in combination.
-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.

7. Sample Collection and Handling

Colonial growth removed from the agar surface of modified Rainbow Agar (mRBA) or tryptic soy agar with 5% sheep blood (SBA) plates is best suited for this testing procedure.

8. Control Procedures

The control reagents provided should be used to check the correct working of the latex reagents each day before routine tests are performed.

The Positive Control suspension must cause visible agglutination with the Antibody-Latex Reagent within one (1) minute.

The Negative Control Suspension should cause no agglutination within one (1) minute.

Do not use the test kit if reactions with the control suspensions are incorrect.

A. Materials Provided

1. Negative Control. Suspension of inactivated E. coli cells (K12) in buffer, 1 vial, 0.5 mL.
2. Positive Control. Suspension of inactivated E. coli O111 cells in buffer, 1 vial, 0.5 mL.
3. PBS (1X), 1 vial, 1.0 mL.
4. Antibody-Latex Beads Reagent. A suspension of red latex particles coupled to specific rabbit IgG to E. Coli O111 serotype, 1.5 mL vial with purple cap (enough for 50 tests).
6. Control Latex Reagent. A suspension of red latex particles sensitized with pre-immune rabbit globulin, 1 vial, 1.0 mL vial with white cap.
7. Test Cards. Disposable reaction cards, 5 cards (10 reactions each).
8. Sample Mixing Sticks (100).
9. Transfer Pipettes (20). Color coded (10 green, 5 blue, 5 red).
10. User's guide.

B. Additional Materials (not provided with the test)

1. Agar Plates.
2. Disinfectant Solution e.g. Sodium hypochloride 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 latex reagent and controls by agitation.

D. Assay Procedure

1. Bring all reagents and samples to room temperature. Make sure the latex suspensions and control are well mixed by vigorous shaking.
2. Using one of the provided transfer pipettes (green), place one drop of PBS onto one (1) circle on the test card. If more than one sample is being tested use additional circles on the test card.
3. Using one of the sample mixing sticks (or an inoculating loop), pick a portion of a suspect colony from the agar plate and thoroughly emulsify in the drop of PBS of one of the circles.
4. Using one of the transfer pipettes (red), add one (1) drop of the Positive Control to a second circle
5. Using another transfer pipette (blue), add one (1) drop of the Negative Control to a third circle.
6. Dispense one (1) free falling drop (with vial held vertically) of the *E. coli* O111 Latex-Antibody bead reagent onto each circle (Positive, Negative, and sample(s))
7. Rotate the test card using a complete circular motion (through 3 planes) for up to one (1) minute or until agglutination is evident, whichever occurs first. Record the results.
8. If agglutination with the test reagent does occur, it is necessary to test a further portion of the colony with the Control Latex Reagent to ensure that the isolate in not an auto-agglutinating strain.

NOTE: All mixing sticks, cards, etc. should be disposed in disinfectant or autoclave waste containers.

E. Interpretation of Results

Agglutination of the test latex within one (1) minute is a positive result. This indicates the presence of *E. coli* serogroup O111.

No agglutination occurring within one (1) minute is a negative result. This indicates the absence of *E. coli* serogroup O111.

NOTE: Some strains of *E. coli* are difficult to emulsify in saline and may give a stringy type reaction with the test reagents. This does not look like true agglutination and should be ignored. If this stringiness is found to be too severe for a correct judgment to be made then the colony should be suspended in 1-2 drops of PBS. Allowing the lumps to settle and re-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.

F. Cross-reactivity Profile

E. Coli **Serotype**	**Agglutination**
O111	"+++"
O26	"-"
O45	"-"
O103	"-"
O145	"-"
O157	"-"
K12	"-"

"+ + +" = Strong positive agglutination
" – " = Negative agglutination

G. 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) Medina, M., Shelver, W., Fratamico, P., Fortis, L., Narang, N., Cray, W. Jr., Esteban, E., Tillman, G., and Debroy, C. Latex agglutination assays for detection of Non-O157 Shiga Toxin-Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121 and O145. Journal of Food Protection 75(5):819-826.

(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 Latex Agglutination Test Kits
Product Code: 541000, 541010, 541020, 541060, 541030, 541040, 541050, 541070
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.