United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-MTH-06		Page 1 of 11
Title: Isolation and Identification of *Escherichia coli* O157 by Immunomagnetic Separation (IMS) and Cultural Methods		
Revision: 07	Replaces: Rv 6 07/01/2010	Effective: 9/1/2011

1. Purpose

This Standard Operating Procedure (SOP) will be used after obtaining a positive PCR result for *Escherichia coli* (*E. coli*) O157:H7 (SOPs MDP MTH-11 and/or MDP MTH-12). This document provides standard procedures for capturing *E. coli* O157 cells by immunomagnetic separation, subculturing to various selective agar media, and identifying isolates by VITEK®. Isolates can be further identified by serotyping.

2. Scope

This SOP shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory. IMS is used as one of the methods in the isolation of *E. coli* O157.

3. Principle

The immunomagnetic separation (IMS) method offers a means to concentrate target bacteria from mixed cultures by physical separation based on an antigen-antibody reaction. Selective capture (concentration) of bacterial cells is achieved by antibodies, specific to the cell surface antigens of the target strain, immobilized on magnetic beads. After washing the beads to remove non-target organisms, the magnetized beads coated with target bacteria are recovered and processed for isolation using various selective agar media and identification by cultural methods and serotyping.

4. Outline of Procedures

5. References

- BAM Online, 8th edition. 2001. Chapter 4. Section: Diarrheagenic *Escherichia coli.* Isolation and confirmation of *E. coli* O157:H7. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM070080 (last accessed 03/11)

- Enrichment of *E. coli* O157, Product information for Dynabeads® anti-*E.coli* 0157 http://www.invitrogen.com/site/us/en/home/brands/Dynal.html? (last accessed 03/11)

- SOP MDP-DATA-01, Microbiological Record Keeping and Results Reporting

- SOP MDP-SHIP-03, Procedures for Packaging, Shipping, and Archiving Microbiological Cultures

- SOP MDP-QA-03, Quality Assurance (QA) Controls

- USDA/FSIS Microbiology Laboratory Guidebook Chapter 5.05, Effective date: 10/1/2010. Detection, isolation, and identification of Escherichia coli O157:H7 and O157 from meat products. http://www.fsis.usda.gov/PDF/MLG_5_05.pdf (last accessed 03/11)

6. Specific Procedures

6.1 Equipment and Materials

- Additional materials needed to perform procedure as listed in the product's user instructions or suggested by the manufacturer
- Laboratories that have Pathatrix may use it in place of the Dynal system. Additional materials needed to perform procedure as listed in the Pathatrix (Matrix MicroSciences) and *E. coli* O157 test kit product instruction or suggested by the manufacturer
- VITEK® (bioMerieux, Inc.) System and Users Manual
- VITEK Cards: GNI+ and GN cards, bioMerieux
- Incubator 35 ± 2°C
- Incubator 42 ± 2°C

6.2 **Media and Reagents**

- Dynabeads® anti-E. coli O157 (Dynal Biotech), Invitrogen
- Optional: Ultra E. coli O157 test kit, Matrix Microsciences
- Wash buffer (PBS Tween): 0.15M NaCl, 0.01M Sodium-Phosphate buffer, pH 7.4, with 0.05% Tween-20; autoclaved at 121ºC for 15 minutes
- CHROMagar™ O157, DRG International, R&F®, (or demonstrated equivalent) with supplements (e.g. tellurite, cefixime, cefsulodin etc.)
- TCSMAC (Tellurite-Cefixime Sorbitol MacConkey Agar) plates
- TP (Tryptone Phosphate) Broth
- Modified Buffered Peptone Water plus pyruvate (mBPWp) with ACV
- TSAYE (Trypticase Soy Agar with Yeast Extract)
- BA (Blood Agar) plates
- Commercially available agglutination test kits for somatic O and flagellar H antisera for *E. coli* O157:H7 (Lenexa or Remel KS or products with demonstrated equivalent)
- Reagents for MUG based test (e.g. ColiComplete Discs, (BioControl Co. Bellevue, WA) or equivalent)
- Kovac's reagent for Indole test (or equivalent such as BBL Dryslide Indole)

6.3 **Controls**: (Specific strains are listed in SOP MDP-QA-03, Attachment 1).

6.3.1 For IMS, at a minimum, carry positive produce control (spiked with MDP 004) from SOP MDP-MTH-11 through this entire procedure. Refer to SOP MDP-LABOP-02 for control setup.

NOTE: Alternatively, it is acceptable for laboratories to set up appropriate media controls (positive, negative, and uninoculated) from pure cultures without using the cultural controls from the screening methods. Each laboratory shall have written procedures for ensuring the appropriate controls are used for this procedure.

6.3.2 If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03.

6.4 **Safety -** *E. coli* O157:H7 is a human pathogen with a low infectious dose. Laboratory personnel should utilize Biosafety Level II (BSL-2) practices for microbiological manipulations of known and potential pathogens. A BSL-2

laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. Material Safety Data Sheets (MSDS) should be obtained from manufacturers for media, chemicals and reagents used in the analysis and personnel who will handle the materials should know the location of and have ready access to the MSDS sheets for reference.

6.5 Isolation

6.5.1 Transfer 10 ml of the pooled PCR-positive UPB sample (identified positive for *E. coli* O157:H7 by SOPs MDP MTH-11 and MTH-12) to one 90ml TP broth and 10ml pooled PCR-positive UPB to one 90ml of mBPWp w/ACV and incubate them at 44 ± 2°C and 42 ± 2°C, respectively, for 18-24 hours. Transfer 10ml of the individual (sprouts) PCR-positive UPB sample to 90ml of TP broth and 10ml individual PCR-positive UPB to 90ml mBPWp w/ACV and incubate at 44 ± 2°C and 42 ± 2°C, respectively, for 18-24 hours.

6.5.2 Extract DNA and repeat BAX® O157:H7 Rt-PCR on TP and mBPWp w/ACV enrichments. If negative results are obtained, return to the UPB enriched positive samples, following procedures in section 6.5.6, this SOP.

6.5.4 Streak the PCR-positive TP and/or mBPWp w/ACV enrichments (pooled and/or individual samples) onto selective agar plates, CHROMagar™ O157 and TCSMAC, for isolation. Incubate one set of selective agar plates at 35 ± 2ºC for 18-24 hours. Incubate a second set of agar plates at 42 ± 2°C for 18-24 hours.

6.5.5 Streak positive and negative control cultures on selective agar plates and incubate the plates at 35 ± 2ºC for 18-24 hours.

***Note: Use of several selective agar plates, both in type and number, will improve the chance of finding the target isolates on plates. Use two types of selective agar plates, CHROMagar O157 and TCSMAC for screening at two different temperatures. BAX® E. coli* O157:H7 realtime *PCR on TP-enriched cultures can be performed to verify the presence of E. coli* O157:H7.**

6.5.6 Perform IMS on all BAX®-positive pooled and individual enrichments (UPB, TP, and/or mBPWp w/ACV) following the manufacturer's instructions or validated IMS procedure. Include positive produce control sample.

6.5.7 Dispense the concentrated samples to several plates of CHROMagar™ O157 and TCSMAC and streak for isolation following Dynal/Pathatrix IMS manufacturer's instructions. Incubate the plates at 35 ± 2ºC and the duplicate plates at 42 ± 2°C for 18-24 hours.

6.5.8 Observe all control and sample plates for typical *E. coli* O157 colonies.

Typical colony characteristics of *E. coli* O157:H7		
Medium/Test	**Colony Characteristics**	**Phenotype**
CHROMagar™ O157	Mauve	Proprietary physiological test for O157
TCSMAC™	Colorless with gray center	Sorbitol negative
Indole Test		Positive

6.5.9. Pick a minimum of 10 typical colonies (if available) from any of the selective agar plates used for isolation. Select presumptive positive colonies and perform latex agglutination for O antigen. Select up to a total of five isolates and streak 'O' positive colonies to BA and/or TSAYE. Include the positive and negative controls. (Aseptically add a ColiComplete disc to the primary streak on the TSAYE agar plates. Incubate TSAYE Agar plates for 18-24 hours at 35±2º C.)

6.5.10 Check for MUG reaction using UV light for fluorescence. (*E. coli* O157:H7 is a MUG negative organism.)

6.5.11 Check Indole test.

6.5.12 Perform latex agglutination test for O and H antigens on typical colonies. Include positive and negative controls and verify GFP absence for positive samples.

Note: Hold CHROMagar and TCSMAC plates at refrigeration temperature until results are confirmed.

6.6 Identification of *E. coli* O157:H7

6.6.1 Select 3-5 MUG negative, Indole positive, O157 antigen positive typical colonies from BA and identify using VITEK® or another official standard method of identification.

6.6.2 Perform real-time PCR to confirm the presence or absence of *stx-1* and *stx-2* genes in the isolates. Refer to SOP MDP MTH-11.

6.6.3 Select one typical colony that has been confirmed by VITEK®, serotyping, and confirmatory Rt-PCR as *E. coli* O157:H7 for archiving and shipping.

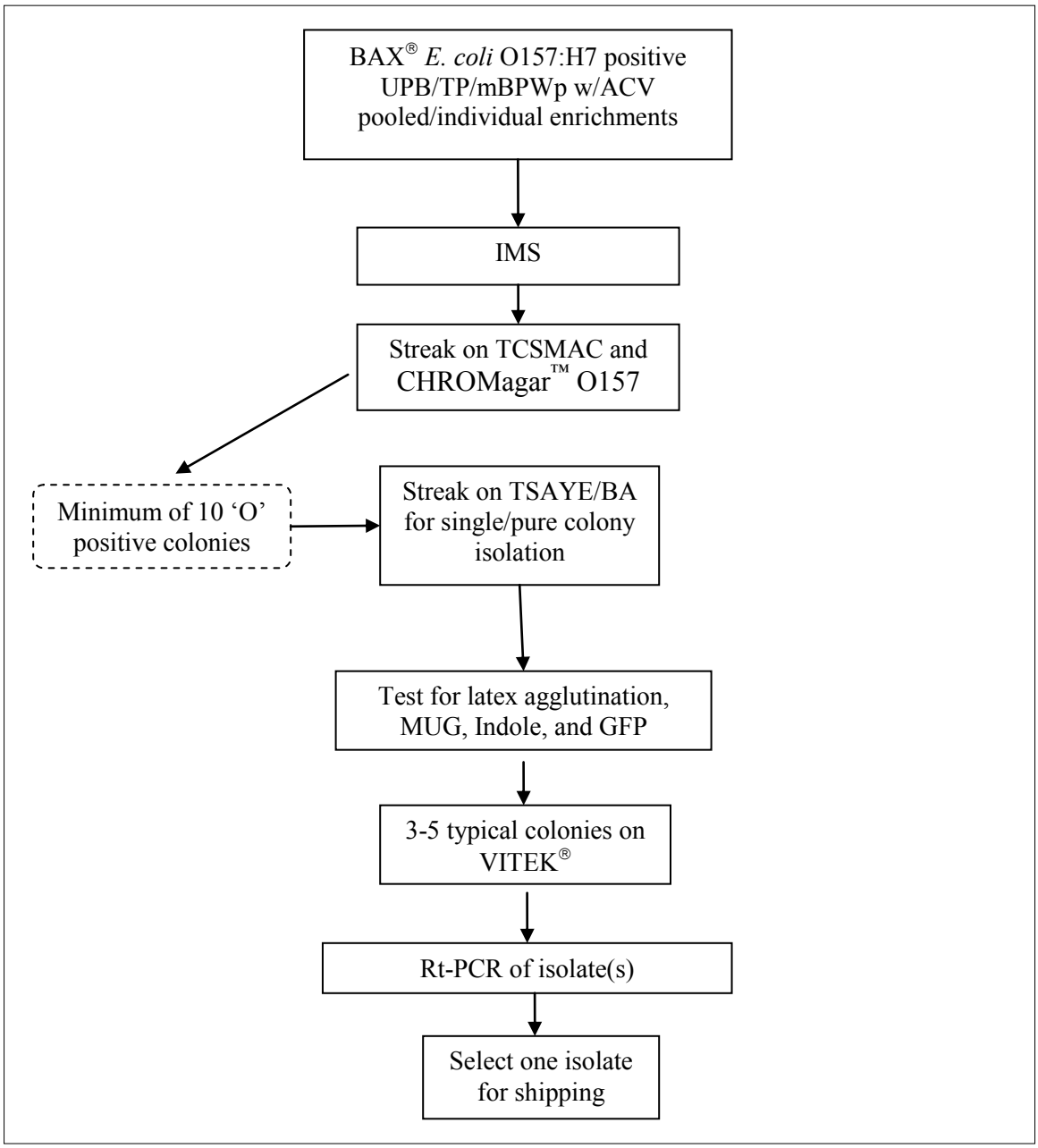
6.7 **Reporting and Shipping**

6.7.1 A final positive result is defined as a culture that is biochemically identified as *E. coli* O157:H7 and serologically confirmed as *E. coli* O157:H7.

6.7.2 IMMEDIATELY following completion of biochemical and serological tests, report confirmed results to MP per SOP MDP-DATA-01 on Attachment 1, Results Notification Form, and prepare (3) isolate slants for shipment to MDP repository for antimicrobial susceptibility testing, and PFGE (if not done by the testing laboratory).

6.7.3 Refer to SOP MDP-SHIP–03 for preparation of cultures for shipment.

E. coli O157:H7 Isolation



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ELECTRONICALLY REPRODUCED SIGNATURES - ORIGINAL SIGNATURE PAGE MAINTAINED BY USDA, AMS, S&T, MONITORING PROGRAMS OFFICE

Revision 07 May 2011 Monitoring Programs Division

- Changed word "Dynabeads®" to beads, Section 3.
- Revised "Equipment and Materials", Section 6.1 by adding words in bold:
    - Additional materials needed to perform procedure as listed in **a product's** user instructions or as suggested by the manufacturer.
    - **Laboratories that have Pathatrix may use it in place of the Dynal system.**
- Deleted "mEC+n" from SOP
- Revised "Media and Reagents", Section 6.2 by adding:
    - Optional: Ultra E. coli O157 test kit, Matrix Microsciences
    - Kovac's reagent for Indole test (or equivalent such as BBL Dryslide Indole)
    - Modified Buffered Peptone Water plus pyruvate (mBPWp) with ACV
- Revised Section 6.3.1 to read as "For IMS, at a minimum, carry positive produce control…"
- Revised old Section 6.5 to be more specific by adding new sub-sections: "6.5.1, 6.5.2, 6.5.3, 6.5.4, 6.5.5" and renumbered old subsections (6.5.2 and 6.5.3) as 6.5.6 and 6.5.7
- Added "Indole Test" to Table of Typcial colony characteristics of E. coli O157:H7.
- Revised and combined old Sections 6.5.5 and 6.5.6 (now 6.5.9) to change minimum number of colonies (in bold) "Pick a minimum of **10** typical colonies (if available) from any of the selective agar plates…."
- Added Section 6.5.11, "Check Indole test."
- Revised old Section 6.5.7 (now 6.5.12) to read as "Perform latex agglutination test for O and H antigens **on typical colonies. Include positive and negative controls and verify GFP absence for positive samples**."
- Revised Section 6.6.1 to read as "Select 3-5 MUG negative, **Indole positive, O157 antigen positive typical colonies** from…."
- Updated "E.coli O157:H7 Isolation" Flowchart

Revision 06 June 2010 Monitoring Programs Office

- Removed words "L-EMB, MacConkey, and CHROMagar™ E. coli plates" from document
- Added the media and reagent: TSAYE and ColiComplete disc
- Expanded the section 6.5 Isolation
- Removed MTH-05 and MTH-07 from reference
- Updated Flowchart

Revision 05 December 2009 Monitoring Programs Office

- Alphabetized and removed numbering from References, Section 5
- Removed numbering from Equipment and Materials, Section 6.1
- Removed numbering from Media and Reagents, Section 6.2

  Changed sentence to "Pick a minimum of 5-10 typical colonies (if available) on the selective agar plates used for isolation or restreak to CHROMagar™…", Section 6.5.5

- Changed Section 6.7.2 to read "IMMEDIATELY following completion of biochemical and serological tests, report confirmed results to MP per SOP MDP-DATA-01 on Atch 01, Results Notification Form, and prepare (3) isolate slants for shipment to ODA for antimicrobial susceptibility testing and, if necessary, PFGE and serotyping.".
- Deleted "Report results according to SOP MDP-DATA-01", Section 6.7.3

Revision 04 March 2009 Monitoring Programs Office

- Added 5.8 to References
- Added "Invitrogen" to 6.2.1.
- Deleted 6.2.3, mEC with Novobiocin
- Added "mEC + n selectively enriched cultures (pooled and individual samples)" to 6.5.1.
- Added "form both the pooled and each individual sample (the 3 samples that tested BAX positive from the pooled sample)" to 6.5.2.
- Deleted transferring UPBt to mEC + n from section 6.5.
- Added "pooled mEC + n and individual" to 6.5.3.
- Added 6.6.2.
- Revised the flowchart and removed reference to UPBt and selective enrichment in mEC + n.

Revision 03 April 2007 Monitoring Programs Office

- Added mEC+n broth for enriching BAX® PCR *E. coli* O157:H7 positive cultures at 42°C and 37°C.
- Added incubation of selective agar plates at two different temperatures, 37 and 42°C.

Revision 02 January 2006 Monitoring Programs Office

- Expanded isolation and identification steps
- Introduced serotyping
- Changed the requirement for IMS processing of a positive sample from one 1.0 mL sample to three to five 1.0 mL samples.
- Changed IMS to one of the methods in the isolation process

Revision 01 January 2005 Monitoring Programs Office

- Changed controls
- Modified to refer to manufacturer's instructions for the IMS protocol